codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 10 **CX/RVDF 06/16/11**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS

Sixteenth Session

Cancun, Quintana Roo, Mexico, 8 -12 May 2006

METHODS OF ANALYSIS FOR RESIDUES OF VETERINARY DRUGS IN FOODS

Comments to CL 2005/10-RVDF submitted by Argentina, Canada, European Community, Pakistan, Thailand and Venezuela

Argentina

Analytical Method Information Summary

A. Descriptive Information:

1. Name of drug or chemical: **1:1 Molar complex of two compounds, HDP (2-hydroxy-4,6-dimethylpyrimidine) and DNC (4,4'-dinitrocarbanilide)**
2. Drug or chemical class: **Coccidiostat (i.e., anti-coccidial)**

   (e.g. antimicrobial, anthelmintic, etc.)
3. Veterinary Use: **Used for the prevention of coccidiosis in poultry, which is an infectious disease caused by an intestinal protozoan parasite.**
4. Analyte(s) measured: **Nicarbazin**

   (specify if metabolite)
5. Intended use of the method:

a. Screening

b. **Routine**

c. Reference

d**. Confirmatory**

6. Test matrix: **Liver.**

   (e.g. muscle, kidney, urine, etc.)
7. Summary of principal steps in sample preparation:

   Liver is cut in chunks and then chopped in an electric grinder until a homogeneous paste is obtained.

Afterwards it is stored in the freezer.

8. Summary of principal steps in extraction procedure:

Nicarbazin is extracted from the liver with ethyl acetate.

Hexane and acetonitrile are added to the extract. The acetonitrile phase is separated by centrifugation.

A second extraction is performed with acetonitrile. Both extracts are combined, evaporated, and reclaimed with methanol.

9. Summary of principal steps in analyte clean-up procedure:

The analyte purification procedure consists of the elimination of the fatty substances, by liquid-liquid partitioning with hexane. The acetonitrile fraction is vacuum-concentrated, to be injected later.

10. Measurement procedure:

a. Chemical

1. Instrumentation: **HPLC**
2. Detector system: **Diode-array detector (DAD)**
3. Chromatographic column: **C- 18**

(if applicable)

b. Immunochemical/Immunoassay

1. Technique: **(not applicable)**

(e.g. Elisa, RIA, Immunochromatog., etc.)

2. Critical reagents: **(not applicable)**

(e.g. antibody specificity and availability)

3. Special equipment required: **(not applicable)**

c. Microbiological

1. Technique: **(not applicable)**
2. Organism: **(not applicable)**
3. Media: **(not applicable)**
4. Special equipment required: **(not applicable)**

11. Sample/Analyte Stability Warning (if applicable):

Store in freezer (at a temperature between -15 ºC [5 ºF] and –20 ºC [-4 ºF])

12. Literature References available:

FSIS (FOOD SAFETY INSPECTION SERVICE 1991)

13. Contact for Information:

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b. Country: **Argentina**

c. Affiliation: ------

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g. E-mail: **ematus@foodscience.com.ar or info@foodscience.com.ar**

B. Method Performance

1. a. Limit of Detection (LOD) (µg/kg): **15**

   How was LOD determined? **It was determined based on the ordinate at the origin of the higher confidence hyperbola for a level of significance of 0.05.**

   b. Limit of Quantification (LOQ) (µg/kg): **41.0**

   How was LOQ determined? **The %CV values for each concentration level are plotted in a graph, and the LOQ is determined as the concentration for which the %CV has a value of 20%.**

   c. Method sensitivity

   (The smallest difference in concentration that can be measured)

2. JECFA MRL **Standard curve, 6.09 area/ng / fortified curve 0.58 area/ng**

3. *Are analytical data corrected for recovery?* ***Yes***

4. How is recovery estimated? **Recovery is estimated with an external standard.**

   (e.g. external standard; internal standard, etc.)

5. Accuracy

a. Concentration(s) tested:	**100.0**	**150.0**	**200.0**	**400.0**	**(µg/kg)**
b. Concentration(s) measured:	**77**	**126.2**	**166.6**	**318.3**	**(µg/kg)**
c. Recovery (%):	**79.6**	**83.3**	**84.1**	**77.0**	

6. Precision using fortified control tissue

a. Concentration(s) tested:	**100.0**	**150.0**	**200.0**	**400.0**	**(µg/kg)**
b. Repeatability (within lab CV):	**11.6 %**	**6.0 %**	**11.1%**	**7.1%**	
c. Reproducibility (between lab CV):	---	---	---	---	

7. Precision using tissue containing incurred drug residues

a. Concentration(s) tested:	**48.9**	**54.2**	**85.5**	**252.5**	**287.0**	**288.5**	**664.0**	**2371.0**
b. Repeatability (within lab CV):	**7.1 %**	**6.1 %**	**3.3 %**	**9.4%**	**16.0%**	**16.3%**	**1.4 %**	**14.5%**
c. Reproducibility (between lab CV)	---	---	---	---	---	---	---	---

8. Selectivity of the method

   This information is often referenced as "Specificity". Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure or drug class or other veterinary drugs that may also be used along with the analyte of interest.

      (Not applicable) Confirmation is achieved by comparing spectra at a wavelength of 340 nm.

   b. Contaminants that are likely to be present in the sample.

      (Not applicable)

9. Type of Validation Studies

a. Single laboratory: **YES. (Inter-laboratory and proficiency testing by the Office of Laboratory and Technical Control Management (*Dirección de Laboratorio y Control Técnico*) from the National Agrifood Quality and Health Service (*Servicio Nacional de Sanidad y Calidad Agroalimentaria,* SENASA)**

b. Multi-laboratory: **NO**

c. AOAC or other official procedure: **NO**

C. Information relevant to laboratory implementation

1. Training and experience recommended for analysts
2. Critical steps in the method
3. Information on availability of unusual reagents or equipment
4. Special reagent or sample stability concerns
5. Reagent handling and safety concerns (if any)
6. Literature references or other useful information

Canada

CHAPTER 1 - CONTAMINANTS

SECTION 6: OXYTETRACYCLINE EXTRACTION AND ANALYSIS PROCEDURE

1.SCOPE AND APPLICATION

1.1This procedure is applicable to a wide range of fresh and frozen fish, shellfish and fish products that have been treated with oxytetracycline, or produced with contaminated raw material. The method has been used to determine oxytetracycline (OTC) concentrations in fish ranging from 0.01 µg/g to over 12 µg/g.

2.PRINCIPLE OF THE METHOD

2.1Fish tissues are blended to homogeneity and the OTC is extracted with 1 N HCl or an extraction solution containing 0.67 N HCl and 0.67 M ammonium sulfate. The extracts are deproteinized with acetonitrile, filtered through a glass wool plug and a portion cleaned up with a methylene chloride:petroleum ether partition.

2.2OTC is analyzed by reversed-phase liquid chromatography with UV detection at 355 nm. The amount of OTC present is calculated by comparison with prepared standards of known concentrations.

3.INTERFERENCES

3.1Retinol, the precursor to Vitamin A, has been reported to cause some interferences with the analysis of OTC in other procedures. To date there has not been any evidence to support that it interferes with this procedure.

3.2In processed products, such as smoked salmon, salmon pâté, and breaded shrimp, there may be some interferences due to the spices or other ingredients used in the formulation. Any problems encountered can generally be alleviated by slight adjustments to the gradient of the routine chromatography or by using the confirmatory HPLC system.

3.3Lobster muscle tissue generally does not pose any problems during analysis. However, hepatopancreas or tissue contaminated with hepatopancreas can make the routine analysis difficult. Some species of fish (e.g., milkfish, tilapia, and Nile perch) and some tissues (e.g., liver) contain compounds which can interfere with the chromatography of OTC. These problems generally can be rectified by altering the gradient conditions to increase the residence time of the analytes on the column or by using the confirmatory HPLC system.

4.SAMPLING PROCEDURE AND STORAGE

4.1Fresh or frozen product.

4.1.1Collect a minimum of 5 animals or enough to yield 150 g of meat for each of 5 sample units.

4.1.2 Samples should be frozen prior to shipping.

4.1.3Pack the samples in a shipping container with adequate protection against mechanical damage and temperature change.

4.1.4Place a description of the sample in the package, i.e., date of collection, time, location, species, and any other pertinent data.

4.1.5 Send to the laboratory without delay.

4.2Canned or packaged product.

4.2.1Take a representative sample (minimum of 5 units) from the product lot and store so as to maintain sample integrity.

5.SAMPLE PREPARATION

5.1 Finfish.

5.1.1Take precautions to prevent contamination of the fish or fish slices by material on the outside of the outer bag or from the processing area.

5.1.2Partially thaw the individual subsamples and remove the skin and backbone.

5.1.3Divide the tissue from each subsample into two approximately equal portions.

5.1.4Prepare a composite sample by placing one portion of each subsample into a Silent Cutter or food processor and comminuting until homogeneous.

5.1.5Refreeze the second portion of each subsample in its original plastic bag in case individual analyses are later required. Wash the cutting board, knife and immediate work area to minimize contamination between subsamples.

5.1.6Remove a significant portion of the composite sample purée from the Silent Cutter and further comminute it in a food processor to ensure better homogeneity.

5.1.7Store a portion of this sample below -20 ^{o}C in a sealable container for later analysis.

5.1.8Ensure the prepared sample is homogeneous prior to weighing the portion for extraction. If liquid separates on thawing, reblend prior to use.

5.2Crustaceans.

5.2.1Remove the meat from the shell, blend until homogeneous and treat as in 5.1.3.

6. APPARATUS

6.1 Polytron homogenizer or equivalent.

6.2 Food processor.

6.3 Silent Cutter or equivalent.

6.4 Pipettors.

6.4.1 Variable volume pipettors with 5 and 10 mL capacity.

6.5 13 mm nylon syringe filters (0.2 µ pore size recommended).

6.6 3 mL disposable syringes.

6.7HPLC pump system able to generate reliable, rapid binary gradients at flow rates up to 1.5 mL/min. and at pressures of at least 3000 psi.

6.8Autosampling system able to communicate with the pump and data system and provide up to 500 µL injection volumes either in one injection or by repeated smaller injections.

6.9Column oven capable of maintaining the column temperature at 50 ^{o}C.

6.10HPLC UV monitor capable of achieving the required sensitivity at 355 nm with minimal refractive index changes. A diode array detector is required for spectral confirmations.

6.11Data-handling system and recording device.

6.12Columns:

6.12.1Routine: Vydac, 201TP54, 25 cm x 4.6 mm i.d. used without a guard column.

6.12.2Routine: Polymer Laboratories PLRP-S, 15 cm x 4.6 mm i.d. used without a guard column.

6.12.3Confirmatory: Partisil, 5 ODS-3 25 cm x 4.6 mm i.d. used without a guard column.

6.13UV spectrophotometer: capable of measuring accurately in the 350 to 370 nm range.

7. REAGENTS

7.1 Methanol, ACS grade.

7.2 Acetonitrile, HPLC grade.

7.3 Petroleum ether, ACS grade.

7.4 Methlyene chloride, ACS grade.

7.5 Glass-distilled water.

7.6 n,n -Dimethylformamide (DMF), ACS grade.

7.7 Oxytetracycline dihydrate.

7.7.1Stock standard solution: Approximately 10.8 mg of oxytetracycline dihydrate is dissolved in methanol (may take several hours to complete) and diluted to 100 mL. The standard is calibrated spectrophotometrically using procedure 8.1 to determine the precise concentration.

7.7.2Spiking standard: (0.010 mg/mL). Pipet an appropriate amount of the stock solution into a 100 mL volumetric flask and dilute to volume with methanol.

7.7.3Working standard: (0.0010 mg/mL). Pipet an appropriate amount of stock solution into a 100 mL volumetric flask and dilute to volume with 0.01 N HCl.

7.7.4Calibration solutions: (approximately 0.020 mg/mL). Pipet 1.0 mL of stock solution and dilute to 5.0 mL with ethanol in a volumetric flask.

7.8Hydrochloric acid (HCl).

7.8.1HCl (1N) solution. 86.2 mL of conc. acid diluted to 1 L with distilled water.

7.8.2HCl (0.01 N) solution. 1.0 mL of 1 N HCl diluted to 100 mL with distilled water.

7.9Oxalic acid.

7.9.1Oxalic acid (0.01 M) solution. Dissolve 0.90 g of oxalic acid in approximately 600 mL of distilled water then dilute to 1 L with distilled water.

7.10Trifluoroacetic acid (TFA). Reagent grade. **Warning !** very corrosive and relatively toxic and volatile.

7.11Ammonium sulfate (ACS grade).

7.12Extraction solution.

7.12.1Weigh 8.8 g of ammonium sulfate and place in a 1 L volumetric flask; add 55 mL concentrated HCl and make up to volume with deionized water.

7.13Antifoam B silicone emulsion.

7.14Sodium decane sulfonate (ACS grade).

7.15Phosphoric acid.

7.16Phosphoric acid (0.02 M) plus decane sulfonate (0.01 M).

7.16.1Pipet 1.3 mL of phosphoric acid and weigh 2.44 g of sodium decane sulfonate into a 1 L volumetric flask. Dilute to volume with glass-distilled water and filter before use (see remarks 11.6).

7.17 Sodium phosphate.

7.17.1Sodium phosphate (monobasic, 2 N). Dissolve 27.6 g of $NaH_2PO_4.H_2O$ in distilled water and dilute to 100 mL.

7.18Mobile phases.

7.18.1Mobile phase (Routine-Vydac column). Solvent A: Glass- distilled water + 0.1 % TFA. Solvent B: Acetonitrile +

0.1 % TFA.

7.18.2Mobile phase (Routine - PLRP-S column). Solvent A: 0.01 M sodium decane sulfonate and 0.02 M phosphoric acid.

Solvent B: Acetonitrile.

7.18.3Mobile phase (Confirmatory - Partisil column). Solvent A: 0.01 M oxalic acid + 6% n,n - Dimethylformamide (DMF). Solvent B: Acetonitrile + 6% DMF.

8. PROCEDURE

8.1Calibration of OTC Standard Solutions.

8.1.1Measure the absorbance of four identically prepared calibration solutions at 370 nm. Average the absorbance readings to obtain the concentration of the calibration solutions by using Beer's Law (A = 0cl) where 0 = 343 at 370 nm. The concentration of the stock OTC solution is calculated by reference to the calibration solutions.

8.2Extraction.

8.2.1Accurately weigh 25 g of remixed tissue purée into a 250 mL Erlenmeyer flask.

8.2.2Blend with an homogenizer in three volumes (mL/g) of

1 N HCl or 2 volumes of extraction solution (2-3 drops of antifoam B are required if the extraction solution is used).

8.2.3Pipet 8.0 mL of the 1 N HCl blend to a 125 mL Erlenmeyer flask containing 32.0 mL of acetonitrile (swirl the sample immediately before transfer to ensure that the sample remains uniform). If the extraction solution has been used quantitatively, transfer the entire contents of the flask to a volumetric flask, allow 5-10 minutes for the foam to break up (additional antifoam B may be required if the extract is very foamy) and dilute to volume with extraction solution. Shake the contents of the flask thoroughly and centrifuge approx. 50 mL of the extract for 5 minutes at 2000 rpm. Pipet 8 mL of the supernatant to a 125 mL flask containing 32.0 mL of acetonitrile.

8.2.4Thoroughly mix this suspension and allow to stand for at least five minutes. Filter the supernatant through a glass wool plug into a 50 mL Erlenmeyer flask.

8.2.5Transfer a 20 mL aliquot to a 125 mL separatory funnel using a disposable tip pipettor.

8.2.6Add 20 mL each of methylene chloride and petroleum ether and mix well with vigorous shaking.

8.2.7Allow the phases to separate and draw off the lower layer quantitatively into a graduated container. Adjust the volume to 4.00 mL with distilled water and mix thoroughly.

8.2.8For convenience and ease of storage, transfer samples to screw-capped culture tubes.

8.2.9For each series of 10 samples, run an internal check sample, repeat samples, spiked samples and a reagent blank.

8.2.10Draw approximately 1.5 mL of sample into a 3 mL disposable syringe and filter through a 0.2 µ syringe filter directly into an autosampler vial and cap.

Caution: see remarks in section 11.9.

8.3HPLC Analysis, Routine.

8.3.1Vydac Column: Equilibrate the system for at least 20 minutes at a column oven temperature of 30 ^{o}C with 100 % solvent A. Adjust the column oven temperature or gradient conditions to obtain a retention time of the standards of between 14 to 15 minutes.

8.3.1.1HPLC Conditions:

Mobile phase -Solvent A -Glass distilled water + 0.1 % TFA.

Solvent B -Acetonitrile + 0.1 % TFA

Gradient - 100 % Solvent A for 2 minutes

75 % Solvent B in 20 minutes

100 % Solvent A in 2 minutes

Equilibrate for 8 minutes

Flow - 1.0 mL/min.

Wavelength - 355 nm

Run time - 32 min.

8.3.2PLRP-S Column: Equilibrate the system for at least 20 minutes at a column oven temperature of 40 ^{o}C with 82 % solvent A. Adjust the column oven or gradient conditions to obtain a retention time of the standards between 20 and 23 minutes.

8.3.2.1HPLC Conditions:

Mobile phase -Solvent A -0.02 M Phosphoric acid and 0.01 M sodium decane sulfonate.

