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Determination of ionophore coccidiostats in feedingstuffs by liquid chromatography–tandem mass spectrometry Part I. Application to targeted feed

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ABSTRACT

A new and fit to purpose multi-analyte method for the determination of six coccidiostats (monensin A, salinomycin, narasin composed of its principal component narasin A and its minor component narasin I, lasalocid, semduramicin and maduramicin) in poultry and cattle compound feed by liquid chromatography tandem mass spectrometry (LC–MS/MS) has been developed and in-house validated. The concentration level of the target analytes at which the validation experiments have been carried out varied between 1 and 9 mg kg^{-1}. The method developed involved a simple extraction of the coccidiostats from the feed samples followed by a clean-up by solid-phase extraction prior to chromatographic analysis. The analytes were quantified either by matrix-matched standards or by the standard addition technique, obtaining the following performance profile of the method for the various analyte/matrix combinations. When quantifying against matrix-matched standards, the concentration independent intermediate precision expressed in terms of relative percentage standard deviation varied between 4 and 10% and the relative percentage recovery rates ranged from 86 to 120%, depending on the target analyte and matrix. When using the standard addition technique, the corresponding values for the intermediate precision varied between 2 and 8% and the relative percentage recovery rate ranged from 73 to 115%. The limit of detection (LOD) and limit of quantification (LOQ) were different for the various analyte/matrix combinations but were in all cases below 0.014 and 0.046 mg kg^{-1}, respectively.

Based on the obtained method performance characteristics, the method is considered suitable for the determination of ionophore coccidiostats in target feed. The main field of application of the validated method is to enforce European legislation regarding the authorisation of coccidiostats, focusing on the measurement at the authorised levels and at low level in feed during the withdrawal period at which the coccidiostats must not be added to the feed. Overall, the method proposed appears to be appropriate as a confirmatory method for the monitoring of these six ionophore coccidiostats and can therefore be considered as complementary to the official HPLC-UV methods.

1. Introduction

During the past years the use of antibiotics as feed additives has been recognised as a major risk for human health by the European legislator mostly because the presence of antibiotic residues in foodstuffs could cause toxic effects, directly in sensitive individuals such as allergic reactions and also indirectly because their widespread usage could be responsible for the promotion of resistant strains of bacteria. Following a ban of a number of antibiotics as feed additives such as tylosin, virginiamycin and spiramycin [1] from 1 January 2006 [2] no antibiotics other than coccidiostats and histomonostats can be marketed and used as feed additives within the European Union. Coccidiostats constitute the main choice to fight against coccidiosis. Coccidiosis is a major disease in poultry as well as in many other hosts. Commission Regulations authorise the use and the conditions of use of specific coccidiostats as feed additives, which are listed in the Community Register of Feed Additives [3]. The conditions of use specify 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 (withdrawal period) before slaughter when the use of these substances is prohibited.

The development, validation and implementation of reliable analytical methods are therefore of key importance to enforce the provisions laid down in the authorisation regulations of coccidiostats. In particular, the analytical methods should allow for

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measuring these substances at inclusion and trace level and preferably designed as multi-analyte method, in order to facilitate use in the frame of official control. Until now, few methods for screening or confirmation of some ionophore coccidiostatsts were published; that were either not sensitive enough for detection at low levels (less than 1 mg kg^{-1}) [4–7] or focussed at one or two ionophore coccidiostats [4,8] or required a derivatisation step [9,10]. Additionally, most multi-analyte methods for the determination of ionophore coccidiostats are targeted to food matrices [11,12]. Since recently a multi-analyte ISO method exists covering monensin, narasin and salinomycin and applying High Performance Liquid Chromatography (HPLC) with post-column derivatisation with vanillin [13].

The objective of this work was to develop and validate a new and fit to purpose multi-analyte method for the simultaneous determination of six ionophore coccidiostats (monensin A, salinomycin, narasin A and I, lasalocid, semduramicin and maduramicin) in animal feed by liquid chromatography tandem mass spectrometry (LC–MS/MS) which constitutes an improvement of the rare published methods that target feed matrices and in general allowing the determination of four coccidiostats [14]. Quantification was performed with matrix-matched standards and by applying the standard addition technique. The minimum authorised levels of the selected coccidiostats cover a quite large range varying from 5 mg kg^{-1} for maduramicin to 20 mg kg^{-1} for semduramicin, narasin and monensin, 60 mg kg^{-1} for salinomycin and 90 mg kg^{-1} for lasolocid [3]. The target concentration of the analytes in feed at trace level was set at 1 mg kg^{-1}.

