AGRICULTURAL MATERIALS

Determination of Decoquinate in Animal Feeds by Liquid Chromatography: Collaborative Study

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The performance characteristics of a liquid chromatographic (LC) method for the analysis of decoquinate (DEC) in supplements, premixes, and complete animal feeds at medicating and trace levels were collaboratively studied. DEC is extracted from ground feed samples with 1% calcium chloride–methanol solution using mechanical agitation for 90 min. After centrifugation for 5 min and dilution (if necessary), an aliquot of the extract is diluted with water. The diluted extracts are filtered and analyzed by reversed-phase LC with fluorescence detection. Suspect positive trace-level samples are confirmed by using an alternate excitation wavelength. Fourteen test samples of medicated feeds, supplement, and medicated premix, along with 8 test samples for trace-level analysis, were sent to 13 collaborators (one in Canada, 4 in Europe, and 8 in the United States). Test samples were analyzed as blind duplicates. Acceptable results were received from 12 laboratories for the medicated test samples and from 13 laboratories for the trace-level samples. Repeatability relative standard deviation estimates ranged from 1.3 to 5.6%. Reproducibility relative standard deviations estimates ranged from 2.8 to 6.1%, and HorRat values ranged from 0.22 to 0.74.

Decoquinate (ethyl 6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate; DEC) is an anticoccidial feed additive approved for use in feeds for broiler chickens, cattle and calves, sheep, and goats. DEC is approved for use in the prevention of coccidiosis in ruminating and nonruminating calves, including veal calves and cattle, caused by *Eimeria bovis* and *Eimeria zeuernii* (1, 2). It is used in the prevention of caecal and intestinal coccidiosis in broiler chickens caused by *Eimeria tenella*, *Eimeria necatrix*, *Eimeria acervulina*, *Eimeria mivati*, *Eimeria maxima*, or *Eimeria burnetti* (1, 2). It is also used as an aid in the prevention of coccidiosis caused by *Eimeria christenseni* and *Eimeria ninakohlyakimovae* in nonlactating young goats, and by *Eimeria bakuensis*, *Eimeria crandallis*, *Eimeria ovinoidalis*, and *Eimeria parva* in nonlactating sheep (2).

The only current official method of analysis for DEC in animal feed is AOAC Official Method **969.55** (3). This method consists of a combination of solvent extraction with 1% calcium chloride–methanol solution, cleanup by liquid–liquid partitioning and Florisil column chromatography followed by quantitative spectrofluorometric determination. This method is time consuming and tedious and does not lend itself to trace-level analysis.

The AOAC Agricultural Materials Community, Feed Additives Group, has identified the need for an official liquid chromatographic (LC) method for the determination of DEC levels in various feeds, supplements, and premixes (4). Methodology is also required for the determination of contamination levels to verify cleanout of feed manufacturing equipment for the prevention of cross-contamination.

The basis of this method is the extraction of DEC from ground feed samples using 1% calcium chloride–methanol solution [same as Official Method **969.55** (3)], followed by a brief centrifugation, dilution, and analysis by reversed-phase LC with fluorescence detection. Suspect positive trace-level samples are confirmed by using an alternate excitation wavelength. Here, we demonstrate the performance characteristics of this method in an interlaboratory study.

Single-Laboratory Validation

Method validation was performed by the authors' laboratory in 2004 (5). The validation emphasized the applicability of the method to medicated and low-level feeds, as well as the development and validation of the confirmation

Submitted for publication June 2008.
The recommendation was approved by the Methods Committee on Feeds, Fertilizer, and Related Agricultural Topics as First Action. *See* "Official Methods Program Actions," (2008) *Inside Laboratory Management*, July/August issue.
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Table 1. Homogeneity testing of test materials: analysis of variance (ANOVA) results

Level	Description	n^a	Avg., mg/kg	F	F_{crit}
Medicated	C, Calf feed, texturized	8	49.2	1.89	3.50
	D, Calf feed	8	27.4	0.90	3.50
	E, Lamb feed	8	15.9	1.47	3.50
	F, Supplement	8	97.6	1.13	3.50
	G, Chicken feed	8	41.0	1.37	3.50
	H, Medicated premix	8	1261	0.72	3.50
Trace	I, Calf feed, texturized	8	4.59	2.83	3.50
	J, Swine feed	8	2.07	1.30	3.50

[a] n = Number of test portions tested.