Solvent B -Acetonitrile

Gradient - 82 % solvent A for 3 minutes

60 % solvent A in 25 minutes

Hold for 5 minutes

End time 30 minutes

Flow -1.0 mL/minute

Wavelength -358 nm

8.3.3Calibrate the instrument by repeated injections of the working standard.

8.3.4Inject 200 µl (500 µl PLRP-S column) of each sample, check sample, 'spiked sample' or blank and measure the resultant peak heights. Peaks are identified by comparison of retention times with recently run standards.

8.4HPLC Analysis, Confirmatory.

8.4.1Partisil Column: Equilibrate the system for at least 20 minutes at a column oven temperature of 50 ^{o}C with 100 % solvent A. Adjust the column oven temperature or the gradient to obtain retention times of the standards of between 12 to 14 minutes.

8.4.2HPLC Conditions:

Mobile phase -Solvent A -0.01 M Oxalic acid + 6 % DMF.

Solvent B - Acetonitrile + 6 % DMF.

Gradient - 100 % Solvent A for 2 minutes

35 % Solvent B in 15 minutes

70 % Solvent B in 5 minutes

100 % Solvent A in 2 minutes

Equilibrate for 8 minutes

Flow -1.0 mL/min.

Wavelength -355 nm

Run time - 32 min.

8.4.3Calibrate the instrument by repeated injections of the working standard.

8.4.4Spectral confirmations diode array: Compare the UV spectrum of the sample peak believed to be OTC with the spectrum of the standard OTC peak. Scan the spectrum from 301 to 500 nm and correct both peaks for background absorption before the comparison is made.

8.4.5Inject 200 µL of each sample onto the HPLC column. Follow each sample run with a 'spiked' run consisting of 200 µL of the sample plus 5 µL of working standard. Inject a standard after each sample and sample plus 'spike' pair to ensure proper retention time comparisons.

9. CALCULATIONS

9.1For a single point calibration, measure peak heights of the standards and calculate the average response factor (height units/µg OTC).

9.2Calculate the average peak height of the duplicate injections of the samples.

9.3Calculate the level of OTC in the sample using the following formula:

$$\mu g\ OTC/g\ ww = \frac{PH \times D}{PHS \times W}$$

PH - average peak height

PHS- peak height per µg OTC standard injected

D - dilution factor

W - weight of the sample extracted

9.4A multi-point calibration may also be used but should not be required if samples are analyzed within the linear range of the method (see 11.8).

10. PRECISION AND ACCURACY

10.1Replicate analysis of standard solutions indicates good reproducibility over the range of concentrations studied with percent relative standard deviations ranging from 2.78 to 8.15 %.

10.2Replicate analysis of salmon tissue samples at residue levels of 0.04 to 0.5 µg/g gave percent relative standard deviations from 9.0 to 23 %. Variations are greatest as the limit of detection is approached.

10.3The detection limit is approximately 0.01 µg/g for the Vydac and Partisil columns and 0.05 µg/g for the PLRP-S column.

11. REMARKS

11.1Representative chromatograms of an OTC standard and a salmon tissue extract run on a Vydac column are shown in Appendix A, Figure 1 while a similar sample run on the Partisil column is shown in Figure 3. A calibration curve of OTC standards is shown in Figure 2. A UV diode array absorption spectra comparing standard OTC and a salmon sample containing OTC is shown in Figure 4. Other reversed-phase columns should provide similar results.

11.2Recoveries of OTC added to tissue samples from a variety of fish species analyzed on the Vydac column over a one year period are shown in Table 1. Recoveries ranged from 81 to 97 % for a variety of species. Recoveries of samples spiked at the 0.2 µg/g level and analyzed by the PLRP-S column ranged from 65 to 95 %.

11.3Initial analyses are performed on sample composites. The individual subsamples are analyzed if the result of the sample composite is in excess of 0.1 µg OTC/g. Samples which approach or exceed this present alert limit should be confirmed either chromatographically or by using diode- array detection.

11.4The OTC standard should be kept at freezer temperatures during storage.

11.5The spiking and working standards must be stored between 0-4 ^{o}C.

11.6Take care when filtering the 0.02 M phosphoric acid, 0.01 M sodium decane sulfonate solution as the solution tends to foam while filtering.

11.7Aqueous extracts are stable for at least one week when stored in the refrigerator at 4 ^{o}C.

11.8Single point standardization can be generally recommended. However, depending on the equipment or columns used, standardization at other levels may be required.

11.9For certain autosamplers and injectors the HCl sample extracts must be 'buffered' before injection onto the HPLC to prevent rapid column deterioration. 1.0 mL of extract is added to 0.5 mL of 2 N $Na_2H_2PO_4$ and mixed. Load at least 1.0 mL into a 3 mL disposable syringe and filter through a 0.2 µ nylon syringe filter into an autosampler vial.

11.10A significant portion of the sample to sample variation may reflect the lack of sample homogeneity rather than method variation. The presence of small bones which are virtually impossible to remove during normal sample processing may greatly affect the results if they are not evenly distributed throughout the sample.

11.11The method can also be applied to the analysis of tetracycline and chlortetracycline in a variety of fish and fish products.

12.REFERENCES

12.1Moats W.A., "Determination of tetracycline antibiotics in tissues and blood serum of cattle and swine by high performance liquid chromatography", (1986), J. Chromatog., 358, pp 255-259.

12.2Association of Official Analytical Chemists, "Official methods of analysis", (1984), 14th edition, 18.001.

12.3Association of Official Analytical Chemists, "Official methods of analysis", (1995), 16th edition, 49.2.02.

12.4Regna P.P., I.A. Solomons, K. Murai, A.E. Timbreck, K.J. Brunings and W.A. Lazier, "The isolation and general properties of terramycin and terramycin salts", (1951),

J. Amer. Chem. Soc. 73, pp 4211-4215.

12.5Bjorklund H., "Determination of oxytetracycline in fish by high performance liquid chromatography", (1988),

J. Chromatog. 432, pp 381-387.

12.6Ibrahim, A. and W.A. Moats, "Effect of cooking procedures on oxytetracycline residues in lamb muscle", (1994),

J. Agric. Food Chem. 42, pp 2561-2563.

APPENDIX A

Table 1. Percent recovery of OTC added to various fish tissue samples.

Sample	OTC added µg/g	% Recovery	# of analyses
Atlantic salmon	0.4	85 ± 11	30
	5.0	97 ± 2	3
Lobster muscle	0.4	85 ± 11	10
hepato	0.4	97 ± 9	3
Pacific salmon	0.4	86	1
Rainbow trout	0.4	86 ± 8	3
Arctic char	0.4	81	1
Shrimp	0.4	95 ± 14	6

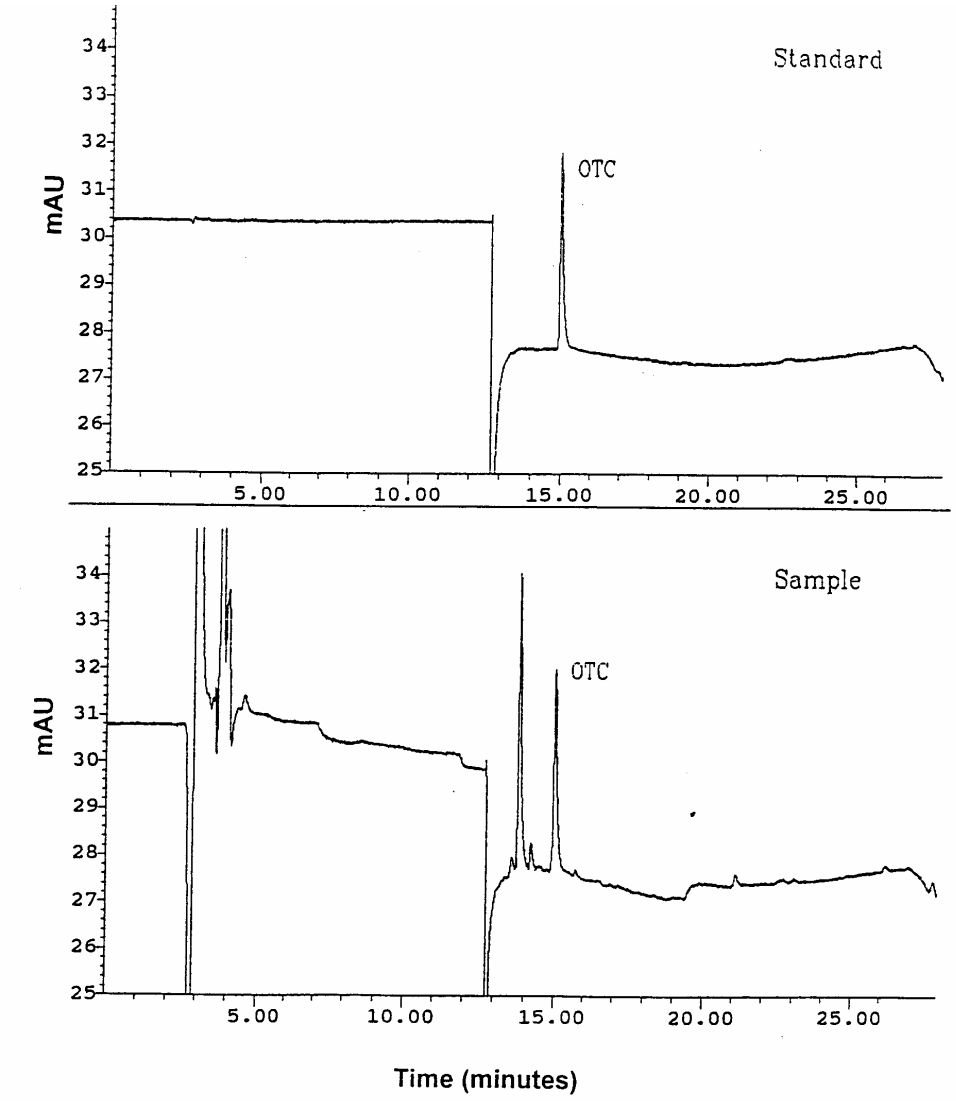


Figure 1. HPLC UV (355 nm) trace of A: standard OTC (19 ng) and B: salmon tissue extract (200 µl) run on a Vydac (25 cm x 4.6 mm i.d., 5µ) column. Operating conditions : flow rate 1.0 mL/min., column oven 30 ^{o}C, program: glass-distilled water plus 0.1 % trifluoroacetic acid (0-2 min.) to 75 % acetonitrile plus 0.1 % trifluoroacetic acid (22 min.).

Figure 2. Standard curve for OTC determinations at 355 nm on the Vydac column. Operating conditions as in Figure 1.

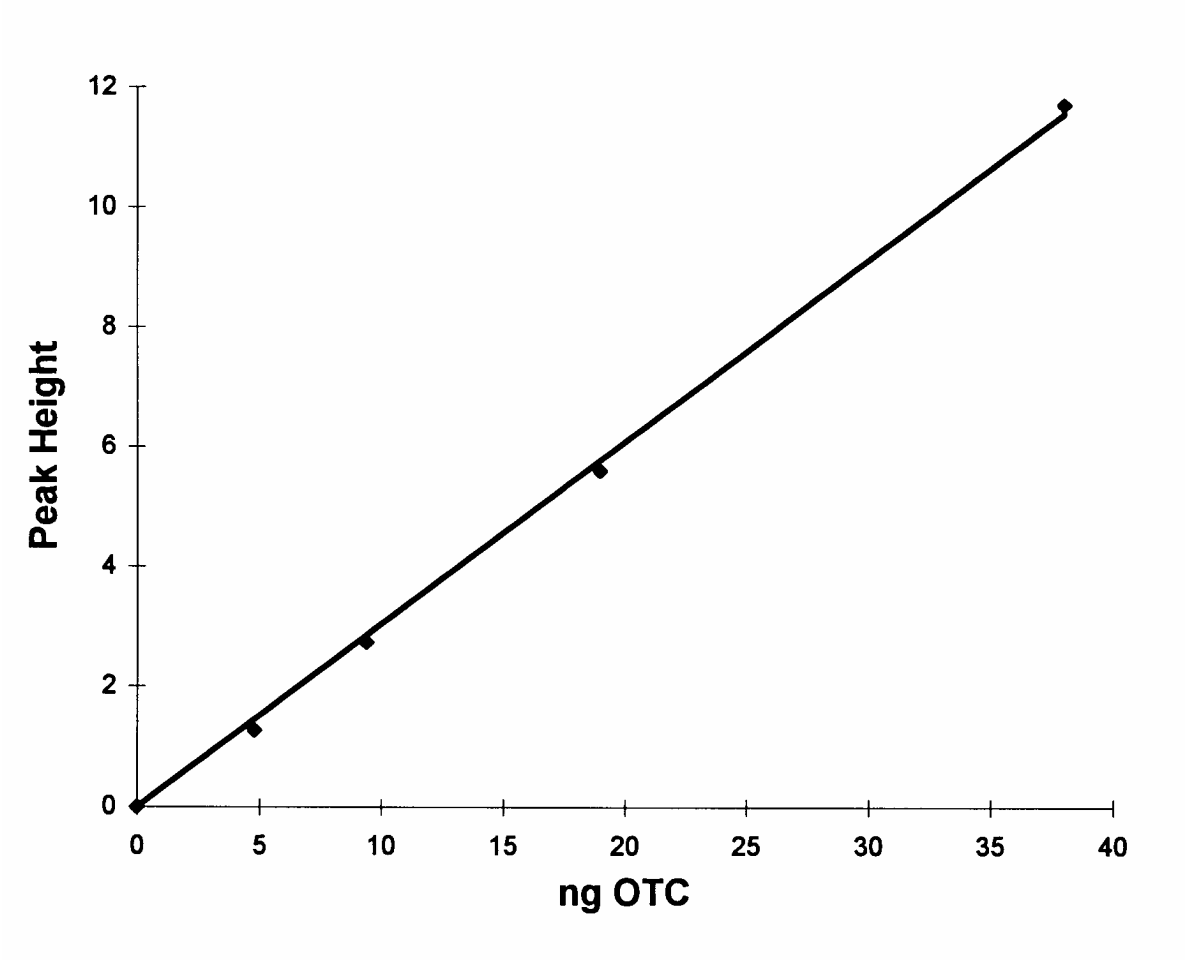
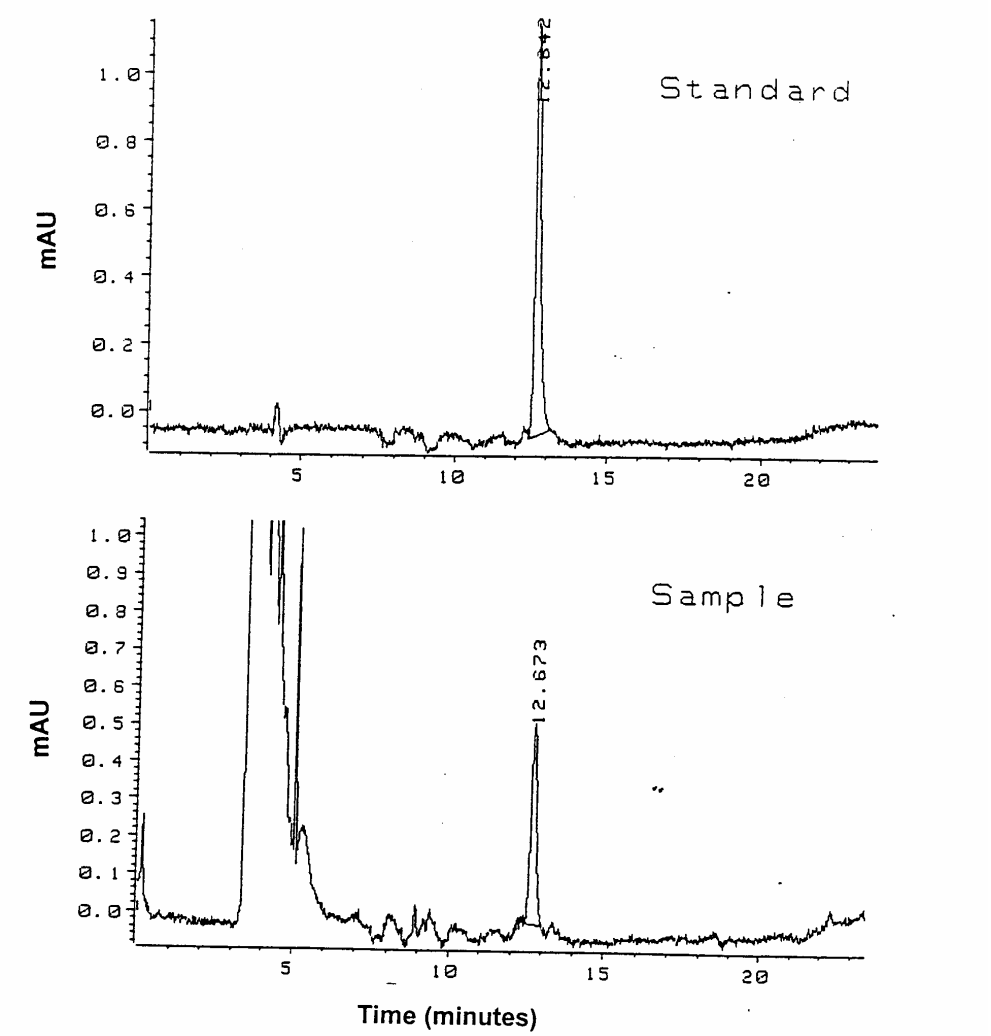
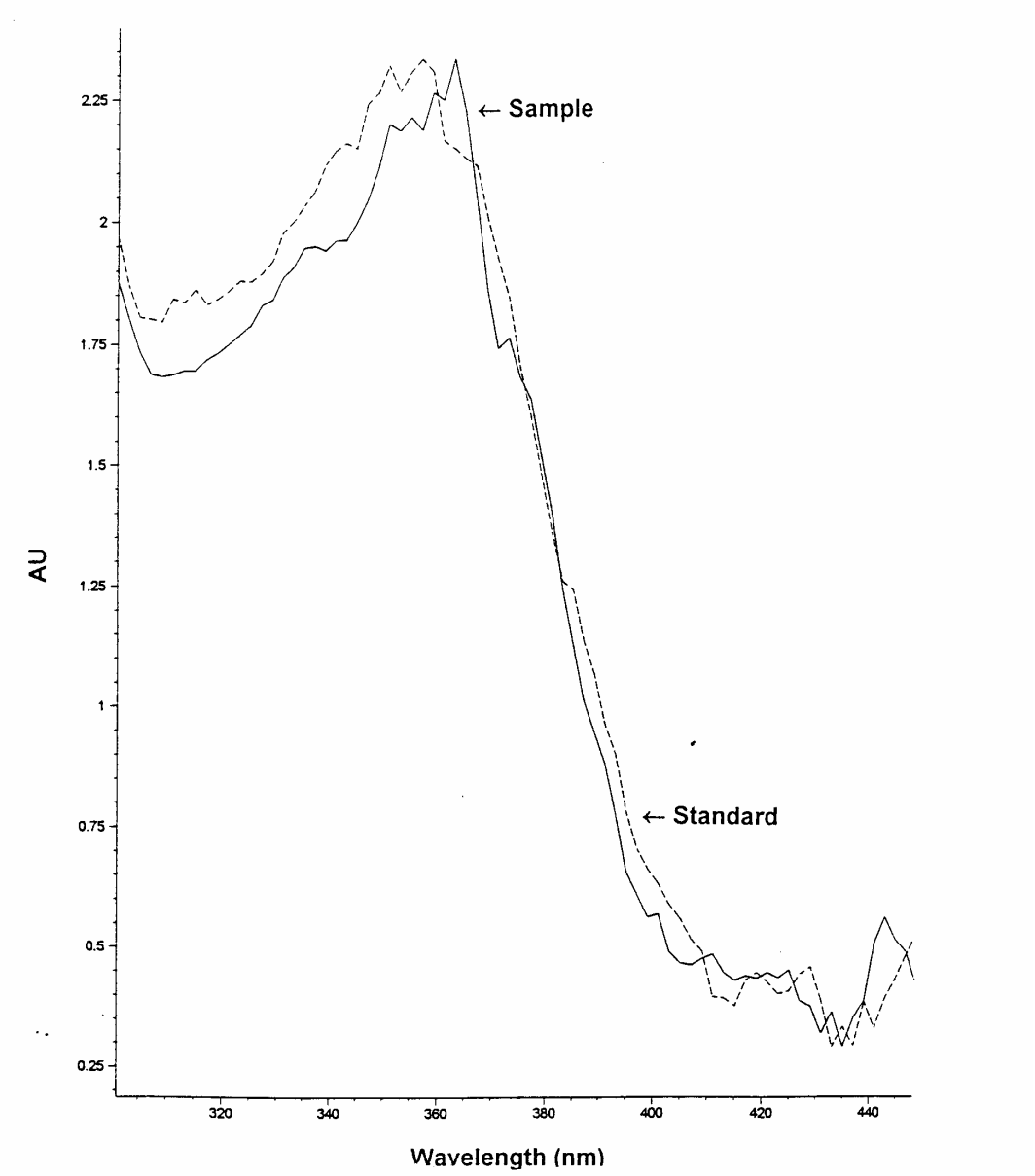