Some authors developed analytical methods specifically for the determination of ionophore coccidiostats [15–19], but these authors focussed on animal tissues, eggs or surface water. These papers were however taken as a basis for the development of our method.

2. Experimental

2.1. Reagents and solvents

All chemicals and solvents used were of analytical purity and suitable for HPLC. For the ionophore coccidiostats, monensin A sodium (MON) was obtained from Calbiochem (Merck Sciences, Darmstadt, Germany), narasin factor I (NAR I) was provided by Eli Lilly (Indianapolis, USA), semduramicin (SEM) by Phibro Animal Health (Fairfield, NJ, USA), maduramicin (MAD) by Alpharma (Willow Island, USA), salinomycin (SAL), narasin A (NAR A), lasalocid (LAS) and nigericin (NIG) (used as internal standard for the LC–MS/MS measurement) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Methanol HPLC grade (MeOH) was from Fluka (Sigma–Aldrich) and formic acid 98% was obtained from Fluka Chemie (Steinheim, Germany). Pure water (H_2O) (18.2 MΩ cm^{-1} quality) used for the preparation of all the aqueous solutions was obtained from a MilliQ Plus 185 System (Millipore, Molsheim, F). Fig. 1 shows the chemical structures of the target compounds. Narasin is depicted in its major component narasin A.

2.2. Test samples

The test materials obtained from the European FP5 project SIMBAG-FEED [20] were compound feedingstuffs for cattle and poultry containing typical ingredients using a realistic recipe. Prior to use, the absence of the target analytes was confirmed by chemical analysis. These blank feed samples were afterwards fortified with the target analytes. The fortified samples were prepared by spiking individually the blank feed samples with target analytes dissolved in methanol, obtaining the target concentrations of the analytes in feed at three levels (1, 5 and 9 mg kg^{-1}). Prior to use the samples were left overnight to ensure a satisfactory penetration of the target ionophore coccidiostats into the feed matrix. The concentration range was selected considering the target level for the withdrawal period and the lowest target authorisation level, which is in this case 5 mg kg^{-1} for maduramicin.

2.3. LC–MS/MS conditions

All chromatographic measurements were performed with a HPLC Waters Alliance 2690 quarternary solvent delivery system (Waters Corporation, Milford, MA, USA) coupled to a Quattro LC triple stage quadrupole instrument from Micromass (Manchester, UK) for the mass spectrometry detection. The chromatographic separation of the ionophore coccidiostats was performed on a reverse phase Nucleosil® C18 (250 mm × 4.6 mm, 5 μm particle diameter) column from Alltech Associate Inc. (Lokeren, Belgium) equipped with a Nucleosil® C18 guard column 7.5 mm × 4.6 mm, 5 μm particle diameter from Alltech Associate Inc. (Lokeren, Belgium). The mobile phase was composed of a 94:6 (v:v) mixture of MeOH containing 0.1% formic acid and H_2O containing 0.1% formic acid. The flow rate of the mobile phase was 1.0 ml min^{-1}. A T-piece splitter (4:1) was used between the LC column and the MS detector in order to introduce 0.25 ml min^{-1} effluent into the ion source of the mass spectrometer. The column temperature was 25 °C and the sample temperature was kept at 4 °C by means of the thermostated carroussel of the LC autosampler. The injection volume in the LC–MS/MS system was 40 μl for all samples injected.

For the detection, the electrospray positive ionisation mode (ESI+) was used and the ions were monitored in the multiple reaction-monitoring (MRM) mode. The following MS conditions were applied. The capillary voltage was set at +3.7 kV, the source block and desolvation temperatures were 120 °C and 400 °C, respectively. The desolvation and nebuliser gas (N_2) flow rates were 600 l h^{-1} and 90 l h^{-1}, respectively. Argon pressure in the collision cell was 2.5×10^{-3} mbar. The cone voltage, the transitions and the collision energy for the MRM acquisitions are given in Table 1 as well as the indicative retention time on the column. The dwell time was 100 ms/transition. Four points were taken into account for the identification, earned by the parent ion and two fragment ions. Since the MRM mode was chosen in this method, two transitions were thus followed for confirmation of the identity of the analysed compound. For quantification, only transition 1 (Table 1) was used.

2.4. Procedures

2.4.1. Sample extraction

5.0 ± 0.1 g of the test sample was extracted with 100 ml of a MeOH:H_2O mixture (90:10, v:v) for 60 min by agitation (GFL 3018, Gesellschaft für Labortechnick mbH, Burgwedel, Germany) followed by 10 min centrifugation at 1850 × *g*.