technique mentioned above. In addition, through the analysis of medicated premixes submitted by feed inspectors and similar premixes provided for the collaborative study, it was also demonstrated that the method was applicable to the analysis of these products. During this validation, the mobile phase composition consisted of methanol–water containing $MgSO_4$. As a result of discussions in late 2006 with a laboratory using a similar method, it was decided to discontinue the use of $MgSO_4$ in the mobile phase and replace it with $CaCl_2$. Both of these compounds cause DEC to fluoresce, but $CaCl_2$ provides advantages in ease of preparation of the mobile phase, and in reducing the possibility of precipitation in the LC system. The method was further validated by the authors' laboratory to examine the replacement of $CaCl_2$ in the mobile phase (6). Results of the validation showed that the change to $CaCl_2$ content in the mobile phase did not affect the following parameters: excitation and emission wavelengths, instrument linearity, DEC retention time, peak asymmetry, or the limit of quantitation (LOQ) and limit of detection (LOD). In addition, the change to $CaCl_2$ in the mobile phase provided an enhanced peak response. Because no changes were made to the extraction procedure and the results obtained in the validation study (6) did not effect to the LOQ and LOD, precision and accuracy, the drug interference study, and other ruggedness test data, it was concluded that the previous validation study conducted with $MgSO_4$ remained valid.

A summary of the single laboratory validation data is as follows:

(**a**) *Drug interference study*.—A drug interference study was conducted with 47 drugs and antibiotics. No interference peaks were observed in the DEC retention time window, except for florfenicol. Florfenicol, a feed additive used only in aquaculture feeds, was eliminated as a false-positive interference with the use of the method's confirmation procedure.

(**b**) *Linearity of standard curve*.—The study showed that a correlation coefficient of >0.999 can be achieved for both medicating level and trace-level standards.

(**c**) *Precision study*.—Results of triplicate analyses conducted with commercial medicated feeds samples containing 14–850 mg/kg and commercial feeds contaminated at 0.5–6 mg/kg showed a mean relative standard deviation (RSD) of 2.8 and 7.1%, respectively.

(**d**) *Recovery studies of spiked quality control samples*.—Results of quadruplicate analyses conducted with samples spiked at 30, 90, and 300 mg/kg generated recoveries ranging from 98.0 to 103.4%, with RSDs ranging between 0.5 and 1.5%. For samples spiked at 0.6, 3, and 9 mg/kg, recoveries ranged from 101.5 to 102.3%, with RSDs ranging between 2.1 and 4.3%.

(**e**) *LOQ*.—The LOQ was determined to be 0.5 mg/kg for all feeds.

Collaborative Study

A collaborative study was organized and carried out in accordance with the *Collaborative Study Guidelines* of AOAC INTERNATIONAL (7). The materials included in this study were representative of animal feeds, premix, and supplement materials that would typically contain DEC. All samples were commercial medicated or exposure-contaminated (trace-level samples) feed products. Concentration for the test samples ranged from trace level (2 mg/kg) to medicated premix (1300 mg/kg). Test samples were prepared by grinding a suitable amount of dry test material with a Retsch ZM1 mill equipped with a 0.75 mm sieve. The ground material was mixed in a V-Mixer for 3 h, and then subdivided into approximately 15 g (medicated level) and 25 g (trace level) test portions using a Retsch rotary PTZ divider. The test portions were subsequently bagged in polyethylene bags, each labeled with a unique sample number.

Sixteen samples from each batch of packaged material were randomly selected and removed for homogeneity testing. Per batch, the 16 samples were paired to produce 8 test portions, which were then mixed thoroughly and analyzed in duplicate using the proposed method. Data produced from the homogeneity testing were evaluated by means of analysis of variance (ANOVA). Study materials were considered to be

homogeneous if the calculated *F*-value (*F*) was less than the critical *F*-value (F_{crit}; $\alpha = 0.05$). The ANOVA results showed that the difference of the between-group variance and the within-group variance regarding all test materials was not significant. The description and homogeneity test results for each material are shown in Table 1. All test materials met the homogeneity criteria.

Study Design

The collaborative study was conducted in 2 phases: a familiarization study phase and a collaborative study phase. Participating laboratories were required to demonstrate proficiency with the method on the familiarization samples prior to analyzing study samples.