Figure 3. HPLC UV (355 nm) trace of A: standard OTC (19 ng) and B: salmon tissue extract (200 µL) run on a Partisil 5 ODS (25 cm x



4.6 mm i.d.) column. Operating conditions: flow rate 1.0 mL/min., column oven 50 ^{o}C, program, 0.01 M oxalic acid plus 6 % dimethyl- formamide (DMF)(0-2 min.) to 35 % acetonitrile plus 6 % dimethylformamide (DMF) (17 min.) to 70 % acetonitrile plus 6 % dimethylformamide (DMF) (22 min.).

Figure 4. UV diode-array absorption spectra from the peaks of standard OTC (A) and a salmon sample containing OTC (B) run on the Partisil column and scanned in the range of 301-501 nm. Operating conditions as in Figure 3.



CHAPTER 1 - CONTAMINANTS

SECTION 7:ROMET 30 AND TRIBRISSEN EXTRACTION AND ANALYSIS PROCEDURES

1.SCOPE AND APPLICATION

1.1These procedures are applicable to a wide range of fresh and frozen fish and shellfish that have been treated with Romet 30 and Tribrissen, or have come into contact with contaminated material. Method B has also been used in the analysis of spiced and breaded products.

2.PRINCIPLE OF THE METHOD

2.1Romet 30 is a mixture of sulfadimethoxine (SDM) and ormetoprim (OMP) in a 5:1 ratio while Tribrissen is a 5:1 mixture of sulfadiazine (SDZ) and trimethoprim (TMP).

2.2Method A is applicable to the determination of the sulfa drugs SDZ and SDM as well as the potentiators OMP and TMP. Method B is applicable to the determination of the sulfa drugs SDZ and SDM only.

2.3Method A:

2.3.1Fish tissues are blended to homogeneity and SDM, SDZ, OMP and TMP are extracted with acetonitrile:water (1:1). The extract is clarified via centrifugation and a portion defatted with hexane. The analytes are partitioned into chloroform and concentrated.

2.3.2SDM, SDZ, OMP and TMP are analyzed by reversed-phase liquid chromatography with UV detection at 285 ∀ 5 nm. The level of the analytes is calculated by comparison with prepared standards of known concentrations.

2.4 Method B:

2.4.1Fish tissues are blended with octadecylsilyl-derivatized (C18) silica packing material. The blended C18/muscle tissue matrix is used to prepare a column that is washed with hexane. Sulfonamides are then eluted with methylene chloride. Extracts are analyzed by HPLC using UV or photodiode array detection at 273 nm.

3.INTERFERENCES

3.1Fishery products that contain added ingredients, such as in patés, and in breaded, smoked, spiced, or marinated products, may contain material that interferes with the HPLC analysis of one or more of the components. Changes to the chromatographic system can sometimes alleviate the problem but in general, analysis of these types of product should be avoided if possible.

4.SAMPLING PROCEDURE AND STORAGE

4.1Fresh or frozen product.

4.1.1Collect a minimum of 5 animals or enough to yield 150 g of meat for each of 5 sample units.

4.1.2Samples should be frozen prior to shipping.

4.1.3Pack the samples in a shipping container with adequate protection against mechanical damage and temperature change.

4.1.4Place a description of the sample in the package, i.e., date of collection, time, location, species, and any other pertinent data.

4.1.5Send to the laboratory without delay.

4.2Canned or packaged product.

4.2.1Take a representative sample (minimum of 5 units) from the product lot and store so as to maintain sample integrity.

5.SAMPLE PREPARATION

5.1Finfish.

5.1.1Take precautions to prevent contamination of the fish or fish slices by material on the outside of the outer bag or from the processing area.

5.1.2Partially thaw the individual subsamples and remove the skin and backbone.

5.1.3Divide the tissue from each subsample into two approximately equal portions.

5.1.4Prepare a composite sample by placing one half of each subsample into a Silent Cutter or food processor and comminuting until homogeneous.

5.1.5Refreeze the second portion of each subsample in its original plastic bag in case individual analyses are later required. Wash the cutting board, knife and immediate work area to minimize contamination between subsamples.

5.1.6If a Silent Cutter has been used, remove a significant portion of the composite sample, purée and further comminute it in a food processor to ensure better homogeneity.

5.1.7Store a portion of this sample below -20 ^{o}C in a sealable container for later analysis.

5.1.8Ensure the prepared sample is homogeneous prior to weighing the portion for extraction. If liquid separates on thawing, reblend prior to use.

5.2Crustaceans.

5.2.1Remove the meat from the shell, blend until homogeneous and treat as in 5.1.3.

6.APPARATUS

6.1Methods A and B.

6.1.1Food processor.

6.1.2 Silent Cutter or equivalent.

6.1.3 Pipettors.

6.1.3.1Variable volume pipettors with 1, 5 and 10 mL capacity.

6.1.4Rotary vacuum evaporator or equivalent.

6.1.5Bench model centrifuge capable of up to 3000 rpm

(Method A) or 12000 rpm (Method B).

6.1.6Autosampling system able to communicate with the pump and data system and provide up to 100 µL injection volumes either in one injection or by repeated smaller injections.

6.1.7Column oven capable of maintaining column temperatures of up to 50 ^{o}C.

6.1.8HPLC UV monitor capable of achieving the required sensitivity between 270-290 nm with minimal refractive index changes.

6.1.9Data-handling system and recording device.

6.2Method A.

6.2.1Polytron homogenizer or equivalent.

6.2.2 N_2 evaporator or equivalent.

6.2.313 mm nylon syringe filters (0.2 µ pore size recommended).

6.2.43 mL disposable syringes.

6.2.5HPLC pump system able to generate reliable, rapid binary gradients at flow rates up to 1.5 mL/min and at pressures of at least 3000 psi.

6.2.6Columns.

6.2.6.1Routine column: Vydac, 201TP54, 25 cm x 4.6 mm i.d. used without a guard column.

6.2.6.2Alternate column: Partisil 5, ODS-3, 25 cm x 4.6 mm i.d. used without a guard column.

6.3Method B.

6.3.1HPLC pump system able to generate reliable, ternary gradients at flow rates up to 1.5 mL/min and at pressures of at least 3000 psi.

6.3.2100 mL glass mortar and pestle.

6.3.3 10 mL glass syringe barrels.

6.3.4 Filter paper No. 1, 1.5 cm.

6.3.5 Solid-phase extraction manifold.

6.3.6 Turbovap concentrator or equivalent.

6.3.7 Ultrasonic bath.

6.3.8Routine column: µBondapak 3.9 x 300 mm used with a µBondapak guard column.

7.REAGENTS

7.1 Methods A and B.

7.1.1 Acetonitrile, HPLC grade.

7.1.2 Glass-distilled or deionized water.

7.1.3 Hexanes, ACS grade.

7.2 Method A.

7.2.1 Chloroform, ACS grade.

7.2.2 SDM, SDZ, OMP and TMP Standards.

7.2.2.1Stock standards (0.1 mg/mL): Weigh 0.0100 g of each standard into separate 100 mL volumetric flasks. Dissolve the standard and bring each flask to volume with acetonitrile. TMP is slow to dissolve in acetonitrile and a drop or two of trifluoroacetic acid may be required to ensure complete dissolution.

7.2.2.2Working standards (0.0010 mg/mL): Pipette 1.0 mL of each stock standard solution into separate 100 mL flasks and dilute each to volume with glass-distilled water:acetonitrile (2:1).

7.2.2.3Mixed standards (0.0010 mg/mL): Pipette 1.0 mL of each of SDZ, SDM, TMP and OMP stock standard solutions into a 100 mL flask and dilute to volume with glass-distilled

water:acetonitrile (2:1).

7.2.3 Mobile phase.

7.2.3.1Solvent A: glass-distilled water. Solvent B: Acetonitrile + 0.1 % TFA.

7.3 Method B:

7.3.1 Acetic acid, reagent grade.

7.3.1.1Acetic acid (0.01 M). Pipet 0.6 mL acetic acid into a

1 L volumetric flask and dilute to volume with deionized water.

7.3.2 Methylene chloride, ACS grade.

7.3.3C18 - 40 µm 18 % load, endcapped, octadecylsilyl-derivatized silica (Varian Analytichem Bondesil part

1221-3012 - other brands tested gave unacceptable results).

7.3.3.1Prepare C18 by washing in a glass column with 2 volumes each of hexane, methylene chloride, and methanol. Dry the C18 in the glass column using a vacuum source. Store the dried C18 in a sealed container until used.

7.3.4 Sulfamerazine (Internal Standard).

7.3.4.1Sulfamerazine (1 mg/mL) stock solution. Weigh 100 mg sulfamerazine, dissolve in methanol and dilute to 100 mL in a volumetric flask.

7.3.4.2Sulfamerazine (50 µg/mL) internal standard solution. Pipet 5 mL of stock sulfamerazine into a 100 mL volumetric flask and dilute to volume with methanol.

7.3.5 SDZ, SDM.

7.3.5.1Stock standards (1 mg/mL): Weigh 100 mg SDZ and SDM into separate 100 mL flasks and dilute to volume with methanol.

7.3.5.2Sulfa mixed standard (10, 25 and 50 µg/mL). Into 3 separate 100 mL volumetric flasks, pipet 1, 2.5 and 5 mL stock SDM and 1, 2.5 and 5 mL stock SDZ and dilute to volume with methanol.

7.3.5.3Sulfa mixed working standards. Into 4 separate 100 mL volumetric flasks pipet 0.2, 0.4, 1.0 and 2.0 mL

50 µg/mL mixed sulfa standard and 2.0 mL 50 µg/mL internal standard and dilute to volume with 0.01 M acetic acid. This represents 0.1, 0.2, 0.5, and 1 µg/mL of each standard and 1 µg/mL of internal standard.

8. PROCEDURE

8.1 Method A.

8.1.1Accurately weigh 5 g of remixed tissue puree into a

50 mL polyethylene centrifuge tube.

8.1.2Add 20 mL of acetonitrile:glass-distilled water (1:1) and blend until uniform.

8.1.3 Centrifuge for 10 minutes at 3000 rpm.

8.1.4Transfer a 10 mL aliquot of the supernatant with a disposable-tipped pipettor into a 50 mL glass centrifuge tube.

8.1.5Extract with 2.0 mL of hexane, centrifuge for 5 minutes at 1000 rpm and draw off and discard the hexane layer.

8.1.6Add 20 mL of chloroform, mix well and centrifuge for 5 minutes at 1000 rpm.

8.1.7Draw off the upper aqueous layer and discard.

8.1.8Quantitatively transfer the lower layer into a 50 mL round-bottomed flask with a small amount of $CHCl_3$.

8.1.9Evaporate to dryness on a rotary evaporator (max. bath temp. 40 ^{o}C) or equivalent.

8.1.10Add approximately 10 mL of methanol and re-evaporate to dryness.

8.1.11Dissolve in 1.0 mL of glass-distilled water:acetonitrile (2:1) and store in the refrigerator at 0-4 ^{o}C in screw- topped culture tubes for HPLC analysis.

8.1.12For each series of 10 samples run an internal check sample, repeat samples, spiked samples and a reagent blank.

8.1.13Draw the sample into a 3 mL disposable syringe and filter through a 0.2 µm syringe filter directly into an autosampler vial and cap.

8.1.14HPLC analysis.

8.1.14.1 Vydac column.

Equilibrate the system for at least 20 minutes at a column oven temperature of 35 ^{o}C with 100 % solvent A. Adjust the column oven temperature or gradient conditions to obtain baseline separation of all of the standards.

8.1.14.1.1 HPLC conditions.

Mobile Phase - Solvent A: Glass-distilled water

Solvent B: Acetonitrile + 0.1 % TFA

Gradient -100 % solvent A for 0.1 minutes

8 % solvent B at 0.1 minutes

15 % solvent B at 7 minutes

70 % solvent B at 24 minutes

100 % solvent A in 2 minutes

Equilibrate for 6 minutes

Flow - 1.0 mL/min.

Wavelength - 285 ∀ 5 nm

Run time - 32 minutes

8.1.14.2Partisil column.

Equilibrate the system for at least 20 minutes at a column oven temperature of 35 ^{o}C with 25 % solvent B. Adjust the column oven temperature or gradient conditions to obtain baseline separation of all the standards.

8.1.14.2.1 HPLC conditions.

Mobile phase - Solvent A: Glass-distilled water

Solvent B: Acetonitrile + 0.1 % TFA

Gradient - 25 % solvent B for 0 minutes

75 % solvent B in 20 minutes

25 % solvent B in 2 minutes

Equilibrate for 6 minutes

Flow - 1.0 mL/min.

Wavelength - 285 ∀ 5 nm

Run time - 32 minutes

8.1.14.3Calibrate the instrument by repeated injections of the working standard.

8.1.14.4Inject 50 µL of each sample, check sample, 'spiked sample' or blank and measure the resultant peak heights. Peaks are identified by comparison of retention times with recently run standards.

8.1.14.5Spectral confirmations: Compare the UV spectrum of the sample peak(s) in question with the standard peak. Scan the spectrum from 210 nm to 350 nm and correct peaks for background absorption before the comparison is made.

8.2 Method B.

8.2.1Weigh 0.50 g of tissue into a 100 mL glass mortar. Add 10 µL of 50 µg/mL sulfamerazine solution (internal standard) and 10 µL methanol. For spiked samples use

10 µL of the appropriate spiking solution instead of the methanol.

8.2.2Add 2 g of washed C18 to the glass mortar. Blend the tissue and C18 with a glass pestle until homogeneous.