2.4.2. SPE clean-up

The silica cartridge (IST Isolute, Mid Glamorgan, United Kingdom), used as a filter, was first conditioned with 2 × 1 ml of MeOH and load with 5 ml of the sample extract. The elution was performed under vacuum with a Vacuum/Pressure station (Bartnant Company, Barington, IL, USA) on an Alltech vacuum Manifold and the filtrate was collected. The target analytes where then eluted with 4 × 1 ml of MeOH collected in the same tube than the filtrate. The resulting eluate was then evaporated up to dryness under a gentle stream of nitrogen at +40 °C. Finally, the dry residue was redissolved in 200 μl of the internal standard solution and 4.8 ml of MeOH with the vortex (Heidolph Type relax top, Germany) and a short centrifugation at 1850 × *g*.

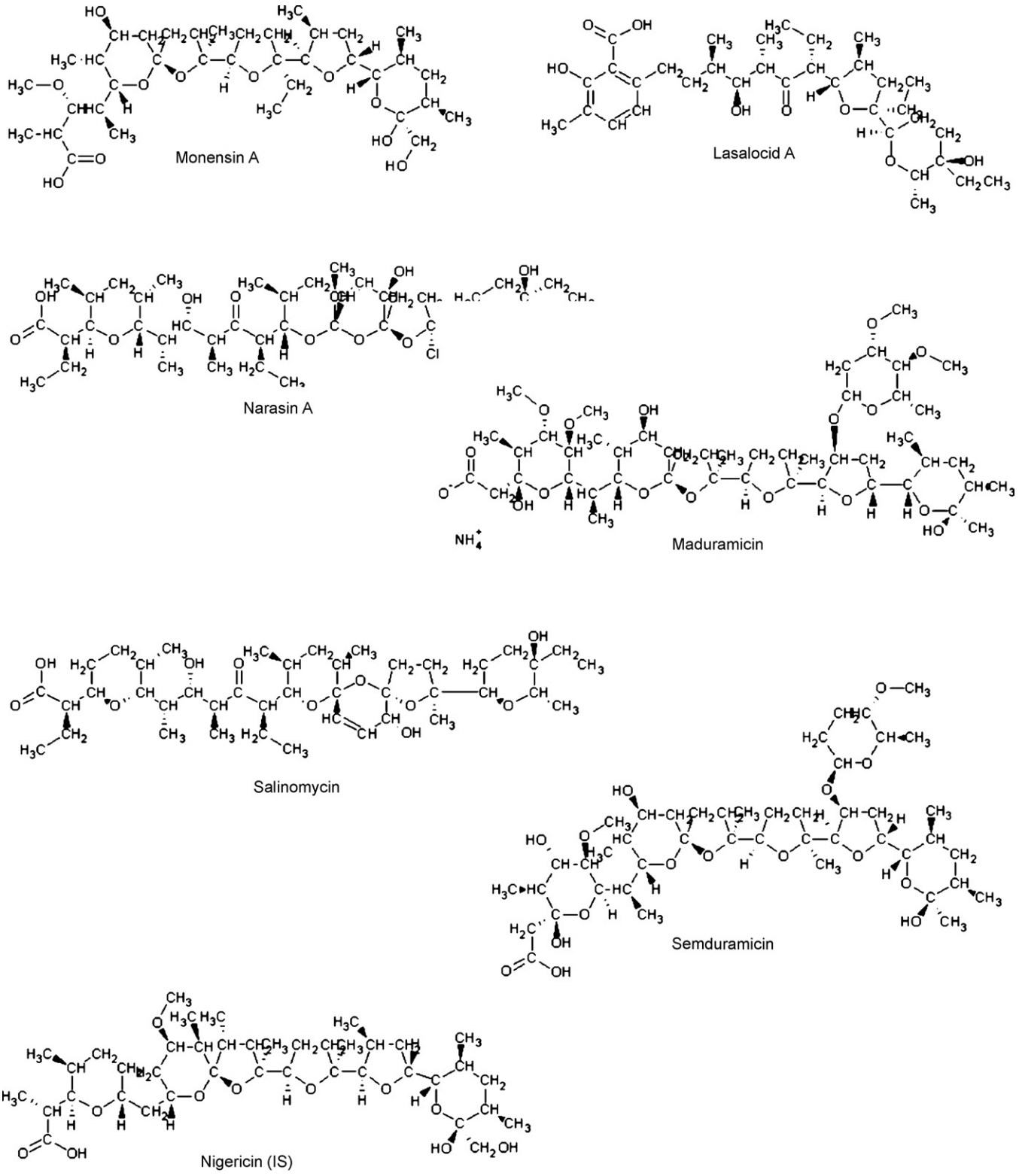

Fig. 1. Chemical structures of the target analytes and of the internal standard.