For the familiarization phase, the laboratories were provided with reference standards (with certified purity of 98.5%) and 10 feed samples (as blind duplicates): 2 blank calf starter feed samples (to be fortified at medicated level, 30 mg/kg, as instructed in the method); 2 medicated-level samples (sample C, Table 1); 4 blank calf starter feed samples (2 to be analyzed as a blank and the remainder to be fortified at trace level, 1.5 mg/kg, as instructed in the method); and 2 trace-level samples (sample I, Table 1). Each participant was also supplied with a set of instructions, a copy of the method to be followed, and a results reporting form (Excel file). As described in the method, laboratories were requested to prepare and calibrate the reference standard solutions, and verify the system suitability and detector linearity. Laboratories were instructed to process the blank, fortified blanks, and test samples on either 2 separate days (a set of trace-level and medicated-level samples prepared and analyzed on separate days) or on 4 separate days (preparation and analysis of a set of trace-level samples on 2 separate days and a medicated-level sample set on another 2 separate days). Collaborators were also instructed to confirm the presence of DEC in sample I by using the confirmation procedure (alternative fluorescence excitation wavelength) described in the method. Thirteen laboratories returned acceptable data within a suitable time frame.

For the collaborative phase of this study, the 13 candidate laboratories were provided with (as blind duplicates): 12 medicated test samples (samples C–H, Table 1); 2 blank calf starter feed samples (to be fortified at medicated level, 30 mg/kg, as instructed in the method); 4 trace-level test samples (samples I and J, Table 1); and 4 blank calf starter feed samples (2 to be analyzed as a blank and the remainder to be fortified at trace level, 1.5 mg/kg, as instructed in the method). Each participant was also supplied with a new set of instructions and results reporting form (Excel file). Instructions for the processing of samples were the same as those given during the familiarization phase of the study (2 or 4 separate days).

AOAC Official Method 2008.08
Decoquinate in Animal Feeds
Liquid Chromatography
First Action 2008

Caution: Refer to *Material Safety Data Sheets*, or equivalent, for each reagent. Dispose of waste solvents in an appropriate manner compatible with applicable environmental rules and regulations. Use all solvents in a fume hood. Wear safety glasses, protective clothing, and avoid skin contact and breathing of dusts or vapors.

See Table **2008.08A** for the results of the interlaboratory study supporting acceptance of the method.

A. Scope

Applicable for the determination of decoquinate (DEC) content in the following types of animal feeds: chicken, calf and cattle, goats and sheep, supplements, and medicated premixes. The limit of quantitation (LOQ) is 0.5 mg/kg. A lower limit may be achievable subject to appropriate validation being conducted by the user laboratory.

B. Principle

DEC is extracted from ground feed samples with 1% calcium chloride–methanol solution using mechanical shaking for 90 min. After centrifugation and dilution, an aliquot is diluted with water, filtered, and analyzed by

Table 2008.08A. Interlaboratory study results of decoquinate in animal feeds

Material	Target, mg/kg	Mean, mg/kg	No. of labs (outliers)	s_r, mg/kg	s_R, mg/kg	RSD_r, %	RSD_R, %	HorRat
C, Calf feed, texturized	49.2	49.8	11 (1)	0.89	1.72	1.78	3.46	0.39
D, Calf feed	27.4	27.4	11 (1)	0.35	0.91	1.29	3.34	0.34
E, Lamb feed	15.9	15.0	10 (2)	0.35	0.92	2.34	6.14	0.58
F, Supplement	97.6	99.3	10 (2)	1.37	2.91	1.38	2.93	0.37
G, Chicken feed	41.0	41.4	11 (1)	0.69	1.47	1.67	3.54	0.39
H, Medicated premix	1260	1310	11 (1)	24.77	52.62	1.89	4.01	0.74
I, Calf feed, texturized	4.6	4.8	13	0.11	0.13	2.23	2.82	0.22
J, Swine feed	2.1	2.0	12 (1)	0.11	0.11	5.55	5.55	0.38

reversed-phase liquid chromatography (LC) with fluorescence detection. Positive trace-level samples are confirmed by LC analysis using an alternate excitation wavelength.

C. Apparatus

Common laboratory apparatus and, in particular, the following:

(**a**) *LC system.*—Consisting of the following: *Pump*.—Pulse free, flow capacity of 0.5–2.0 mL/min. *Injection system*.—Manual or autosampler, with loop suitable for 50 µL injections. *Fluorescence detector*.—For operation at 266 and 318 nm excitation and 394 nm emission wavelengths. *Integrator or computer data system*. *Analytical column*.—Waters Corp. (Milford, MA) Symmetry C_{18}, 5 µm, 0.46 × 25 cm, or equivalent, with C_{18} guard column.