8.2.3Transfer the blended sample-C18 into a 10 mL syringe barrel containing 2 filter paper disks. Place 2 more filter disks on top of the sample-C18 mixture. Compress the mixture until it is packed solidly (about 3 to 4 mL) using a syringe plunger with rubber end and pointed plastic tip removed.

8.2.4 Place the syringe barrel on a vacuum manifold. Add 8 mL hexane to the syringe barrel; with the vacuum on, start flow at a rate of 1-2 drops per second (3-6 mL/minute). After all the hexane has passed through, stop the flow.

8.2.5 Place test tubes (16 mm x 100 mm) in the rack and place in the manifold to collect. Add 8 mL methylene chloride to the syringe barrel; with vacuum on, start flow at a rate of 1-2 drops per second, collect the eluate. After all the methylene chloride has passed through, stop the flow.

8.2.6 Place the test tube containing the methylene chloride extract in the Turbovap and evaporate under nitrogen at max. 45 ^{o}C.

8.2.7 To the dry extract add 50 µL methanol and 200 µL 0.01 M acetic acid and mix using a vortex mixer. Place the test tube containing the sample in an ultrasonic bath for 5 minutes. Transfer the sample to a centrifuge tube and centrifuge for 5 minutes at 12000 rpm. Remove the lower portion for analysis by HPLC (any fat remaining will be in the upper layer). Do not filter.

8.2.8 HPLC conditions:

t = 0 min.	80 %	0.01 M acetic acid
	10 %	acetonitrile
	10 %	methanol

Linear gradient to:

t = 15 min.	30 %	0.01 M acetic acid
	60 %	acetonitrile
	10 %	methanol

Run time:	18 min.
Flow:	1 mL/min.
Wavelength:	273 nm
Injection volume:	50 µL (photodiode array detection)

9. CALCULATIONS

9.1 Method A.

9.1.1 Measure the peak height (peak area) of each analyte from the standard injections, and calculate the average response factor (height units/µg) for each analyte.

9.1.2 Calculate the average peak height of the duplicate injections of the samples.

9.1.3 Calculate the level of SDM, SDZ, OMP or TMP in the sample using the following formula:

$$\mu g/g \text{ ww} = \frac{PH \times D}{PHS \times W}$$

where:

PH =Average peak height of the analyte peak in the sample

PHS =Response factor of the standard in height units per µg

W =Weight of the sample extracted

D =Dilution factor

9.2 Method B.

9.2.1 Prepare a calibration curve by plotting the peak area ratios of the standard to the internal standard versus their weight ratios. Determine the amount of sulfadimethoxine and sulfadiazine in the sample solution from the calibration curve by comparing the peak area ratios of the sample solution to the internal standard versus their weight ratios.

9.2.2Determine the sulfadimethoxine and sulfadiazine concentration in the fish tissue by using the following equation:

$$C1\ (\mu g/g\ tissue) = \frac{C2}{W}$$

where:

C1 = sulfa concentration in tissue sample (μg/g)

C2 = sulfa amount in the sample solution (μg)

W = weight of tissue (0.5 g)

10. PRECISION AND ACCURACY

10.1 Method A.

10.1.1Replicate analysis of salmon tissue at residue levels ranging from 0.03 to 0.19 μg/g gave percent relative standard deviations from 1.5 to 12. Variations were the highest as the limit of detection was approached.

10.1.2The limit of detection in the tissue is approximately 0.01 μg/g for SDM, 0.02 μg/g for OMP and SDZ and between 0.02 and 0.03 for TMP.

10.2 Method B.

10.2.1The method gives a detection limit of 0.05 μg/g for sulfadimethoxine and sulfadiazine.

11.REMARKS

11.1Method A (Tables and Figures - Appendix B).

11.1.1Representative chromatograms of standard SDM, SDZ, OMP and TMP and spiked and control salmon tissue extracts run on a Vydac column are shown in Figures 1 and 2. A chromatogram of SDM, SDZ, OMP and TMP standards run on a Partisil column is shown in Figure 3, while standard curves for the components of Romet 30 and Tribrissen are shown in Figures 4 and 5.

11.1.2Recoveries of SDZ, SDM, OMP and TMP from fortified samples of salmon tissue are shown in Table 1. Mean recoveries from tissue samples fortified at the 0.05 to 2.0 μg/g levels were 90, 70, 73 and 85 % for SDZ, TMP, OMP and SDM respectively.

11.1.3Initial analyses are performed on sample composites. The individual sub-samples are analyzed if the result of the sample composite is in excess of the regulatory limit for any of the components. Samples which approach or exceed this present alert limit should be confirmed either chromatographically and/or by using diode array detection.

11.1.4The quality of water for the mobile phase is important. Impurities in the water can cause peaks in the chromatograms that interfere with the quantitation of OMP.

11.1.5Romet 30 and Tribrissen stock, and working standards must be stored in a refrigerator between 0-4 ^{o}C.

11.2Method B (Figures - Appendix B).

11.2.1Representative chromatograms of a standard solution

(1 μg/mL sulfadiazine, sulfamerazine, and sulfadimethoxine), a salmon tissue extract and a spiked salmon tissue extract run on a μBondapak column are shown in Figures 1, 2, and 3 respectively.

11.2.2Recoveries for sulfadimethoxine at 0.2 μg/g averaged

87 % with a standard deviation of 11.9 %; for sulfadiazine at 0.2 μg/g averaged 97 % with a standard deviation of 8.7 % (n=35). These spiked tissues were

90 % salmon and the remainder other species (mainly shrimp).

11.2.3Other sulfa drugs (sulfathiazole, sulfamethazine, sulfamethoxine, and sulfisoxazole) can be determined by this method; however, interference from the fish tissue matrix has not been explored for these compounds.

11.2.4The relative retention time for sulfadiazine is 0.88 and for sulfadimethoxine is 1.44 when compared to the internal standard sulfamerazine.

11.2.5It is not possible to do a reagent blank with this method; tissue must be used to produce acceptable results as without tissue the chromatograms are very poor.

11.2.6Filtering the extract tends to give poor recoveries (cause unknown); this does not appear to affect the life of the column.

12. REFERENCES

12.1Association of Official Analytical Chemists, Official methods of analysis, 14th edition, (1984), 18.001.

12.2Burns, B.G., J.G. Landry, J.M. van de Riet and M.W. Gilgan, "The simultaneous determination of the residual components of Romet-30 and Tribrissen in salmonid products by HPLC", Can. Tech. Rep. Fish. Aquat. Sci., (1996), 2008: vi + 18 pages.

12.3Long, A.R. et al., "Multiresidue Method for the Determination of Sulfonamides in Pork Tissue", J. Agric. Food Chem., 38, (1990), p 423-426.

12.4Long, A.R. et al., "Matrix Solid Phase Dispersion Isolation and Liquid Chromatographic Determination of Sulfadimethoxine in Catfish (Ictalurus punctatus) Muscle Tissue", J. Assoc. Off. Anal. Chem. 73, (1990), p 868-871.

APPENDIX A

Table 1:Percent recovery of SDZ, SDM, OMP and TMP added to salmon muscle tissue using Method A.

Sample	Fortification Level				Percent Recovery		
(µg/g)	SDZ	TMP	OMP	SDM			
1	0		-	-	-	-	
2	0.05		90	64	70	86	
3	0.10		97	74	84	83	
4	0.20		92	75	78	87	
5	1.0		89	70	70	85	
6	2.0		80	65	61	83	
	Mean recoveries:			90	70	73	85

Figure 1: Liquid chromatogram of 50 ng of each of SDZ, TMP, OMP and SDM. Chromatographic conditions: column: Vydac 201TP54 (25 cm x 4.6 mm i.d.); mobile phase: glass- distilled water (A) and acetonitrile + 0.1 % trifluoroacetic acid (B). System stabilized at 35 ^{o}C with 100 % solvent A at a flow rate of 1.0 mL/min. Held for 0.1 min then to 8 % B. Gradient immediately started to 15 % B at 7 min. then to 70 % B in 24 min. UV detection at 288 nm.

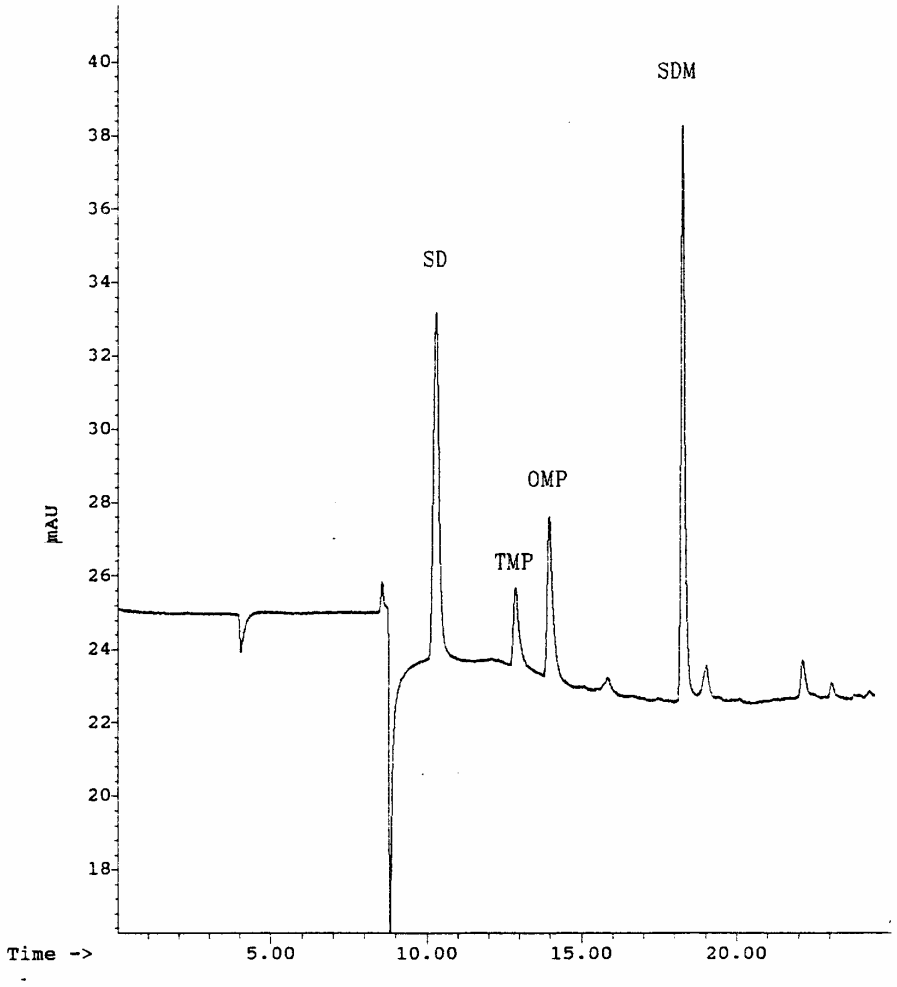


Figure 2: Liquid chromatograms of: (A) control salmon tissue sample spiked at the 1.0 µg/g level; and (B) a control salmon tissue sample. Chromatographic conditions as in Figure 1.

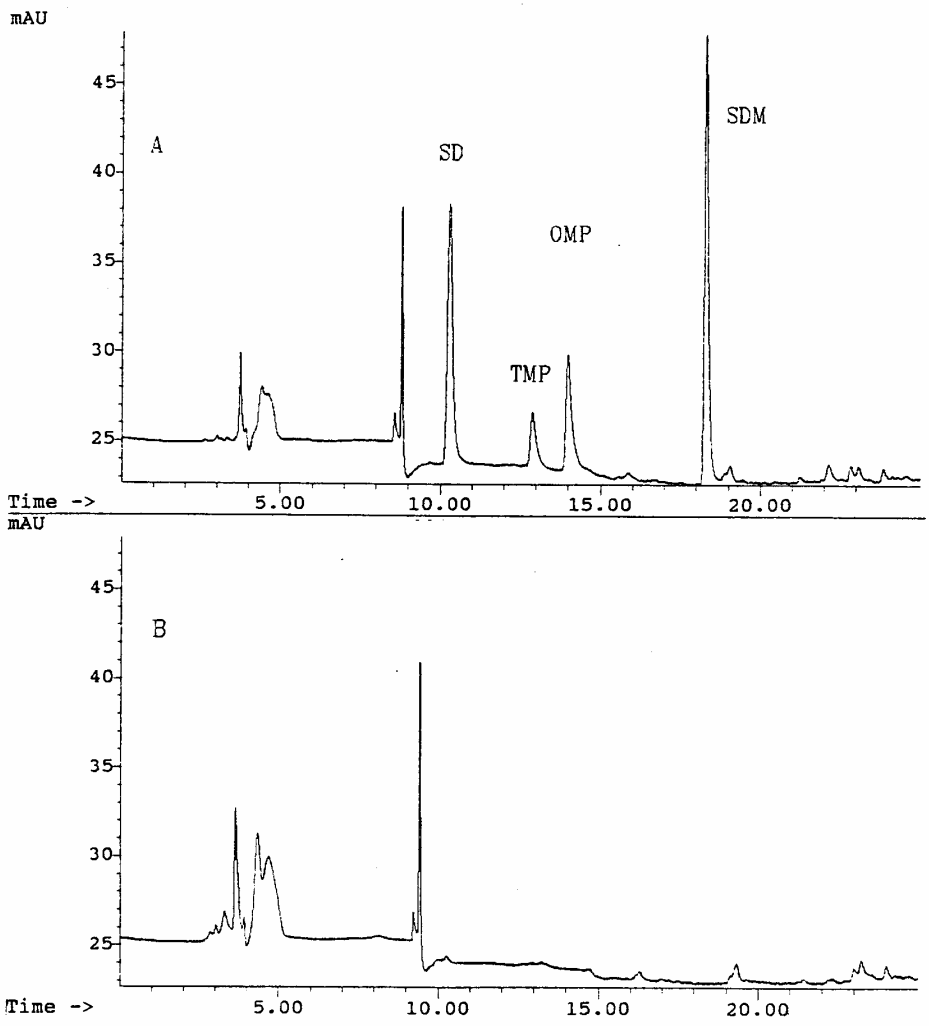
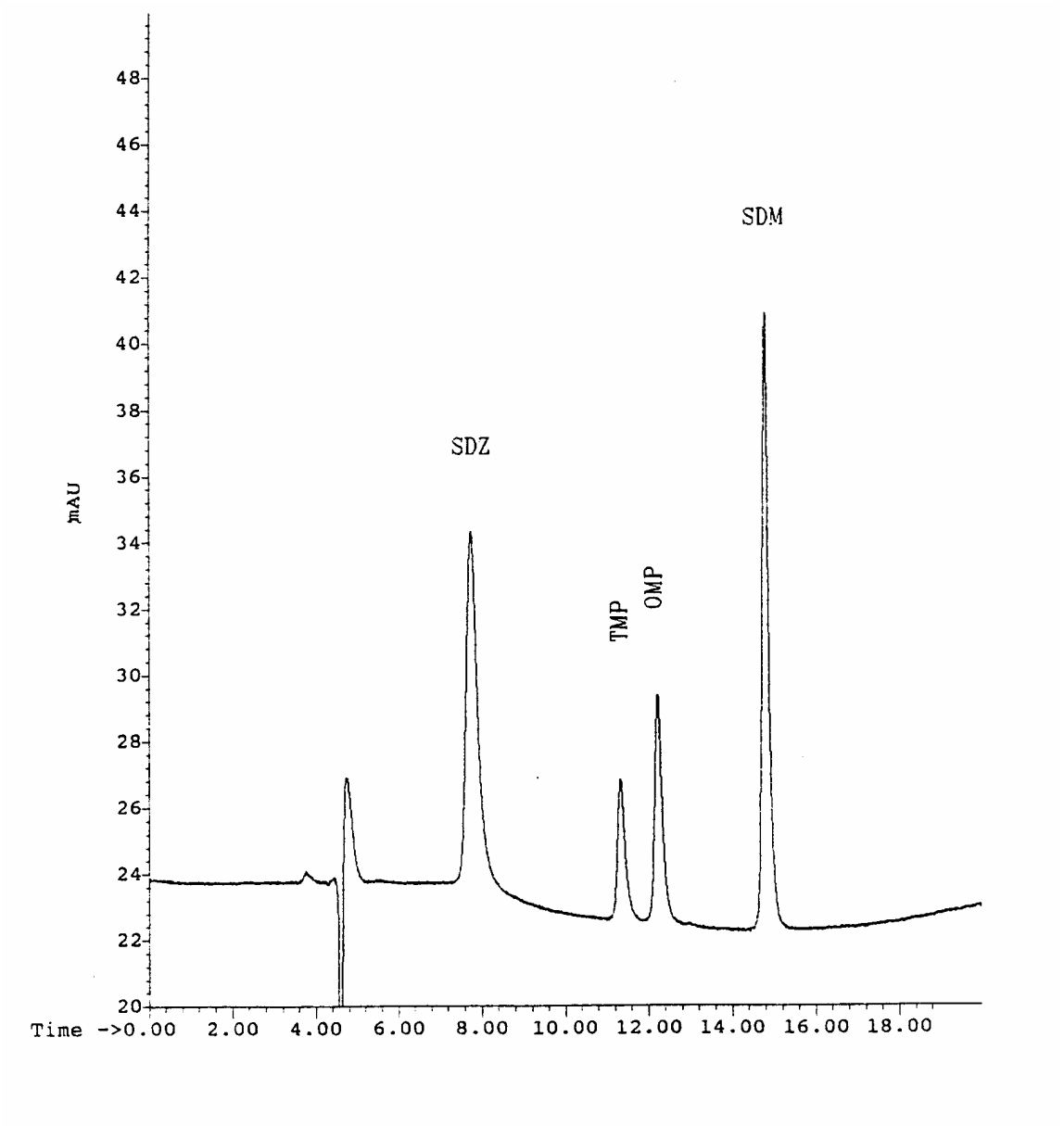


Figure 3: Liquid chromatogram of 50 ng of each of SDZ, TMP, OMP and SDM. Chromatographic conditions: column: Partisil 5 ODS-3 (25 cm x 4.6 mm i.d.); mobile phase: glass- distilled water (A) and acetonitrile + 0.1 % trifluoroacetic acid (B). System stabilized with 25 % B in A at 35 °C with a flow rate of 1.0 mL/min. Gradient immediately started to 75 % B at 20 mins. UV detection at 280 nm.