A chromatogram of the LC–MS/MS analysis of a fortified cattle feed sample is shown in Fig. 2.

During the development phase of the method we also tested the suitability of an alumina column which however failed since lasalocid could not be eluted from the cartridge. Therefore the silica cartridge was selected.

2.4.3. Calibration solutions and quantification

Matrix-matched standards were prepared by submitting 5.0 ± 0.1 g of the blank feedingstuffs to the sample preparation procedure. The dry residue was re-suspended in 5 ml of MeOH. From this solution the calibration dilutions were prepared containing the target compounds at various concentrations ranging from 0.4 to

Table 1
MS and MS/MS conditions for the acquisition in MRM ESI positive mode for the target six ionophore coccidiostats

	Parent ion	Transition 1	Transition 2	Cone voltage (V)	Collision energy (eV)
Semduramicin	895.42	833.66	851.67	35	35
Monensin A	693.44	461.38	479.15	50	55
Lasalocid A	613.34	376.67	359.36	50	40
Maduramicin	939.55	877.61	719.11	35	30
Salinomycin	773.48	431.45	431.32	50	50
Nigericin (IS)	749.51	729.51	703.47	50	40
Narasin A	787.40	431.39	531.35	50	55
Narasin I	801.50	531.28	431.32	50	50

10 mg l^{-1} and the internal standard at a constant concentration of 0.2 mg l^{-1}. The values for the MS response of each of the target analytes were divided by the corresponding response of the internal standard and the ratio was plotted against the concentration of the target analytes. The calibration was performed by weighted regression.

When developing the method we also compared the quantification of the analytes with standard calibration curves and matrix-matched standards. The results revealed a strong matrix effect, since standard calibration curves yielded high recoveries, e.g. for semduramicin above 250%. Therefore the validation experiments were carried out using matrix-matched standards.

2.4.4. Standard addition technique

The performance of this technique was evaluated at three concentration levels of the target analytes, namely 1, 3 and 5 mg kg^{-1} (fortification level). Four samples were prepared separately for *each* concentration level by fortifying individually the samples applying the procedure explained under the paragraph "test samples". For the application of the standard addition technique, one of the fortified samples was analysed as such, whereas the other three samples of the same fortification level were spiked prior to analysis with the target analytes at increasing 3 levels of concentrations, which were also 1, 3 and 5 mg kg^{-1} (standard addition levels). All four fractions were submitted to the sample preparation procedure and the internal standard was added before determination by LC–MS/MS. The ratios of the peak areas of the target analytes and the internal standard were plotted versus the concentration and the intercept of this regression line with the *x*-axis gave the initial analyte concentration in the sample.

3. In-house validation

Analytical methods for the determination of coccidiostats suitable for official control within the European Union need to comply with legislation or with commonly accepted criteria. Performance