(**b**) *Shaker*.—Rotary or wrist-action.

(**c**) *Balances*.—Analytical balance of 10 g capacity or greater with 0.1 mg readability, and top-loading of 100 g capacity or greater with 0.01 g readability.

(**d**) *Centrifuge*.—Capable of holding and spinning 50 mL centrifuge tubes at 200 *g*.

(**e**) *Centrifuge tube*.—50 mL polypropylene, or equivalent.

(**f**) *Erlenmeyer flasks*.—250 mL with glass stoppers.

(**g**) *Solvent filtration system*.—All-glass filter apparatus suitable for 47 mm filter and 47 mm diameter nylon filters with pore size of 0.45 µm.

(**h**) *Test solution filters*.—Equipped with nylon filter with pore size of 0.45 µm.

D. Reagents

Use only ACS grade reagents, unless otherwise specified.

(**a**) *Methanol*.—LC grade, or equivalent.

(**b**) *Water*.—LC grade, or equivalent.

(**c**) *Calcium chloride ($CaCl_2$), dihydrate*.—Minimum 99% purity, or equivalent.

(**d**) *Extraction solvent, 1% calcium chloride–methanol (w/v)*.—Dissolve 14.7 g calcium chloride, (**c**), in 1 L methanol, (**a**). Mix well.

(**e**) *Mobile phase*.—Dissolve 5.0 g of calcium chloride, (**c**), in 1 L methanol–water (825 + 175), (**f**). Mix well, and filter under vacuum using the solvent filtration system, **C**(**g**).

(**f**) *Methanol–water (825 + 175)*.—Mix 825 mL methanol, (**a**), with 175 mL water, (**b**). Mix well, and filter under vacuum using the solvent filtration system, **C**(**g**).

(**g**) *DEC reference standard*.—Alpharma Inc. (Toronto, ON, Canada). Composition and potency are required for each lot of reference standard. Record all standard solution concentrations to at least 3 significant figures.

(**h**) *Stock standard solution*.—Ca 300 µg/mL. Accurately weigh, to the nearest 0.1 mg, 30 mg reference standard, (**g**), into a 100 mL volumetric flask. Dissolve in 1% calcium chloride–methanol, (**d**). Use an ultrasonic bath if necessary to aid dissolution, and dilute to volume with the same solution. Mix well. Calculate the concentration based on the purity of the reference standard. Prepare fresh monthly and store in a refrigerator.

$$\text{Stock standard, } \mu g / mL = \frac{\text{weight (g)} \times \text{potency} \times 1000000\ (\mu g / g)}{\text{volume, mL}}$$

(**i**) *Trace-level spiking standard solution*.—Ca 30 µg/mL. Transfer by pipet 5.0 mL stock standard, (**h**), into a 50 mL volumetric flask. Dilute to volume with 1% calcium chloride–methanol, (**d**). Mix well. Prepare fresh monthly and store in a refrigerator.

(**j**) *Intermediate standard solution*.—Ca 30 µg/mL. Transfer by pipet 10.0 mL stock standard, (**h**), into a 100 mL volumetric flask. Dilute to volume with methanol–water (825 + 175), (**f**). Mix well. Prepare fresh monthly and store in a refrigerator.

(**k**) *LC standard solutions (medicating level)*.—As specified in Table **2008.08B**, transfer by pipet the required volume of intermediate standard, (**j**), or LC standard, **D**(**k**)(*3*), into volumetric flasks, and dilute to volume with methanol–water (825 + 175), (**f**). Mix well. Prepare fresh monthly and store in a refrigerator.

Note: A small degradation peak may appear in the chromatogram at the front of the DEC peak by the expiration date; the peak should not be present in fresh solutions.

(**l**) *LC standard solutions (trace level)*.—As specified in Table **2008.08C**, transfer by pipet the required volume of intermediate standard, (**j**), or LC standard, **D**(**k**)(*3*), into volumetric flasks, and dilute to volume with methanol–water (825 + 175), (**f**). Mix well. Prepare fresh monthly and store in a refrigerator.

E. Preparation of Test Samples

Sampling.—Grind the laboratory sample (>200 g) so that it passes completely through a sieve with 1 mm apertures, taking care to avoid the generation of heat. Mix thoroughly.