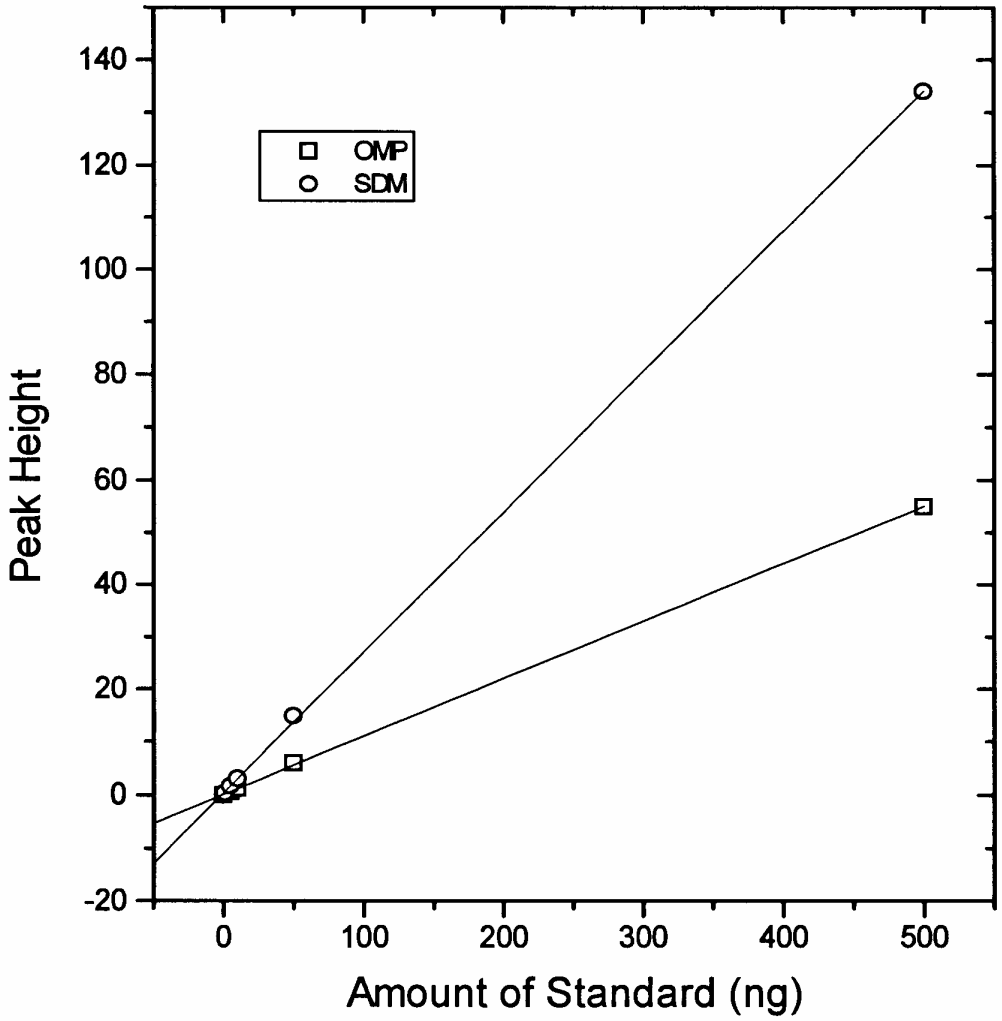


Figure 4: Standard calibration curve for SDM and OMP (Romet 30)

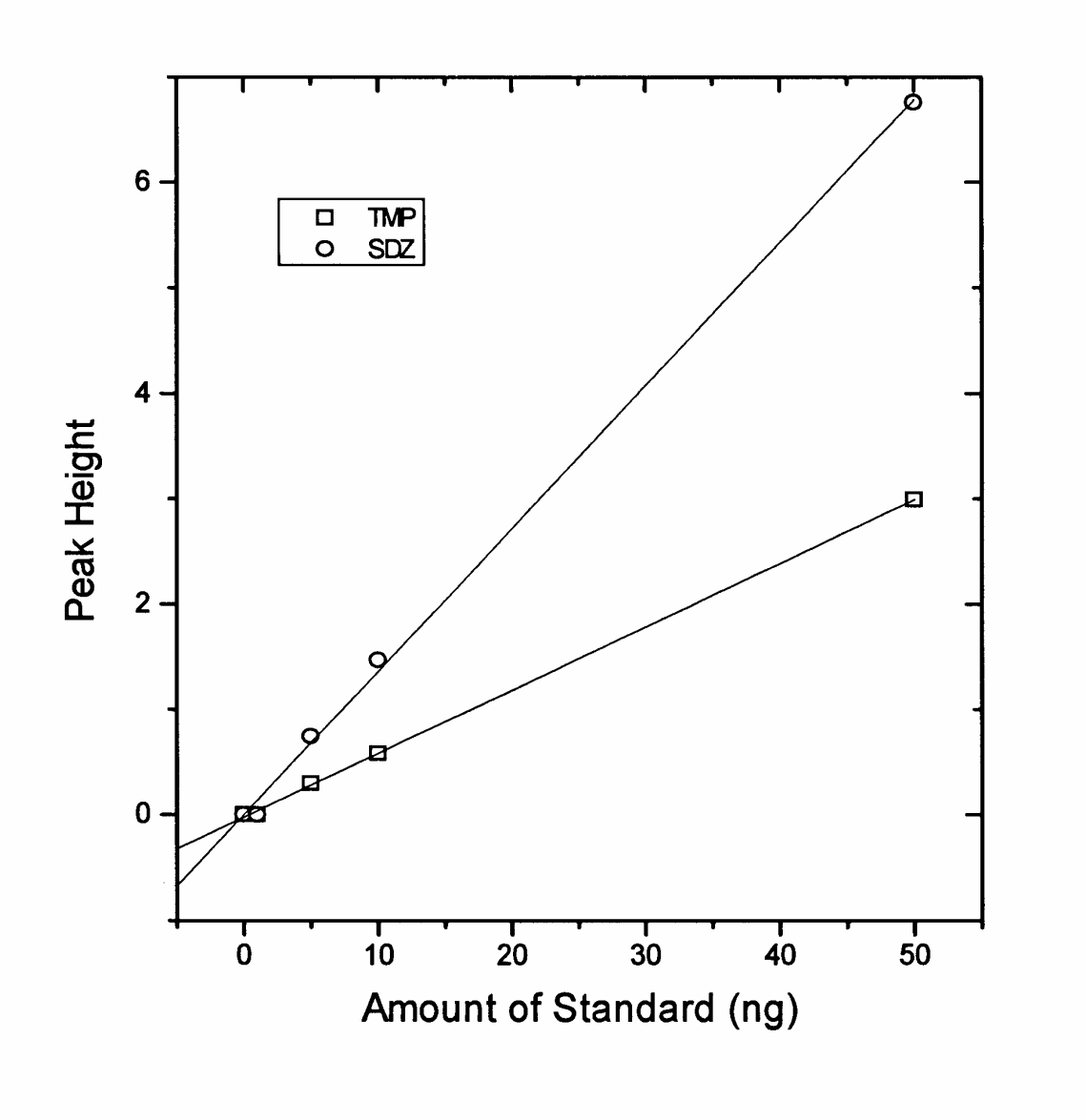


Figure 5: Standard curve for SDZ and TMP (Tribrissen)

APPENDIX B

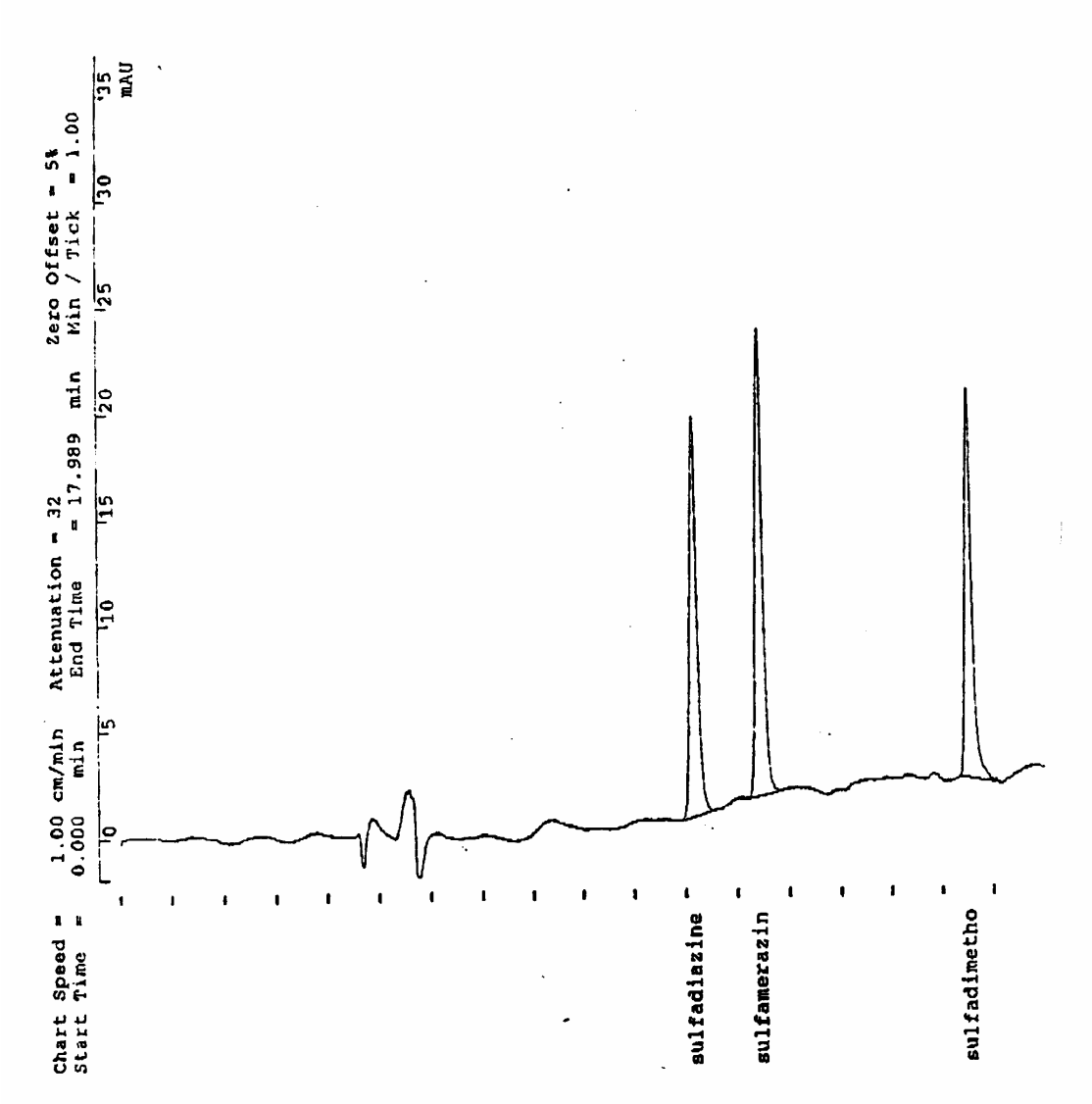


Figure 1: Liquid chromatogram of 50 ng of each of sulfadiazine, sulfamerazine and sulfadimethoxine. Chromatographic conditions: column: µBondapak (3.9 mm x 300 mm); mobile phase: 0.01 M acetic acid (A), methanol (B), and acetonitrile (C). Initial conditions 85 % A, 10 % B, and 5 % C. Gradient started at t=0 min. continuing to t=15 min.: conditions 30 % A, 10 % B, and 60 % C. End time 18 min. Column temperature 30 ^{o}C. UV detection at 273 nm.

Figure 2: Liquid chromatogram of a control salmon tissue containing sulfamerazine internal standard. Chromatographic conditions are as in Figure 1.

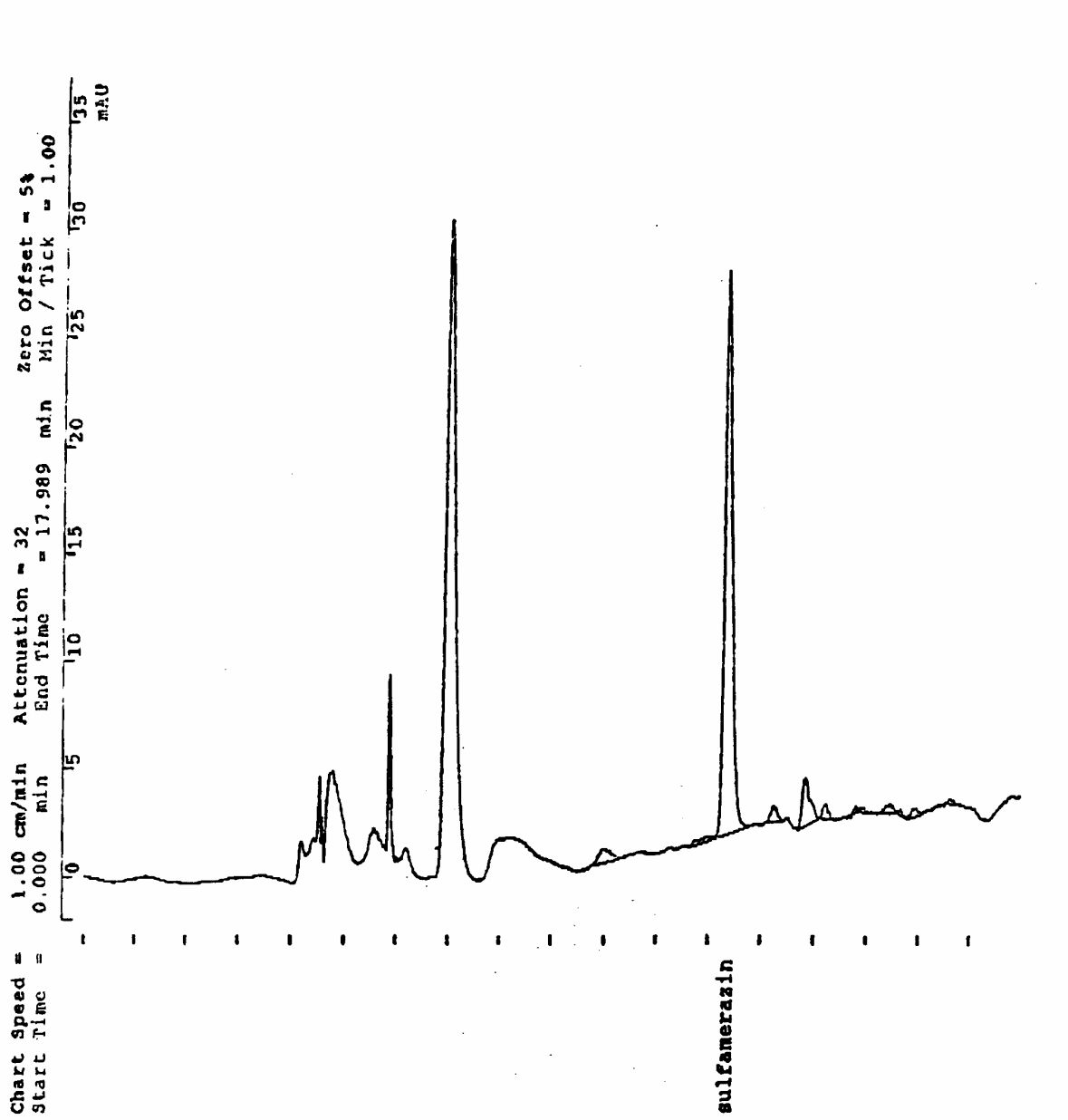
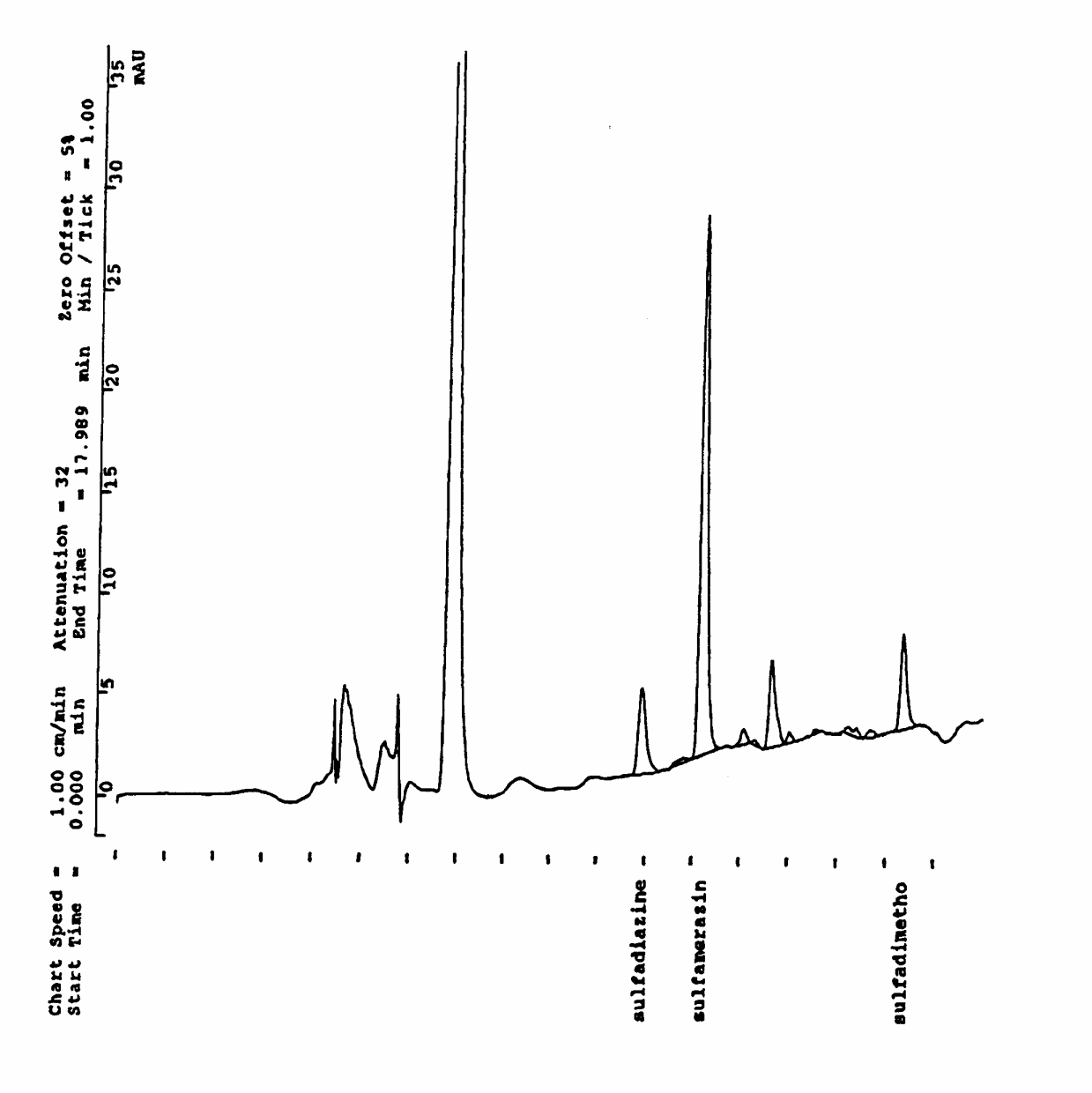