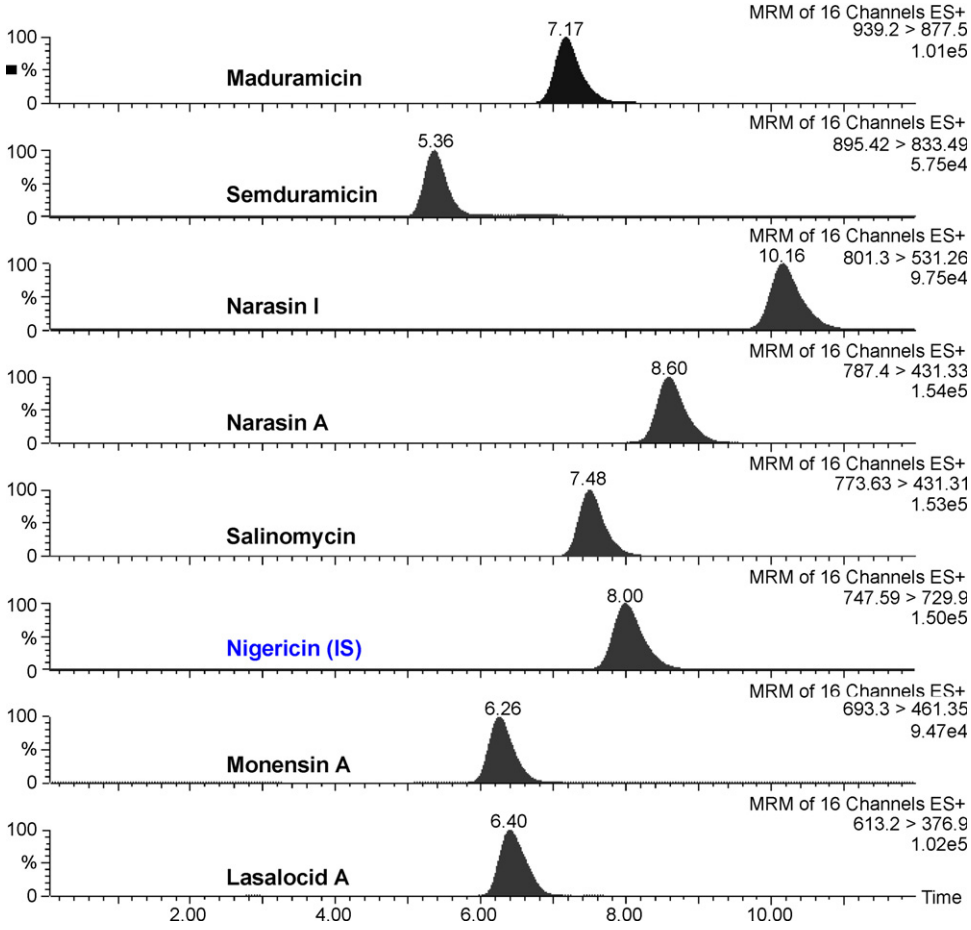

Fig. 2. LC–MS/MS separation of six ionophore coccidiostats after extraction and clean-up on silica SPE cartridge of a cattle feed sample containing the analytes at a concentration of 1 mg kg^{-1}.

criteria are set by Commission Decision 657/2002 [21]. However, the application of this Commission Decision is confined to specific *food matrices*, whereas a corresponding document specifically designed for the analysis of *feed matrices* does not exist. Nevertheless important aspects of this document were taken into account for the validation of the method as well as other internationally recognised guidelines [22,23] on single laboratory validation. In addition, some criteria of the above-mentioned Commission Decision have been utilised when the impact of the different matrices (feed and not food) was considered minor. The validation of the method included the estimation of the limit of detection (LOD), limit of quantification (LOQ), precision under repeatability and intermediate conditions and the trueness. The concentration range included in this validation study was below the target authorisation level of some of the analytes (e.g. 90 mg kg^{-1} for lasolocid). However, the results of this validation study also apply to the higher concentrations, if the extracts are appropriately diluted prior to analysis.

3.1. Sensitivity

The limits of detection (LODs) and limits of quantification (LOQs) were determined using matrix-matched calibration curves established at appropriate low concentration levels of the target analytes in feed. In detail, three samples of each type of feed were individually fortified with different amounts of the target analytes and subsequently subjected to the whole analytical procedure. The fortification level of the target analytes was close to the assumed LODs and LOQs. Based on these measurements calibration curves for each analyte were established which were then utilised to calculate the LODs and LOQs [24]. As shown in Table 2 all LODs and LOQs were below 0.014 and 0.046 mg kg^{-1} and therefore well below the target concentration of 1 mg kg^{-1}, demonstrating that the developed method is sensitive enough for the intended purpose.

Table 2
Limits of detection (LODs) and of quantification (LOQs) obtained for six ionophore coccidiostats in cattle and poultry feed

	Poultry		Cattle	
	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
Monensin	0.003	0.010	0.001	0.004
Salinomycin	0.007	0.023	0.006	0.019
Narasin A	0.002	0.007	0.005	0.016
Narasin I	0.001	0.005	0.002	0.005
Lasalocid	0.003	0.009	0.008	0.026
Maduramicin	0.014	0.046	0.005	0.017
Semduramicin	0.006	0.020	0.006	0.020

Note: LOD is given by $(3 \times Sy/x)/b$ and LOQ by$(10 \times Sy/x)/b$ for each analyte, where Sy/x corresponds to the standard deviation of the residuals and b is the slope of the calibration curve [24].