F. Procedure

(**a**) *Preparation of quality control samples*.—The use of a quality control sample is recommended. Analyze a control sample with each sample set. For medicated samples, include a control sample spiked at approximately 30 mg/kg (add 1.0 mL stock standard solution, **D**(**h**), to 10 g blank feed, and mix well). Alternatively, the medicated control sample could

Table 2008.08B. Medicating-level LC standard solutions

Standard to dilute			LC standards	
Identification	Volume, mL	Final volume, mL	Approximate concn, µg/mL	Identification
D(**j**)	10.0	50	6.0	**D**(**k**)(*1*)
D(**j**)	15.0	100	4.5	**D**(**k**)(*2*)
D(**j**)	10.0	100	3.0	**D**(**k**)(*3*)
D(**j**)	5.0	100	1.5	**D**(**k**)(*4*)
D(**k**)(*3*)	25.0	100	0.75	**D**(**k**)(*5*)

Table 2008.08C. Trace-level LC standard solutions

Standard to dilute		LC standards		
Identification	Volume, mL	Final volume, mL	Approximate concn, μg/mL	Identification
D(**j**)	5.0	100	1.5	**D**(**k**)(*4*)[a]
D(**j**)	4.0	100	1.2	**D**(**l**)(*2*)
D(**k**)(*3*)	25.0	100	0.75	**D**(**k**)(*5*)[a]
D(**k**)(*3*)	15.0	100	0.45	**D**(**l**)(*4*)
D(**k**)(*3*)	3.0	100	0.09	**D**(**l**)(*5*)

[a] Same standard solution as in Table **2008.08B**.

Table 2008.08D. Medicated-level sample extract dilution

	Dilution in **F**(**b**)		
Target level, mg/kg	Extract aliquot, mL	Volumetric flask, mL	Dilution factor (DF)
<50	None	None	1
50 to 100	5.0	10	2
101 to 250	5.0	25	5
251 to 500	5.0	50	10
501 to 1000	5.0	100	20
1001 to 2000	5.0	200	40

be 10 g medicated feed containing 25–50 mg/kg DEC. For trace levels, include a control sample spiked at approximately 1.5 mg/kg (add 1.0 mL trace-level spiking standard, **D**(**i**), to 20 g blank feed and mix well). Add by graduated cylinder 100 mL extraction solvent, **D**(**d**). Stopper and shake as in (**b**). Expected recoveries for medicated and trace-level control samples are 95–105%.

(**b**) *Extraction*.—Accurately weigh a test portion of each sample into a 250 mL Erlenmeyer flask. For medicated samples (≥10 mg/kg), weigh 10 g and for trace level samples, (<10 mg/kg) weigh 20 g. Record the test portion weight to 2 decimal places. Add by graduated cylinder 100 mL extraction solvent, **D**(**d**). Stopper the flask and shake for 90 min on a shaker, **C**(**b**). Shaking can be started at the end of the day; let the extracts sit overnight, and then shake for 5 min.

Transfer at least 40 mL of the extract to a 50 mL centrifuge tube, **C**(**e**). Cap the tube tightly and centrifuge for 5 min at 200 *g* (approximately 1500 rpm).

Dilute the extract to approximately 3 μg/mL using extraction solvent, **D**(**d**), according to Table **2008.08D**. Measure all volumes by pipet and volumetric flasks. After dilution, transfer by pipet an 8.0 mL aliquot of the above extract to a 10 mL volumetric flask and dilute to volume with water, **D**(**b**). Mix well. Filter through a 0.45 μm filter, **C**(**h**), (discard the first 0.5 mL) into an autosampler vial before proceeding to the LC analysis.

(**c**) *LC analysis*.—(*1*) *LC conditions*.—(*a*) *Primary procedure*.—Autosampler syringe flush solvent, methanol, **D**(**a**); column and guard column as in **C**(**a**); mobile phase as in **D**(**e**); flow rate, 1.1 mL/min; injection volume, 10 μL; detector parameters: excitation wavelength, 266 nm, and emission wavelength, 394 nm; run time, 12 min. (*b*) *Confirmation procedure*.—Autosampler syringe flush solvent, methanol, **D**(**a**); column and guard column as in **C**(**a**); mobile phase as in **D**(**e**); flow rate, 1.1 mL/min; injection volume, 50 μL; detector parameters: excitation wavelength, 318 nm, and emission wavelength, 394 nm; run time, 12 min.