Figure 3: Liquid chromatogram of a salmon tissue containing internal standard and spiked with 0.2 µg/g sulfadiazine and 0.2 µg/g sulfadimethoxine. Chromatographic conditions as in Figure 1.



Ad Hoc Working Group on Methods of Analysis and Sampling

Analytical Method Information Summary

A. Descriptive Information

1. Name of drug or chemical: **Emamectin and Ivermectin**
2. Drug or chemical class: Avermectin family
   (e.g. antimicrobial, anthelmintic, etc)
3. Veterinary Use: Broad Spectrum Insecticide
4. Analyte(s) measured: Emamectin (EMA) and Ivermectin (IVR)
   (specify if metabolite)
5. Intended use of the method:
   a. Screening _____
   b. Routine_____x_____
   c. Reference_____
   d. Confirmatory_____
6. Test matrix : Atlantic Salmon (muscle / edible tissue)
   (e.g. muscle, kidney, urine, etc)
7. Summary of principal steps in sample preparation:
   Fish samples are filleted, de-boned and homogenized.
8. Summary of principal steps in extraction procedure:
   Samples homogenized with acetonitrile and extracted by centrifugation. Supernatant is diluted with water.
9. Summary of principal steps in analyte clean-up procedure:
   Extract is clean-up with C-18 solid phase extraction followed by derivitization with 1-methylimidazole and trifluoroacetic acid.
10. Measurement procedure:
    a. Chemical
       1. Instrumentation HPLC
       2. Detector system FLD λex 365nm λem 470nm
       3. Chromatographic column Supelcosil LC-18-DB, 5µm, 25cm x 4.6mm id
          (if applicable)
    b. Immunochemical/Immunoassay
       1. Technique: _____
          (e.g. Elisa, RIA, Immunochromatog, etc)
       2. Critical reagents: _____
          _____
          (e.g. antibody specificity and availability)
       3. Special equipment required: _____
    c. Microbiological

1. Technique: _____
2. Organism: _____
3. Media:_____
4. Special equipment required:_____

11. Sample/Analyte Stability

Warning (if applicable): Samples should be stored frozen. Standards should be kept refrigerated.

12. Literature References available:

van de Riet et al. 2001. Journal of AOAC International. 84 (5). 1358-1362.

13. Contact for Information:

a. Name Garth Burns _____
b. Country Canada_____
c. Affiliation Canadian Food Inspection Agency_____
d. Address 1992 Agency Dr. Dartmouth, NS, B3B 1Y9_____
e. Telephone (902) 426-1982_____
f. FAX (902) 426-0314_____
g. Email burnsbg@inspection.gc.ca_____

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) 5.0×10^{-4} for EMA and IVR

How was LOD determined? 3 times baseline noise_____

_____

b. Limit of Quantification (LOQ) (mg/kg) 1.5×10^{-3} for EMA and IVR

How was LOQ determined? 3 times LOD_____

_____

c. Method sensitivity uncertainty of measurement 43% for EMA and 36% for IVR

(The smallest difference in concentration that can be measured)

2. JECFA MRL_____IVR not permitted

EMA 42ng/g

3. Are analytical data corrected for recovery? Yes__x__ No_____
4. How is recovery estimated _____external standard / fortification

(e.g. external standard; internal standard. etc)

5. Accuracy

EMA

a. Concentration(s) tested	80 ng/g	40 ng/g	5 ng/g
b. Concentration(s) measured	72.8 ng/g	42 ng/g	4.6 ng/g
c. Recovery (%)±% RSD	91% ±7	104% ±5	92% ±5

IVR

a. Concentration(s) tested	80 ng/g	40 ng/g	5 ng/g
b. Concentration(s) measured	72 ng/g	33.6 ng/g	4.2 ng/g

c. Recovery (%)±% RSD	90% ±6	84% ±4	83% ±5

6. Precision using fortified control tissue

	EMA	IVR
a. Concentration(s) tested (ng/g)	38ng/g	5.2
b. Repeatability (within lab CV) %RSD	4	7
c. Reproducibility (between lab CV)	__	__

7. Precision using tissue containing incurred drug residues

a. Concentration(s) tested ____NA_____

b. Repeatability (within lab CV) ____NA_____

c. Reproducibility (between lab CV) ____NA_____

8. Selectivity of the method

This information is often referenced as "Specificity". Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure _____
or drug class or other veterinary _____
drugs that may also be used along _____
with the analyte of interest
_____.

b. Contaminants that are likely ___tetracyclines, fenicols, nitrofuran metabolites
to be present in the sample ____oxolinic acid, malachite green_____

9. Type of Validation studies

Single laboratory __x__

a. Multi-laboratory _____

b. AOAC or other official procedure _____

C. Information relevant to laboratory implementation

1. Training and experience recommended for analysts
   -See attached document
2. Critical steps in the method
   -See attached document
3. Information on availability of unusual reagents or equipment
   -See attached document
4. Special reagent or sample stability concerns
   -See attached document
5. Reagent handling and safety concerns (if any)
   -See attached document
6. Literature references or other useful information

-See attached document

Ad Hoc Working Group on Methods of Analysis and Sampling

Analytical Method Information Summary

A. Descriptive Information

1. Name of drug or chemical: Fenicols
2. Drug or chemical class: Antimicrobial

   (e.g. antimicrobial, anthelmintic, etc)
3. Veterinary Use: Broad spectrum antibiotic
4. Analyte(s) measured: Chloramphenicol (CAP), Thiamphenicol (TAP), Florfenicol (FLR) and Florfenicol Amine (FFA) (metabolite of FLR)

   (specify if metabolite)
5. Intended use of the method:

   e. Screening _____

   f. Routine_____x_____

   g. Reference_____

   h. Confirmatory__x_____
6. Test matrix : Atlantic Salmon (muscle / edible tissue)

   (e.g. muscle, kidney, urine, etc)
7. Summary of principal steps in sample preparation:

   Fish samples are filleted, de-boned and homogenized.
8. Summary of principal steps in extraction procedure:

   Samples homogenized with actetone/water and extracted by centrifugation
9. Summary of principal steps in analyte clean-up procedure:

   Extract is partitioned with Dichloromethane and evaporated to dryness. Samples are re-suspended in dilute acetic acid and further cleaned-up with hexane. Aqueous portion injected on LC/MS
10. Measurement procedure:

    a. Chemical

       4. Instrumentation LC/MS
       5. Detector system MS
       6. Chromatographic column Hypersil C-18 BD, 5 µ, 15cm x 2 mm ID or equivalent (if applicable)

    b. Immunochemical/Immunoassay

       3. Technique: _____

          (e.g. Elisa, RIA, Immunochromatog, etc)

       2. Critical reagents: _____

          _____

          (e.g. antibody specificity and availability)

       3. Special equipment required: _____

    c. Microbiological

       1. Technique: _____

       4. Organism: _____

3. Media:_____
4. Special equipment required:_____

11. Sample/Analyte Stability

Warning (if applicable): Samples should be stored frozen. Standards should be kept refrigerated.

12. Literature References available:

van de Riet et al. Journal of AOAC International. Vol. 86 (3). 2003.

_____

13. Contact for Information:

a. Name Garth Burns _____
b. Country Canada_____
c. Affiliation Canadian Food Inspection Agency_____
d. Address 1992 Agency Dr. Dartmouth, NS, B3B 1Y9_____
e. Telephone (902) 426-1982_____
f. FAX (902) 426-0314_____
g. Email burnsbg@inspection.gc.ca_____

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) 1.0 x 10^{-4} CAP, TAP, FLR and 8.0 x10^{-4} FFA

How was LOD determined? 3 times baseline noise_____

_____

b. Limit of Quantification (LOQ) (mg/kg) 3.0 x 10^{-4} CAP, TAP, FLR and 2.0 x10^{-3} FFA

How was LOQ determined? 3 times LOD_____

_____

c. Method sensitivity uncertainty of measurement 28% FFA, 33% TAP, 32% FFC and 29% CAP

(The smallest difference in concentration that can be measured)

2. JECFA MRL_____ FFA and FLR- sum cannot exceed 800ng/g

CAP and TAP-banned, no MRL

3. Are analytical data corrected for recovery? Yes__x__ No_____
4. How is recovery estimated _____external standard / fortification

(e.g. external standard; internal standard. etc)

5. Accuracy

FFA

a. Concentration(s) tested	2 ng/g	50 ng/g	100 ng/g
b. Concentration(s) measured	1.42 ng/g	28 ng/g	57 ng/g
c. Recovery (%)	70.8% ±9	56% ±9	57% ±7

TAP

a. Concentration(s) tested	2 ng/g	50 ng/g	100 ng/g
b. Concentration(s) measured	1.64 ng/g	34 ng/g	66 ng/g
c. Recovery (%)	82% ±11	68% ±3	66% ±8

FFC

a. Concentration(s) tested	2ng/g	50 ng/g	100 ng/g	
b. Concentration(s) measured	2.14 ng/g	46.5 ng/g	90 ng/g	
c. Recovery (%)	107% ±9	93% ±3	90% ±7	

CAP

a. Concentration(s) tested	2 ng/g	50 ng/g	100 ng/g	
b. Concentration(s) measured	1.74ng/g	41 ng/g	84 ng/g	
c. Recovery (%) ± %RSD		87.2% ±9	82% ±4	84% ±10

6. Precision using fortified control tissue

	FFA	TAP	FFC	CAP
a. Concentration(s) tested (ng/g)	0.58	0.76	0.68	0.49
b. Repeatability (within lab CV) %RSD	44	13	13	13
c. Reproducibility (between lab CV)	__	__	__	__

7. Precision using tissue containing incurred drug residues

a. Concentration(s) tested ____NA_____

b. Repeatability (within lab CV) ____NA_____

c. Reproducibility (between lab CV) ____NA_____

8. Selectivity of the method

This information is often referenced as "Specificity". Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

b. Drugs of similar structure _____
or drug class or other veterinary _____
drugs that may also be used along _____
with the analyte of interest
_____.

b. Contaminants that are likely ___tetracyclines, sulfonamides, nitrofuran metabolites
to be present in the sample ____oxolinic acid, malachite green_____

9. Type of Validation studies

c. Single laboratory __x__

d. Multi-laboratory _____

e. AOAC or other official procedure _____

C. Information relevant to laboratory implementation

1. Training and experience recommended for analysts

-See attached document

2. Critical steps in the method

-See attached document

3. Information on availability of unusual reagents or equipment

   -See attached document

4. Special reagent or sample stability concerns

   -See attached document

5. Reagent handling and safety concerns (if any)

   -See attached document

6. Literature references or other useful information

   -See attached document

Ad Hoc Working Group on Methods of Analysis and Sampling

Analytical Method Information Summary

A. Descriptive Information

1. Name of drug or chemical: Nitrofuran metabolites
2. Drug or chemical class: Antibiotic

   (e.g. antimicrobial, anthelmintic, etc)
3. Veterinary Use: Use for treatment of gastrointestinal infections in cattle, pigs and poultry. Also used as broad spectrum antibiotic in shrimp and other species.
4. Analyte(s) measured: 3-Amino-2-oxazolidinone (AOZ) : metabolite of furazolidone

   1-Aminohydantoin hydrochloride (AHD): metabolite of nitrofurantoin

   3-Amino-5-morpholinomethyl-oxazolidin-2-one (AMOZ): metabolite of furaltadone

   Semicarbazide: metabolite of nitrofurazone

   (specify if metabolite)
5. Intended use of the method:
   a. Screening _____
   b. Routine_____x_____
   c. Reference_____
   d. Confirmatory__x_____
6. Test matrix : Atlantic Salmon (muscle / edible tissue)

   (e.g. muscle, kidney, urine, etc)
7. Summary of principal steps in sample preparation:

   Fish samples are filleted, de-boned and homogenized.
8. Summary of principal steps in extraction procedure:

   Samples washed with increasing concentrations of methanol to remove components which are not protein bound. Washed samples are derivatized with 2-nitrobenzaldehyde under acidic conditions. Derivatized samples are neutralized and extracted with ethyl acetate.
9. Summary of principal steps in analyte clean-up procedure:

   Samples are evaporated, re-suspended and filtered before LC/MS/MS analysis.
10. Measurement procedure:
    a. Chemical
       1. Instrumentation LC/MS/MS
       2. Detector system MS/MS
       3. Chromatographic column Luna C-18, 3µm, 15cm x 2 mm ID or equivalent

          (if applicable)
    b. Immunochemical/Immunoassay
       1. Technique: _____

          (e.g. Elisa, RIA, Immunochromatog, etc)
       2. Critical reagents: _____

          _____

(e.g. antibody specificity and availability)

3. Special equipment required: _____

c. Microbiological

1. Technique: _____
2. Organism: _____
3. Media:_____
4. Special equipment required:_____

11. Sample/Analyte Stability

Warning (if applicable): Samples should be stored frozen. Standards should be kept refrigerated.

12. Literature References available:

Muscle of poultry, rabbit and aquaculture products - Detection and identification of residues of metabolites of furazolidone, furaltadone, nitrofurantoin and nitrofurazone LC/MS/MS confirmatory analysis. State Institite for Quality Control for Agricultural Products(RIKILT)-27-06-2002

Determination of total nitrofuran residues in tissue using LC-MS/MS. Standard Operating Procedure of the Department of Agriculture and Rural DevelopmentVeterinary Sciences Division, Republic of Ireland

13. Contact for Information:

a. Name Garth Burns _____

b. Country Canada_____

c. Affiliation Canadian Food Inspection Agency_____

d. Address 1992 Agency Dr. Dartmouth, NS, B3B 1Y9_____

e. Telephone (902) 426-1982_____

f. FAX (902) 426-0314_____

g. Email burnsbg@inspection.gc.ca_____

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) 1.0 x 10^{-4} AMOZ, AHD and AOZ and 4.0 x10^{-4} SEM

How was LOD determined? 3 times baseline noise_____

_____

b. Limit of Quantification (LOQ) (mg/kg) 1.0 x 10^{-4} AMOZ, AHD and AOZ and 4.0 x10^{-4} SEM
How was LOQ determined? Same as LOD – Metabolites are banned substances in food producing animals. The lowest level that can be detected and quantified is reported.