3.2. Precision

The precision of the method was determined according to ISO standard 5725-3 [23] under different circumstances, namely repeatability conditions where the experiments were carried out on the same day and intermediate conditions where the experiments were distributed over different days. All experiments were conducted by the same technician using the same instrumentation. The experiments were carried out at three concentrations of the analyte in the feed, which were 1, 5 and 9 mg kg^{-1}. This exercise was applied for cattle and poultry feed, respectively. Fitness for purpose criteria for the precision were taken from Commission Decision 2002/657 [21], specifying that the intermediate precision

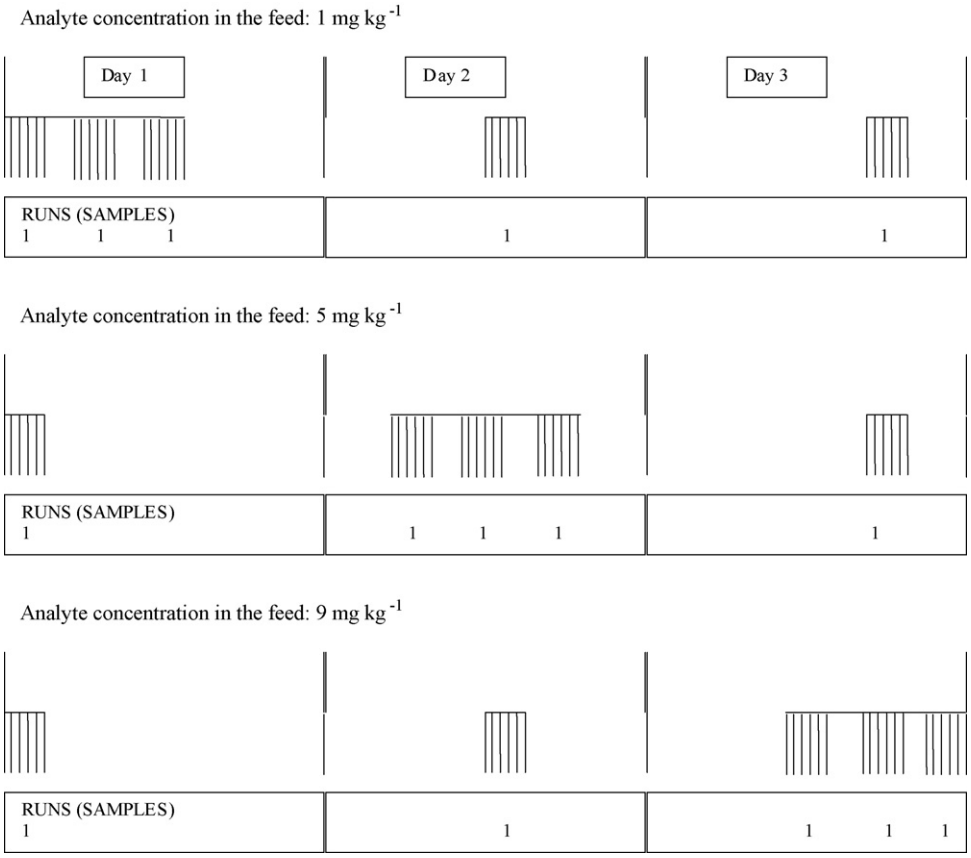

Fig. 3. Design of the experiments for calculating the precision and the trueness of the method.

Table 3
Results from the statistical evaluation for the target coccidiostats in poultry feed

	Target value (mg kg^{-1})	Mean value (mg kg^{-1})	RR (%)	S_r	RSD_r, %	$S_{Int.}$	$RSD_{Int.}$, %	Horrat value
Maduramicin	1	0.9	89	0.04	4.2	0.05	6.0	0.4
	5	4.8	96	0.27	5.6	0.38	7.9	0.6
	9	8.0	89	0.19	2.4	0.91	11.4	1.0
Semduramicin	1	1.0	104	0.08	7.4	0.08	7.6	0.5
	5	4.8	96	0.33	6.8	0.35	7.3	0.6
	9	8.4	93	0.49	5.8	0.73	8.7	0.7
Narasin I	1	0.8	84	0.03	3.6	0.04	4.7	0.3
	5	4.5	89	0.14	3.1	0.34	7.5	0.6
	9	7.8	86	0.17	2.2	0.58	7.5	0.6
Narasin A	1	0.9	90	0.02	2.0	0.08	8.5	0.5
	5	4.6	93	0.13	2.7	0.21	4.5	0.4
	9	7.9	87	0.16	2.1	0.56	7.1	0.6
Salinomycin	1	0.9	88	0.02	2.5	0.06	6.3	0.4
	5	4.4	89	0.1	2.2	0.14	3.1	0.2
	9	7.9	87	0.17	2.2	0.29	3.6	0.3
Lasalocid	1	1.0	101	0.03	2.5	0.16	16.0	1.0
	5	5.0	100	0.29	5.7	0.42	8.3	0.7
	9	8.6	95	0.31	3.6	0.82	9.5	0.8
Monensin	1	0.9	90	0.02	2.2	0.07	7.7	0.5
	5	4.5	91	0.11	2.4	0.3	6.6	0.5
	9	7.7	86	0.15	2.0	0.6	7.8	0.7

S_r, standard deviation for repeatability; RSD_r (%), relative standard deviation for repeatability; $S_{Int.}$, standard deviation for intermediate precision; $RSD_{Int.}$ (%), relative standard deviation for intermediate precision. The mean value is calculated from the 30 results obtained for each analyte/concentration/matrix combination. RR (%), mean percentage recovery rate.

were considered acceptable when these value were not larger than the precision calculated by the Horwitz equation [25]. This condition was expressed by the HORRAT value which is the ratio of the experimentally obtained values of the standard deviation and the target standard deviation calculated by the Horwitz equation [25]. As a fitness for purpose criterion this value should not be above 1.