(*2*) *System suitability*.—(*a*) Retention time should be 7–10 min; tailing factor, T_f [U.S. Pharmacopeia (Rockville, MD) tailing factor or European Pharmacopoeia (Strasbourg, France) symmetry factor] must be <1.5.

(*3*) *Calibration curve*.—Verify the LC system's linearity by injecting 10 μL of 5 LC standard solutions (Tables **2008.08B** and **C**) at the start and end of the sample set, where applicable. Use the average peak area of each standard for the preparation of the calibration curve. Plot the average peak areas vs DEC standard concentrations (μg/mL). For medicated-level analyses, inject each LC standard in Table **2008.08B**. For trace-level analyses, inject each standard in Table **2008.08C**. The correlation coefficient of the regression must be ≥0.999 and the 95% confidence interval of the *y*-intercept must include zero. The confidence interval for the *y*-intercept (b) is calculated as:

$$b \pm t_{n-2}\, s_b$$

where t_{n-2} is the *t*-statistic for *n*-2 degrees of freedom and s_b is the standard error of the *y*-intercept. For a 95% confidence interval of the *y*-intercept estimate with 5 calibration points, use $t_{n-2} = 3.18$.

Note: If the calibration curve for the standards in Tables **2008.08B** and **C** is not linear and curvature occurs for the most concentrated standard(s), it is possible that the photomultiplier (PMT) is overloaded. Reducing the PMT voltage may correct the problem. If reducing the PMT voltage does not correct the problem, dilute the standards and sample extracts, prior to LC analysis, by a factor of 2 using methanol–water (825 + 175), **D**(**f**).

(**d**) *Determination*.—(*1*) *Medicated samples (≥10 mg/kg)*.—Using the *Primary Procedure* described in (**c**)(*1*)(*a*), make triplicate 10 μL injections of LC standard, **D**(**k**)(*3*), and verify that peak area repeatability is <2 %. Inject 10 μL of each LC standard in Table **2008.08B**. Inject 10 μL of each test solution. Reinject LC standard, **D**(**k**)(*3*), after every 4 or 5 test solution injections to check that the instrument response has not changed significantly. Reinject 10 μL of the 5 LC standards in Table **2008.08B** at the end of the sample set. The test solution is quantified by using the calibration curve. Use the average peak area of each standard for preparation of the calibration curve.

(*2*) *Trace-level samples (<10 mg/kg)*.—Using the *Primary Procedure* described in (**c**)(*1*)(*a*), make triplicate 10 μL

injections of LC standard, **D**(**k**)(*5*), and verify that peak area repeatability is <2%. Inject 10 μL of each LC standard in Table **2008.08C**. Inject 10 μL of each test solution. Reinject LC standard, **D**(**k**)(*5*), after every 4 or 5 test solution injections to check that the instrument response has not changed significantly. Reinject 10 μL of the 5 LC standards in Table **2008.08C** at the end of the sample set. The test solution is quantified by using the calibration curve. Use the average peak area of each standard for preparation of the calibration curve.

(**e**) *Confirmation of suspect positive trace-level samples (≥0.5 mg/kg)*.—Using the *Confirmation Procedure* described in (**c**)(*1*)(*b*), make triplicate 50 μL injections of LC standard, **D**(**k**)(*5*), and verify that peak area repeatability is <2%. Inject 50 μL of each LC standard in Table **2008.08C**. Inject 50 μL of each suspect positive test solution from (**d**)(*2*). Reinject LC standard, **D**(**k**)(*5*), after every 4 or 5 test solution injections to check for drift. The presence of DEC is confirmed if the emission peak area ratio (266/318 nm) of the test solution agrees to within 5% of the emission peak area ratio (266/318 nm) of LC standard, **D**(**k**)(*5*).

(**f**) *LC system shutdown*.—When the injection sequence is finished, wash the system with methanol–water (825 + 175), **D**(**f**), at a flow rate of 1.0 mL/min for a minimum of 30 min. For pumps equipped with back-flush seals, rinse the piston with water at each shutdown to remove any salts.

G. Calculations

Formula (using a calibration curve):

$$\text{Decoquinate, mg / kg} = \frac{(A-b)}{m} \times \frac{V}{W} \times \frac{10.0}{8.0} \times DF$$

where A = peak area of DEC in the test solution; b = *y*-intercept of the calibration curve; m = slope of the calibration curve; V = volume of extraction solution, 100 mL (+spiking standard volume, if applicable); W = test portion mass (g); DF = dilution factor.