_____

c. Method sensitivity uncertainty of measurement 19% AMOZ, 32% SEM, 23% AHD and 21% AOZ

(The smallest difference in concentration that can be measured)

2. JECFA MRL_____Substances are banned in Canada
3. Are analytical data corrected for recovery? Yes__x__ No_____
4. How is recovery estimated _____external standard / fortification

(e.g. external standard; internal standard. etc)

5. Accuracy

AMOZ

a. Concentration(s) tested	11.8 ng/g	2.37 ng/g	0.47 ng/g
b. Concentration(s) measured	9.7 ng/g	1.97 ng/g	0.37 ng/g
c. Recovery (%)± %RSD	82% ±5	83% ±6	78% ±

SEM

a. Concentration(s) tested	15.0 ng/g	2.99 ng/g	0.60 ng/g
b. Concentration(s) measured	11.1 ng/g	2.12 ng/g	0.44 ng/g
c. Recovery (%)± %RSD	74% ±5	71% ±4	73±15

AHD

a. Concentration(s) tested	13.0 ng/g	2.59 ng/g	0.52 ng/g
b. Concentration(s) measured	8.8 ng/g	1.79 ng/g	0.31 ng/g
c. Recovery (%)± %RSD	68% ±6	69% ±4	60% ±14

AOZ

a. Concentration(s) tested	11.8 ng/g	2.37 ng/g	0.47 ng/g
b. Concentration(s) measured	6.8 ng/g	1.35 ng/g	0.26 ng/g
c. Recovery (%) ± %RSD	58% ±5		57% ±3

56% ±11

6. Precision using fortified control tissue

	AMOZ	SEM	AHD	AOZ
a. Concentration(s) tested (ng/g)	0.95	0.75	0.80	0.65
b. Repeatability (within lab CV) %RSD	16	17	20	17
c. Reproducibility (between lab CV)	__	__	__	__

7. Precision using tissue containing incurred drug residues

a. Concentration(s) tested ____NA_____

b. Repeatability (within lab CV) ____NA_____

c. Reproducibility (between lab CV) ____NA_____

8. Selectivity of the method

This information is often referenced as “Specificity”. Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure _____
or drug class or other veterinary _____
drugs that may also be used along _____
with the analyte of interest
_____.

b. Contaminants that are likely ___tetracyclines, sulfonamides, nitrofuran metabolites
to be present in the sample ____oxolinic acid, malachite green_____

9. Type of Validation studies
    a. Single laboratory __x__
    b. Multi-laboratory _____
    c. AOAC or other
       official procedure _____

C. Information relevant to laboratory implementation

1. Training and experience recommended for analysts
   -See attached document
2. Critical steps in the method
   -See attached document
3. Information on availability of unusual reagents or equipment
   -See attached document
4. Special reagent or sample stability concerns
   -See attached document
5. Reagent handling and safety concerns (if any)
   -See attached document
6. Literature references or other useful information
   -See attached document

Ad Hoc Working Group on Methods of Analysis and Sampling

Analytical Method Information Summary

A. Descriptive Information

1. Name of drug or chemical: Sulfathiazole
2. Drug or chemical class: Antimicrobial
   (e.g. antimicrobial, anthelmintic, etc)
3. Veterinary Use: Broad spectrum antibiotic
4. Analyte(s) measured: Sulfathiazole (STZ)
   (specify if metabolite)
5. Intended use of the method:
   a. Screening _____
   b. Routine_____x_____
   c. Reference_____
   d. Confirmatory__x_____
6. Test matrix : Atlantic Salmon (muscle / edible tissue)
   (e.g. muscle, kidney, urine, etc)
7. Summary of principal steps in sample preparation:
   Fish samples are filleted, de-boned and homogenized.
8. Summary of principal steps in extraction procedure:
   Samples homogenized with acetonitrile/water and extracted by centrifugation
9. Summary of principal steps in analyte clean-up procedure:
   Extract is partitioned with hexane and then by chloroform and evaporated to dryness. Samples are re-suspended with acetonitrile/water.
10. Measurement procedure:
    a. Chemical
       1. Instrumentation LC/MS/MS
       2. Detector system MS/MS
       3. Chromatographic column Luna C-18, 3µm, 15cm x 2 mm ID or equivalent
          (if applicable)
    b. Immunochemical/Immunoassay
       1. Technique: _____
          (e.g. Elisa, RIA, Immunochromatog, etc)
       2. Critical reagents: _____
          _____
          (e.g. antibody specificity and availability)
       3. Special equipment required: _____
    c. Microbiological
       1. Technique: _____
       2. Organism: _____

3. Media:_____
4. Special equipment required:_____

11. Sample/Analyte Stability

Warning (if applicable): Samples should be stored frozen. Standards should be kept refrigerated.

12. Literature References available:

Burns, BG., van de Riet, J.M. Landry, J.G., and Gilgan, M.W. 1996. Can. Tech. Rep. Fish. And Aquat. Sci. 2008: vi + 18 pp.

13. Contact for Information:

a. Name Garth Burns _____
b. Country Canada_____
c. Affiliation Canadian Food Inspection Agency_____
d. Address 1992 Agency Dr. Dartmouth, NS, B3B 1Y9_____
e. Telephone (902) 426-1982_____
f. FAX (902) 426-0314_____
g. Email burnsbg@inspection.gc.ca_____

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) 4.0×10^{-3}

How was LOD determined? 3 times baseline noise_____

_____

b. Limit of Quantification (LOQ) (mg/kg) 1.3×10^{-2}

How was LOQ determined? 3 times LOD_____

_____

c. Method sensitivity uncertainty of measurement 31%

(The smallest difference in concentration that can be measured)

2. JECFA MRL_____not permitted
3. Are analytical data corrected for recovery? Yes__x__ No_____
4. How is recovery estimated _____external standard / fortification

(e.g. external standard; internal standard. etc)

5. Accuracy

STZ

a. Concentration(s) tested	200 ng/g	100 ng/g	10 ng/g
b. Concentration(s) measured	142 ng/g	74 ng/g	6.0 ng/g
c. Recovery (%)±% RSD	71% ±6	74% ±11	60% ±16

6. Precision using fortified control tissue

	STZ
a. Concentration(s) tested (ng/g)	136ng/g
b. Repeatability (within lab CV) %RSD	7
c. Reproducibility (between lab CV)	__ __ __ __

7. Precision using tissue containing
   incurred drug residues
   a. Concentration(s) tested ____NA_____
   b. Repeatability (within lab CV) ____NA_____
   c. Reproducibility (between lab CV) ____NA_____
8. Selectivity of the method

   This information is often referenced as "Specificity". Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure sulfonamides_____
   or drug class or other veterinary _____
   drugs that may also be used along _____
   with the analyte of interest
   _____.

   b. Contaminants that are likely ___tetracyclines, fenicols, nitrofuran metabolites
   to be present in the sample ____oxolinic acid, malachite green_____

9. Type of Validation studies

Single laboratory __x__

   a. Multi-laboratory _____
   b. AOAC or other
   official procedure _____

C. Information relevant to laboratory implementation

1. Training and experience recommended for analysts
   -See attached document
2. Critical steps in the method
   -See attached document
3. Information on availability of unusual reagents or equipment
   -See attached document
4. Special reagent or sample stability concerns
   -See attached document
5. Reagent handling and safety concerns (if any)
   -See attached document
6. Literature references or other useful information
   -See attached document

European Community

The European Community supports the drafting of a list of analytical methods suitable for adoption as CODEX methods for the determination of veterinary drug residues and thanks the Chairperson of the *ad hoc* Working group on Methods of analysis and sampling for having prepared this list including all of the methods recommendations identified from JECFA and CCRVDF Working group. The EC considers that validation of methods according to performance criteria implemented under assurance of quality allows for technical progress whilst ensuring the methods meet the required quality. However, a non-exhaustive list of recommended methods assessed on the basis of a summary supplied by the laboratories can also be useful. In addition, the European Community supports the establishment of a list of contact points responsible for keeping up to date the information on analytical methods on particular substances to complete the list of analytical methods.

In general it must be stated that an evaluation of the methods laid down in Annex 1 of CL 2005-10 RVDF as such could not be carried out since the necessary methodical details were not provided. Therefore the comments refer mainly to the MRLs in comparison to EU MRLs according to Regulation (EC) No 2377/90. The limit of quantification of the analytical methods should not be greater than half the MRL.[1]

The detection techniques could not be checked with regard to their compliance with analytical performance criteria (e.g. that established in the EU by Commission Decision 2002/657/EC) as they were not indicated in most cases.

Finally, the European Community also supports the establishment of a list of international contact points of experts or laboratories in the analysis of particular substances. This was also discussed at the Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL (Bangkok, 24 – 26 August 2004), conclusions and recommendations No 9: "*to facilitate transparency and the sharing of scientific analytical methods for the control of residues, it is recommended that FAO, in cooperation with other international agencies, develop an international network among official residue control laboratories*".

Annex 1

Anthelmintics[2]

Avermectines

- **<u>Abamectin</u>**

  The MRL values for cattle liver, -kidney und -fat range considerably above those laid down in Council Regulation 2377/90/EC.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
liver	100	***20***
kidney	50	**-**
fat	100	***10***

  Milk as an important matrix is missing.

  The LOQs are sufficient for the MRL control.

- **<u>Doramectin</u>**

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1 As it is laid down in Volume 8, (Notice to applicants and Note for guidance for Veterinary medicinal products) on the establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin, the limit of quantification of the analytical methods should not be greater than half the MRL.
The limit of quantification corresponds to the smallest measured content of an analyte above which a determination of the analyte can be made with a specified degree of accuracy and precision. In its "region of" quantification" (at and above the limit of quantification and over a range of analyte concentration suitable for the enforcement of the MRL) the method has to meet certain requirements of accuracy and precision.

2 CRL Berlin can be contacted for information on anthelmintics, coccidiostats, NSAIDs and Beta-agonists.

The MRL values for cattle liver, -kidney, -muscle and –fat are in accordance with CR 2377/90.

However, a few deviations can be stated regarding the values for pigs.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
liver	100	***50***
kidney	30	30
muscle	5	***20***
fat	150	***100***

There is no European MRL for doramectin in milk (cattle).

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
milk	15	-

The LOQs are sufficient for the MRL control.

- **Eprinomectin**

Deviations can be stated regarding the MRL values for cattle liver and –fat.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
liver	2000	***1500***
kidney	300	300
muscle	100	***50***
fat	250	***250***
milk	20	20

The LOQ are sufficient for the MRL control.

- **Ivermectin**

The MRL-values for cattle and pig liver and –fat are in accordance with CR 2377/90.

There is no European MRL for ivermectin in milk (cattle).

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
milk	10	-

The LOQ are sufficient for the MRL control except for ivermectin in milk, where no values were provided.

- **Moxidectin**

Deviations can be stated regarding the MRL values for muscle of cattle and deer as well as for milk.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
muscle (cattle, deer)	20	***50***
milk	-	***40***

The LOQ are sufficient for the MRL control.

Benzimidazoles

- **Albendazole**

Albendazole sulfoxide and albendazole sulfone are not taken into account as marker substances.

Whereas the MRL values for cattle and sheep liver and –kidney are higher by a factor of 5 or 10 respectively, the other values are in accordance with CR 2377/90.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
liver (cattle, deer)	5000	***1000***
kidney (cattle, deer)	5000	***50***

The matrix selection is adequate.

The LOQ were not indicated.

- **Febantel/Fenbendazole**

The marker substance is in accordance with CR 2377/90.

Deviations by a factor of 2 can be stated regarding the MRL values for kidney, muscle and fat, deviations by a factor of 10 for milk. The values for liver are in accordance with CR 2377/90.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
kidney (cattle, sheep, pig, horse, goat)	100	***50***
muscle (cattle, sheep, pig, horse, goat)	100	***50***
fat (cattle, sheep, pig, horse, goat)	100	***50***
milk	100	***10***

The matrix selection is adequate.

The LOQ are sufficient for the MRL control.

- **Flubendazole**

Deviations can be stated regarding the MRL values for pig and poultry liver and –muscle. The values for egg are in accordance with CR 2377/90.

	Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
	liver (pig)	10	***50***
(poultry)	liver	500	***400***
(pig)	muscle	10	***50***
(poultry)	muscle	200	***50***

The matrix selection is adequate.

The LOQ were not indicated.

- **Thiabendazole**

  The MRL values for liver, kidney, muscle, fat and milk are in accordance with CR 2377/90.

  In contrast to CODEX, CR 2377/90 does not lay down any MRL values for pig, sheep and goat.

  The marker substances are in accordance.

  The matrix selection is adequate.

- **Mebendazole, Oxibendazole and Triclabendazole**: No data provided.

Others

- **Closantel**

  The MRL values for cattle matrices are in accordance with CR 2377/90, while for sheep deviations can be stated.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
fat (sheep)	3000	***2000***
kidney (sheep)	5000	***3000***

  The LOQ were not indicated.

- **Levamisole**

  The MRL values for cattle liver, -kidney, -muscle and -fat are in accordance with CR 2377/90. For pig, sheep and poultry no MRLs have been laid down in the EU.

  The LOQ were not indicated.

Coccidiostats

Ionophores

No data were provided for ionophores. In CR 2377/90 an MRL for lasalocid can be found.

Chemical Coccidiostats

- **Clopidol**

  No MRL has been laid down, but there is a high LOQ of 100 µg/kg.

  LC-UV is not suitable for confirmatory purposes.

- **Diclazuril**

  In the EU diclazuril has been rated as ANNEX II substance..

  The MRL values for sheep, poultry und rabbit liver, -kidney, -muscle and –fat are very high.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
liver (sheep, poultry, rabbit)	3000	-
kidney (sheep, poultry, rabbit)	2000	-
muscle (sheep, poultry, rabbit)	500	-
fat (sheep, poultry, rabbit)	1000	-

  No data were provided for egg (poultry).

- **Imidocarb**

The MRL values for cattle liver, -kidney, -muscle, -fat and -milk are in accordance with CR 2377/90.

The LOQ are sufficient for the MRL control.

- **Nicarbazin**

  In the EU no MRL has been laid down for nicarbazin; it is exclusively used together with narasin.

  The MRL values for chicken liver, -kidney, -muscle and -skin seems relatively high (200 µg/kg).

  No data were provided for egg.

- Apart from the ionophores, other important analytes like halofuginone, toltrazuril and amprolium are also missing.

Beta-agonists

- **Clenbuterol**

The MRL values deviate from those of CR 2377/90.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
liver (cattle)	0,6	***0,5***
kidney (cattle)	0,6	***0,5***
muscle (cattle)	0,2	***0,1***
milk (cattle)	0,05	***0,05***

The LOQ were not indicated.

GC-MS is suitable for confirmatory purposes.

- **Ractopamine**

Ractopamine is a banned substance in the EU.

High MRL values are stated.

Matrix	MRL Codex [µg/kg]	MRL 2377/90 [µg/kg]
liver (cattle)	40	-
kidney (cattle)	90	-
muscle (cattle)	10	-
fat	10	-

Nitroimidazoles

- **Dimetridazole**

  Dimetridazole is classified as a banned substance in the EU (Annex IV of CR 2377/90/EC).

  For dimetridazole no CODEX MRL, no LOQ and no method recommendations exist.

- **Ipronidazole**

  Ipronidazole is classified as a banned substance in the EU (Annex IV of CR 2377/90/EC).

  For ipronidazole no CODEX MRL, no LOQ and no method recommendations exist.

- **Metronidazole**

  Metronidazole is classified as a banned substance in the EU (Annex IV of CR 2377/90/EC).

  For metronidazole no CODEX MRL, no LOQ and no method recommendations exist.

- **Ronidazole**

  Ronidazole is classified as a banned substance in the EU (Annex IV of CR 2377/90/EC). For ronidazole no CODEX MRL, no LOQ and no method recommendations exist.

  Due to the missing data it was not possible to carry out an evaluation for this group.

- **Azaperone**

  Not fully (multiple laboratory) validated methods currently are included. The references listed are still suitable methods, but relative old. Currently, some methods are published using mass spectrometry. Adam (J. AOAC international (1999), Vol. 82, 815) published a partly validated method for swine liver and Fluchard *et al* (J. Chrom. B 744 (2000) 139) a method for pig muscle and kidney based on LC-MS and Kaufmann *et al*. (rapid comm. Mass spec. (2001) 15 1747) a LC-MS method. These methods can be considered reasonably validated.

  The CRL Bilthoven[3] (RIVM) has a method for sedatives in pig kidney, inclusive azaperol and *azaperone*, based on LC-MS, which is not yet published. The method was in house validated. There are no activities know to use with respect to the analyses of kidney-fat. To our knowledge, residues in this matrix are not likely.

- **Bovine and porcine Somatotropin**

  In spite of the work ongoing in several research institutes, RIVM[3] inclusive, there currently are no know methods available that can be recommended at this time.

- **Carazolol**

  Situation as for Azaperone, with relative old methods provisionally recommended. The methods mentioned above (Fluchard *et al*. and Kaufamnn *et al*) also include carazolol for muscle and/or kidney. Also the CRL Bilthoven[3] -method includes this compound.

- **Chlorpromazine**

  Currently Annex I does not contain a recommended method for Chlorpromazine. Of the methods mentioned above only the method by Kaufmann *et al.* and the CRL-method include chlorpromazine. However, additional work still is necessary for full (in house) validation as banned (Group A) compound.

- **Dexamethasone**

  No species or matrix indicated in annex I. The technique most frequently used recently is LC-MS. In house validated methods currently are available, amongst others at the CRL Bilthoven[3].