3.2.1. Statistical model

The experimental design is shown in Fig. 3. Five samples containing the target analytes at the same concentration were distributed over 3 days, analysing three samples on the same day and two samples on 2 other days. Each sample was injected six times, obtaining 30 results for each analyte/concentration/matrix combination. Therefore, the experiment is a 3 factor-staggered-nested design as specified in the ISO standard [23]. The total variability of the analytical results can be attributed to the following levels of variability,

- between-days variability (D_i),
- between-samples variability analysed on the same day [within-days] (S_{ij}),
- between-injection variability [within-samples] (W_{ijk}).

The between injection and between samples variability contribute to the precision under repeatability conditions, whereas all components including the between days variability give the intermediate precision.

The model that underlies the analysis of variance of the data collected by the staggered nested design is that each of the measurement, Y_{ijk} is defined as the sum of 3 variance components plus true value (TV) of standard material measured (fixed quantity) and these 3 components are estimated as follows:

$$Y_{ijk} = \mathrm{TV} + D_i + S_{ij} + W_{ijk}$$

For the calculation of the repeatability standard deviation and intermediate precision the analytical results were subjected to analysis of variance (ANOVA) using the software package MINITAB^{TM} Statistical Software for Windows (Version 15).

3.2.2. Results of the statistical assessment

The results of the statistical evaluation are shown in Table 3 for poultry feed and Table 4 for cattle feed, respectively. The obtained relative standard deviation for repeatability varied from 1.8 to 7.8 and the relative standard deviation of intermediate precision ranged from 3 to 16%, depending on the target analyte, concentration and feedingstuff. However, in all cases the HORRAT values were equal or below 1, thus indicating that the precision of the method fulfils the criteria.

3.2.3. Estimation of the trueness and precision of the method independent of the analyte concentration

As recommended by ISO standard 5725 [23] the data were also evaluated to establish whether a concentration independent trueness and precision of the method for each compound and matrix could be calculated. Plotting the *absolute* standard deviation of intermediate precision against the analyte concentration revealed a strong dependence, whereas the corresponding *relative* standard deviation was not much affected by the analyte concentration. Therefore all measured concentrations were first divided by the respective mean values given in Tables 3 and 4 in order to obtain normalised data. Since the normalised data do not depend anymore on the respective concentrations, they can be pooled to one data set per analyte and matrix. These data were subsequently subjected to statistical analysis. The obtained standard deviations of these normalised concentrations are identical to the *relative* standard deviations of the original measurements (i.e. *absolute* concentrations) and are shown in the aggregated Table 5. The statistical evaluation revealed satisfactory values for the intermediate precision that are *independent* of the concentration within the frame of this study.

Table 4
Results from the statistical evaluation for the target coccidiostats in cattle feed

	Target value (mg kg^{-1})	Mean value (mg kg^{-1})	RR (%)	S_r	RSD$_r$, %	$S_{Int.}$	RSD$_{Int.}$, %	Horrat value
Maduramicin	1	1.0	100	0.04	4.2	0.08	7.5	0.5
	5	5.0	100	0.30	6.0	0.3	6.0	0.5
	9	8.4	93	0.32	3.8	0.54	6.4	0.6
Semduramicin	1	1.3	130	0.10	7.2	0.1	7.2	0.5
	5	5.9	118	0.16	2.7	0.4	6.8	0.6
	9	10	111	0.24	2.3	0.81	7.9	0.7
Narasin I	1	1.0	100	0.04	3.7	0.08	7.9	0.5
	5	4.7	94	0.11	2.2	0.15	3.3	0.3
	9	8.2	91	0.18	2.2	0.39	4.7	0.4
Narasin A	1	1.0	100	0.03	3.2	0.07	7.3	0.5
	5	4.8	96	0.18	3.7	0.18	3.7	0.3
	9	8.3	92	0.17	2.0	0.36	4.3	0.4
Salinomycin	1	1.0	100	0.04	4.1	0.04	4.2	0.3
	5	4.8	96	0.15	3.1	0.15	3.2	0.3
	9	8.3	92	0.15	1.8	0.4	4.8	0.4
Lasalocid	1	1.1	110	0.05	4.2	0.07	6.8	0.4
	5	4.9	98	0.34	7.0	0.5	10	0.8
	9	7.6	84	0.25	3.3	0.85	11	0.9
Monensin	1	1.0	100	0.04	3.9	0.06	5.5	0.3
	5	4.8	96	0.18	3.8	0.18	3.8	0.3
	9	8.4	93	0.17	2.0	0.4	4.8	0.4