Examples.—Medicating-level sample: A = 2 210 565, b = 2039, m = 568 190, V = 100 mL, W = 10.04 g, DF = 1

$$\text{Decoquinate, mg / kg} = \frac{(2210565 - 2039)}{568190} \times \frac{100}{10.04} \times \frac{10.0}{8.0} \times 1 = 48.4$$

Trace-level sample: A = 140 680, b = 3407, m = 570 692, V = 100 mL, W = 20.01 g, DF = 1

$$\text{Decoquinate, mg / kg} = \frac{(140680 - 3407)}{570692} \times \frac{100}{20.01} \times \frac{10.0}{8.0} \times 1 = 1.50$$

Report the final results to 3 significant figures for values ≥10 mg/kg, and 2 significant figures for values <10 mg/kg. Suspect positive trace-level samples must be confirmed, **F**(**e**). For trace-level samples found to contain <0.5 mg/kg DEC, report <0.5 mg/kg.

Reference: *J. AOAC Int*. **91**, 685(2008).

Results and Discussion

Statistical Analysis of Results

Twelve laboratories returned acceptable data for the medicated-level test samples (one laboratory did not meet the system suitability requirements) and 13 laboratories returned acceptable data for the trace-level samples. For all laboratories, the blank sample was reported as not detected or below the method's LOQ (<0.5 mg/kg). Reported recoveries for spiked control samples ranged from 95 to 111% for the medicating level and 96 to 118% for the trace-level spiked control samples. By procedures described in the *Collaborative Study Guidelines* (7), the study results were examined for evidence of individual systematic error using Cochran's and Grubbs' tests progressively. Statistical calculations for the determination of the repeatability standard deviation (s_r) and corresponding relative standard deviation (RSD_r), reproducibility standard deviation (s_R) and corresponding relative standard deviation (RSD_R), and HorRat values were carried out after the removal of outliers as defined by the guidelines. HorRat values between 0.5 and 1.5 are the expected range for performance acceptability (7). All outlier removal and statistics were calculated using the AOAC Interlaboratory Study Workbook for Blind (Unpaired) Replicates Statistical Program, Version 2.0, 2006, provided by AOAC. The calculated method performance data are presented in Table **2008.08A**. The data reported by the participating laboratories for the blind duplicate medicated and trace-level samples are presented in Tables 2 and 3, respectively.

The results of the analyses on the medicated materials from the 12 laboratories are shown in Table 2. The results of analyses from the 13 laboratories on the trace-level materials are shown in Table 3. Outlier tests were run as recommended by AOAC INTERNATIONAL (7), except in one instance as noted in Table 3 (Material J, swine feed). In this case, Laboratory 4340 was not designated as an outlier since it was determined to be a marginal Cochran's test statistic and the authors chose to err on the side of higher statistics.

The results shown in Tables 2 and 3 were used to generate the statistics presented in Table **2008.08A**. RSD_r values ranged between 1.3 and 5.6%; RSD_R values ranged between 2.8 and 6.1%. The mean results were within 5.7% relative of the target values. HorRat values ranged between 0.22 and 0.74. All HorRat values were below the expected 1.5. It is notable that the HorRat values for 6 out the 8 materials tested were <0.5. A random sample of participants was contacted to enquire if they took part in consultations with other participating collaborators and/or if any of the reported results submitted were averaged. All of the participants that were contacted indicated that they did not average any results or consult with other collaborators. Therefore, the <0.5 HorRat values were considered acceptable after determining there was study independence and no unreported averaging or consultations occurred (7, 8).