S_r, standard deviation for repeatability; RSD$_r$ (%), relative standard deviation for repeatability; $S_{Int.}$, standard deviation for intermediate precision; RSD$_{Int.}$ (%), relative standard deviation for intermediate precision. The mean value is calculated from the 30 results obtained for each analyte/concentration/matrix combination. RR (%), mean percentage recovery rate.

3.3. Trueness estimated from the obtained recovery rates

The trueness of the method was calculated from the mean values of the results from all trials of the experimental design shown in Fig. 3 and expressed in terms of the percentage recovery rate. The obtained values for the recovery rate are given in Table 3 for poultry feed and in Table 4 for cattle feed. The values ranged from 84 to 130%, the latter for semduramicin at 1 mg kg^{-1} in cattle feed. Given the complexity of the feed matrix, the obtained estimates were considered acceptable.

3.4. Trueness and precision data applying the standard addition technique

Trials as described in the experimental part were conducted on samples containing the target analytes at 1, 3 and 5 mg kg^{-1} in order to estimate the trueness and precision of the method when applying the standard addition technique. Table 6 shows the results obtained on the three levels expressed in terms of the mean recovery rates and the corresponding relative standard deviations, reflecting intermediate precision, since the experiments were carried out at different days for the three concentrations. With the exception of semduramicin in poultry the trueness of the method showed good results for all compound/matrix combinations, since the obtained percentage recovery rates varied between 86 and 120%. As observed in the experiments utilising matrix-matched standards (Table 5), the values for the percentage recovery rates for the target analytes in cattle feed where somewhat higher compared to the corresponding values in poultry feed. The obtained values for the relative percentage standard deviation ranged from 2 to 8% which were somewhat lower compared to the corresponding precision of the matrix-matched calibration approach.

Table 5
Results from the statistical evaluation of the results for each compound/matrix, with the matrix-matched calibration curves and in which the results from the three concentrations (1, 5 and 9 mg kg^{-1}) have been pooled

	Poultry feed		Cattle feed	
	RR (%)	RSD$_{Int.}$ (%)	RR (%)	RSD$_{Int.}$ (%)
Maduramicin	91	8.0	98	5.5
Semduramicin	98	7.4	120	6.7
Narasin I	86	6.0	95	4.8
Narasin A	90	5.9	96	4.7
Salinomycin	88	4.1	96	3.8
Lasalocid	99	10	97	8.2
Monensin	89	6.5	96	4.2

RR (%), mean percentage recovery rate; RSD$_{Int.}$ (%), relative standard deviation for intermediate precision.

Table 6
Results from the standard addition experiments conducted on fortified samples containing 1, 3 and 5 mg kg^{-1} of the target analytes

	Poultry feed		Cattle feed	
	RR (%)	RSD$_{Int.}$ (%)	RR (%)	RSD$_{Int.}$ (%)
Maduramicin	87	4.6	105	6.2
Semduramicin	73	2.7	111	8.3
Narasin I	112	1.9	105	4.3
Narasin A	96	7.9	104	5.4
Salinomycin	102	5.9	109	4.0
Lasalocid	98	5.3	115	5.2
Monensin	100	8.1	111	3.1

RR (%), mean percentage recovery rate of the recovery rates obtained on the three concentrations; RSD$_{Int.}$ (%), relative standard deviation for intermediate precision.

4. Conclusion

A new LC–MS/MS method fit for the purpose of detection and confirmation of semduramicin, maduramicin, narasin A and I, lasalocid A, salinomycin and monensin A ionophore coccidiostats in poultry and cattle feed has been developed and single laboratory validated. The quantification was carried out using two different approaches, namely the use of matrix standards and the application of the standard addition technique. The results of the validation study confirmed satisfactory values for the sensitivity, precision

and trueness of developed method, regardless of which calibration technique is applied. Therefore it is shown that the developed method is fit for the purpose to be used in the frame of official control for the control of the presence of the coccidiostats included in this study at target and trace level.

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