Table 2. Medicated sample data: duplicate samples

Material	Target, mg/kg	Results, mg/kg, by laboratory number											
		3734	3779	3841	3955	4203	4340	4497	4598	5094	5203	5373	5474
C, Calf starter feed, texturized	49.2	49.2	47.6	52.1	48.0	50.3	51.1	53.0	50.2	47.9	50.1	46.6	41.2[a]
		50.2	48.1	50.4	49.2	50.0	52.1	50.8	49.4	49.7	51.4	47.1	41.9[a]
D, Calf starter feed	27.4	27.7	25.8	29.0	27.3	27.4	28.3	27.7	27.7	26.9	28.2	25.6	22.6[a]
		27.7	26.1	27.7	26.9	27.1	28.5	27.4	27.9	26.5	28.2	26.2	23.4[a]
E, Lamb feed	15.9	12.4[b]	14.6	15.9	15.8	14.9	15.9	22.8[b]	15.6	15.0	15.5	14.0	13.3
		15.4[b]	15.1	15.2	15.3	15.1	16.0	15.7[b]	15.1	15.0	15.6	14.6	12.4
F, Supplement	97.6	100.6	96.9	103.4	99.7	100.9	115.6[b]	101.0	97.4	95.0	102.0	93.8	85.4[a]
		99.3	96.9	99.1	100.0	102.6	106.5[b]	101.3	99.4	96.3	104.1	95.9	85.0[a]
G, Chicken feed	41.0	39.8	39.7	41.3	39.4	42.2	42.8	43.0	41.2	39.2	41.5	44.9	33.9[a]
		40.2	40.1	41.0	41.6	41.9	42.8	42.0	40.7	40.4	42.0	43.4	34.2[a]
H, Drug premix	1260	1320	1260	1340	1280	1300	1430	1360	1270	1260	1310	1410	1070[a]
		1270	1260	1280	1280	1290	1360	1330	1260	1270	1340	1390	1040[a]

[a] Single Grubbs' test outlier.
[b] Cochran's test outlier.

Table 3. Trace-level sample data: duplicate samples

Material	Target, mg/kg	Results, mg/kg, by laboratory number												
		3734	3779	3841	3955	4203	4340	4497	4598	5049	5094	5203	5373	5474
I, Calf feed, texturized	4.6	4.8	4.7	4.9	4.8	4.7	4.8	4.8	4.8	4.8	4.8	4.6	4.5	4.9
		4.9	4.5	4.8	4.7	4.8	4.8	4.6	4.8	4.9	4.6	4.8	4.7	5.1
J, Swine feed	2.1	2.0	2.1	1.9	2.0	1.9	1.9[a]	2.0	2.0	2.0	1.9	2.0	1.8	2.8[b]
		1.9	1.9	2.0	2.0	2.0	2.3[a]	2.0	2.0	2.1	1.8	2.0	2.0	2.2[b]

[a] Marginal Cochran's test; not designated as outlier (*see Results and Discussion* section).
[b] Single Grubbs' test outlier.

Collaborators' Comments

In the familiarization phase of the study, 2 laboratories experienced difficulty in meeting system suitability: standard calibration curves were not linear and curvature occurred for the most concentrated standards. In both cases, the detector's photomultiplier tube (PMT) was found to be overloaded, and reducing the PMT voltage corrected the problem for one laboratory. The PMT value could not be reduced for the other laboratory; therefore, the laboratory was instructed to dilute the standards and the test sample extracts prior to LC analysis. The final results were unaffected by the extra dilution since all system suitability requirements were met.

Two laboratories overlooked the final test sample extract dilution (8.0 mL of extract diluted to 10 mL with water) for one of test sample sets. Once the oversight was caught, the laboratories diluted the extracts accordingly.

Recommendations

The data show that the method is valid for determining the presence and quantity of DEC in animal feeds, supplements, and premixes. It is also valid for determining the presence of decoquinate at trace levels in animal feeds. The collaborative study demonstrated the performance of the method from 2 to 1300 mg/kg. It is recommended that the method be approved as AOAC Official First Action.

Acknowledgments

We thank the following collaborators and their associates for their cooperation and participation in this study:

Jaroslava Petrova and Lydie Dudiková, UKZUZ, Praha, Oddeleni Laboratori, Prana, Czech Republic

Renaud Le Bouquin, Laboratoire de Recherche Alimentaire, Talhouët, France

Christophe Genouel, Marie-Catherine Rues, and Michèle Joubert, Laboratoire de la DGCCRF, Rennes, France

Clara Cruz and Sara Moura, Laboratório Nacional de Investigação Veterinária, Lisboa, Portugal

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Vicki Murley, Ryan Lerch, and Dave Warrick, Oklahoma Department of Agriculture, Oklahoma City, OK

Kristen Elson, Canadian Food Inspection Agency, Ottawa Laboratory (Carling), Ottawa, ON, Canada

We thank the following for assistance in providing reference standards and/or test materials:

Maurice W. Smith, Alpharma Inc., Toronto, ON, Canada

Jay Johnston, Ritchie Feed & Seed Inc., Ottawa, ON, Canada

We thank Philippe Desjardins for preparing the test samples and Aaron Price for the statistical evaluation of the test data [both are of the Canadian Food Inspection Agency, Ottawa Laboratory (Carling), Ottawa, ON, Canada].

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