- **Estradiol 17-ß**

  No method currently recommended. No species of matrix indicated. Validated methods for urine and muscle tissue are available at the CRL Bilthoven[3]. **Melengestrol acetate**

  Recommended method fully validated, with reference to CRL Bilthoven[3] method for regulatory purposes.

- **Trenbolone acetate**

  The table gives a recommended method. Probably there is an error in the column marker residue (β-Trenbolone should be the marker for muscle, α-Trenbolone for liver. The method is partly validated. Its suitability for EU regulatory purposes is not clear from the table. Alternative methods are available too. None of these is "fully validated" under the current definition.

- **Zeranol**

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[3] CRL RIVM Bilthoven can be contacted for information on this substance.

Fully validated method recommended. Alternative methods, recently developed, are available too. None of these are "fully validated" under the current definition.

Antibiotics[4]:

Certain listed methods are obsolete and should be removed from the list:

- **Chloramphenicol** by GC
- **Carazolol** by LC

Other methods should be introduced to the list

- **Carbadox and Olaquindox** metabolites (QCA, MQCA) in pig muscle by LC/MSMS
- **Chloramphenicol** in animal matrices by GC/MS or LC/MSMS
- **Malachite green** in fish flesh by LC/MSMS
- **(Fluoro)quinolones** in muscle and kidney by LC/fluorimetry or by LC/MSMS
- **Nitrofurans** metabolites by LC/MSMS

Pakistan

1. The document contains the information regarding the MRL of different veterinary drugs in edible tissue i.e. fat, liver, kidney and muscle. In Asian countries including Pakistan, other tissues, like heart and lungs are also preferred and commonly used in the diet. The information and analytical methods for these tissues would definitely help the countries where these products are used.
2. Further MRL information is also required for muscle with adhering skin particularly for poultry and fish.
3. Information is not available for goat species.
4. In the document no method is recommended for sulphathiazole Multi residue method i.e. Automated dialysis is available. In this method GILSON "ASTED" equipment is used for the analysis of a range of sulphonamide antibiotic residue.
    a. This issues with approval of the Director SDC.

Thailand

Analytical Method Information Summary

A. Descriptive Information

1. Name of drug or chemical: **Chloramphenicol**
2. Drug or chemical class: Antimicrobial (e.g. antimicrobial, anthelmintic, etc)
3. Veterinary Use: Microbial disease treatment
4. Analyte(s) measured: Chloramphenicol (specify if metabolite)
5. Intended use of the method:
    a. Screening
    b. Routine
    c. Reference
    d. Confirmatory ✓
6. Test matrix: Muscle (e.g. muscle, kidney, urine, etc)
7. Summary of principal steps in sample preparation: Muscle tissue is cut into small pieces, blended and homogenized with phosphate buffer solution.

[4] AFSSA-Fougères (Agence Française de securité sanitaire des aliments) laboratory can be contacted for information on methods for antibiotics.

8. Summary of principal steps in extraction procedure: Chloramphenicol is extracted from muscle with acetonitrile, defatted with hexane. Chloramphenicol is extracted by liquid-liquid partition into ethyl acetate phase.

9. Summary of principal steps in analyte clean-up procedure: The analyte is cleaned up using SPE C18 column and residue is reconstituted in methanol: water 50:50 and injected to LC-MS-MS.

10. Measurement procedure:

a. Chemical

1. Instrumentation: LC-MS-MS

2. Detector system : Triple quadrupole mass spectrometer, electro spray in negative mode.

3. Chromatographic column : Phenomenex Luna 3 μm C18, 150×2 mm (if applicable)

b. Immunochemical / Immunoassay

1. Technique: e.g. Elisa, RIA, Immunochromatog, etc)

2. Critical reagents: (e.g. antibody specificity and availability)

3. Special equipment required:

c. Microbiological

1. Technique:

2. Organism:

3. Media:

4. Special equipment required:

11. Sample / Analyte Stability Warning (if applicable):

12. Literature References available:

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B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) CC∝ = 0.04 μg/kg

How was LOD determined? In this case CC∝ (Decision Limit) was determined. The concentration of CCα corresponding to: Signal = intercept + 2.33 × SE of the within laboratory reproducibility of the intercept.

b. Limit of Quantification (LOQ) (mg/kg) CCβ = 0.05 μg/kg

How was LOQ determined? In this case CCβ (Detection Capability) was determined. The concentration of CCβ corresponding to: Signal at CC∝ + 1.64 × within laboratory reproducibility at CC∝.

c. Method sensitivity : 0.03 μg/kg (The smallest difference in concentration that can be measured)

2. JECFA MRL NONE

3. Are analytical data corrected for recovery? Yes_✓ NO

4. How is recovery estimated? Internal standard (e.g. external standard; internal standard, etc)

5. Accuracy

a. Concentration(s) tested	0.03	0.045	0.06	0.075
b. Concentration(s) measured	0.034	0.052	0.068	0.082
c. Recovery (%)	113.2	115.1	113.9	109.7

6. Precision using fortified control tissue

a. Concentration(s) tested	0.03	0.045	0.06	0.075
b. Repeatability (within lab CV)	11.8%	7.6%	7.2%	9.3%
c. Reproducibility (between lab CV)	-	-	-	-

7. Precision using tissue containing incurred drug residues

   a. Concentration(s) tested

   b. Repeatability (within lab CV)

   c. Reproducibility (between lab CV)

8. Selectivity of the method This information is often referenced as "Specificity". Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure or drug class or other veterinary drugs that may also be used along with the analyte of interest. Peaks of drugs in amphenicol class can be differentiated by retention time. The RT of Thiamphenicol , Florfenicol and Chloramphenicol are 5.24, 8.47 and 9.29 min. respectively.

   b. Contaminants that are likely to be present in the sample

9. Type of validation studies

   a. Single laboratory ✓

   b. Multi-laboratory

   c. AOAC or other official procedure

C. Information relevant to laboratory implementation

1. Training and experience recommended for analyst : Analysts should have knowledge and experience in operation of LC-MS-MS and Chromatogram interpretation.

2. Critical steps in the method : Chromatogram interpretation.

3. Information on availability of unusual reagents or equipment : D5-CAP, used as internal standard, is rarely available and very expensive.

4. Special reagent or sample stability concerns

5. Reagent handling and safety concerns (if any)

6. Literature references or other useful information: Proficiency test result and additional data can be provided.

***Ad Hoc* Working Group on Methods of Analysis and Sampling**

Analytical Method Information Summary

A. Descriptive Information

1. Name of drug or chemical: **Furazolidone, Furaltadone, Nitrofurantoin, Nitrofurazone**

2. Drug or chemical class: Antimicrobial (e.g. antimicrobial, anthelmintic, etc)
3. Veterinary Use:Treatment of microbial infection diseases.
4. Analyte(s) measured: Tissue-bound metabolite forms:- AOZ, AMOZ, AHD, SEM (specify if metabolite)
5. Intended use of the method:

   a. Screening

   b. Routine

   c. Reference

   d. Confirmatory ✓
6. Test matrix : Muscle (e.g. muscle, kidney, urine, etc)
7. Summary of principal steps in sample preparation: The method should be recommended to raw animal tissue only and should not applicable to processed food.
8. Summary of principal steps in extraction procedure: Tissues are subjected to a series of solvent extractions. AOZ, AHD, AMOZ and SEM are extracted and detected in the residual tissue pellet (protein-bound residues). The metabolites are simultaneously released from tissue drug residues and derivatised in acid medium containing 2-nitrobenzaldehyde. The derivatives produced are NPAOZ, NPAHD, NPAMOZ and NPSEM. They are detected and quantified using HPLC ESP tandem mass spectrometry.
9. Summary of principal steps in analyte clean-up procedure:
10. Measurement procedure:

    a. Chemical

        1. Instrumentation: HPLC-MS-MS

        2. Detector system : Electrospray (ESI) tandem mass spetrometry.

        3. Chromatographic column : Zorbax SB C18, 150×4.6 mm, 3 μm. (if applicable)

    b. Immunochemical / Immunoassay

        1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)

        2. Critical reagents: (e.g. antibody specificity and availability)

        3. Special equipment required:

    c. Microbiological

        1. Technique:

        2. Organism:

        3. Media:

        4. Special equipment required:
11. Sample / Analyte Stability Warning (if applicable):
12. Literature References available: 1) McCracken, R., Kennedy, G., j. Chromatogr. B 691(1997) p 87-94; 2) Leitner, A., Zollner, P., Lindner, W., J. Chromatogr. A, 939(2001) p 49-58
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B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg), CC∝ for AOZ = 0.04, AMOZ = 0.02, AHD = 0.1, SEM = 0.15

How was LOD determined? In this case CC∝ (Decision Limit) was determined. The concentration of CCα corresponding to: Signal = intercept + 2.33 × SE of the within laboratory reproducibility of the intercept.

b. Limit of Quantification (LOQ) (mg/kg) CCβ for AOZ = 0.06, AMOZ = 0.03, AHD = 0.15, SEM = 0.25

How was LOQ determined? In this case CCβ (Detection Capability) was determined. The concentration of CCβ corresponding to: Signal at CC∝ + 1.64 × within laboratory reproducibility at CC∝.

c. Method sensitivity AOZ = 0.04, AMOZ = 0.02, AHD = 0.1, SEM = 0.15 (The smallest difference in concentration that can be measured)

2. JECFA MRL NONE

3. Are analytical data corrected for recovery? Yes ✓ NO

4. How is recovery estimated? Internal standard (e.g. external standard; internal standard, etc)

5. Accuracy

	AOZ	AMOZ	AHD	SEM
a. Concentration(s)tested	0.2	0.2	0.4	1.0
b. Concentration(s) measured	0.199	0.19	0.45	0.88
c. Recovery (%)	99.7%	93.0%	111.8%	88.2%

	AOZ	AMOZ	AHD	SEM
a. Concentration(s)tested	0.2	0.2	0.4	1.0
b. Repeatability (within lab CV)	5.0%	4.9%	6.9%	8.8 %
c. Reproducibility (between lab CV)	-	-	-	-

7. Precision using tissue containing incurred drug residues

a. Concentration(s) tested

b. Repeatability (within lab CV)

c. Reproducibility (between lab CV)

8. Selectivity of the method This information is often referenced as "Specificity". Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure or drug class or other veterinary drugs that may also be used along with the analyte of interest

   b. Contaminants that are likely to be present in the sample:- Azodicarbonamide, Biurea, Urazol, chemical reagents involve in food processing could be source of SEM, the marker residue of Nitrofurazone.

9. Type of validation studies

   a. Single laboratory ✓

   b. Multi-laboratory

   c. AOAC or other official procedure

C. Information relevant to laboratory implementation

1. Training and experience recommended for analyst: This confirmation method for nitrofurans is recommended to determine only tissue-bound residues.
2. Critical steps in the method:
3. Information on availability of unusual reagents or equipment: Deuterated internal standards are rarely available and extremely expensive.
4. Special reagent or sample stability concerns
5. Reagent handling and safety concerns (if any)
6. Literature references or other useful information: Report on joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs without ADI/MRL. Bangkok, 24-26 Aug 2004. Proficiency test and additional data can be provided.

Ad Hoc Working Group on Methods of Analysis and Sampling

Analytical Method Information Summary

A. Descriptive Information

1. Name of drug or chemical : **Oxolinic acid**
2. Drug of chemical class : Antibiotic (e.g. antimicrobial , anathematic, etc)
3. Veterinary Use: _____marine_____
4. Analyst(s) measured : -
5. Intended use of the method:

   a. Screening

   b. Routine ✓

   c. Reference

   d. Confirmatory

6. Test matrix : Muscle (e.g. muscle , kidney, urine, etc)
7. Summary of principal steps in sample preparation: Weight 2.00 ±0.02 g of sample into 50 polypropylene centrifuge tube and added 2 g of sodium sulfate, anhydrous.
8. Summary of principal steps in extraction procedure: Homogenized sample with 12 ml dried ethyl acetate , shake 10 min and centrifuge at 3,000 rpm 10 min , the ethyl acetate fraction is collected and the ethyl acetate extraction procedure is repeated twice The combined ethyl acetate fraction is evaporated to dryness.

9. Summary of principal steps in analyst clean-up procedure: The residue is redissolved in 2 ml of 0.01M oxalic acid(pH3). After washing with 2 ml n-hexane and cleaned uo by filter nylon 0.45 µm.

10. Measurement procedure:

a. Chemical

1. Instrumentation HPLC

2. Detector system Fluorescent

3. Chromatographic column C18 (Hypersil gold

b. Immunochemical/Immunoassay

1. Technique: -(e.g. ELISA, RIA, Immunochromatog, etc)

2. Critical reagents: - (eg. Antibody specificity and availability)

3. Special equipment required: -

c. Microbiological

1. Technique: -

2. Organism: -

3. Media: -

4. Special equipment required: -

11. Sample/Analyst Stability

Warning(if applicable): : Sample should be kept _frozen until analyzed

12. Literature References available: J, ASSOC OFF ANAL CHEM 74 (4) 1091,608-611

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B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) 1.00 µg/kg

How was LOD determined? signal to noise ratio (S/N>3)

b. Limit of Quantification (LOQ)(mg/kg) 5.00 µg/kg

How was LOQ determined? _using to concentration of detection limit

c. Method sensitivity 0.31 µg/kg

2. JECFA MRL -

3. Are analytical data corrected for recovery? Yes ____/____No

4. How is recovery estimated : External standard (e.g. external standard; internal standard etc)

5. Accuracy

a. Concentration(s) tested	5 µg/kg	10 µg/kg	20 µg/kg
b. Concentration(s) measured	4.39	9.54	18.64
c. Recovery(%)	87.76	95.43	93.18

6. Precision using fortified Control tissue

a. Concentration(s) tested	5 µg/kg	10 µg/kg	20 µg/kg
b. Repeatability(within lab CV)	3.11	4.16	1.95
c. Reproducibility(between lab CV)	-	-	-

7. Precision using tissue containing incurred drug residues

   a. Concentration(s) tested

   b. Repeatability (within lab CV)

   c. Reproducibility (between lab CV)

8. Selectivity of the method This information is often referenced as "Specificity". Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure _____

      Or drug class or other veterinary _____

      Drugs that may also be used along _____

      With the analyst of interest_____

   b. Contaminants that are likely _____

      To be present in the sample _____

9. Type of validation studies

   a. Single laboratory ✓

   b. Multi-laboratory

   c. AOAC or other official procedure

   Official procedure

C. Information relevant to laboratory implementation

1. Training and experience recommended for analyst. Analyst should train using HPLC
2. Critical steps in the method Extract sample and cleanness
3. Information on availability of unusual reagents of equipment FLD detector ; λ_{ex}327nm, λ_{em}369nm
4. Special reagent of sample stability concerns Sample should be kept frozen until analyzed
5. Reagent handling and safety concerns (if any) Preparation of extraction and mobile phase should be done in chemical fume hood
6. Literature references or other useful information *J. ASSOC OFF ANAL CHEM* 74(1) 1991,608 - 611

Ad Hoc Working Group on Method of Analysis and Sampling

Analytical Method Information Summary

A. Descriptive Information

1. Name of drug or chemical: **Oxytetracycline hydrochloride**
2. Drug or chemical class: antibiotic (e.g. antimicrobial, anthelmintic, etc)
3. Veterinary Use:
4. Analyte(s) measured: OTC (specify if metabolite)
5. Intended use of the method:

   a. Screening

   b. Routine for monitoring and service in shrimp.

   c. Reference

   d. Confirmatory
6. Test matrix muscle (e.g. muscle, kidney, urine, etc)
7. Summary of principal steps in sample procedure: Oxytetracycline (OTC) is extracted from tissue with McIlvain buffer (pH4)/EDTA, sample extract (supernatant) is clean up on C18 solid phase extraction column, and elute from the column by the methanolic oxalic acid. Determine OTC by using HPLC-UV detector wavelength at 350 nm.
8. Summary of principal steps in extraction procedure: Add 20 ml McIlvain buffer (pH4))/EDTA solution to sample blend with homogenizer, centrifuge and filter and re-extract 2 times with McIlvain buffer (pH4))/EDTA 20 and 10 ml repectively.
9. Summary of principal steps in analyte clean-up procedure: The supernatant is cleaned up on C18 solid phase extraction column and elute from the column by the methanolic oxalic acid.
10. Measurement procedure:

    a. Chemical

       1. Instrumentation HPLC
       2. Detector system UV 350 nm.
       3. Chromatographic column Lichrosorb RP-18 ; 5 micron 250 x 4.6 mm.

    b. Immunochemical/Immunoassay

       1. Technique: - (e.g. Elisa, RIA, Immunochromatog, etc)
       2. Critical reagent: - (e.g. antibody specify and availability)
       3. Special equipment required:-

    c. Microbiological

       1. Technique: -
       2. Organism: -
       3. Media: -
       4. Special equipment required: -
11. Sample/Analyte Stability

    Warning (if applicable): Sample should be kept frozen until analyzed and the entire extraction-cleanup procedure should be completed in one-day.

12. Literature References available: AOAC Official Method 995.09, Chlortetracycline, Oxytetracycline, and Tetracycline in Edible Animal Tissues; Liquid Chromatographic Method. AOAC International, 2000: Chapter 23 p.20-23.
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B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) 0.01

How was LOD determined? LOD = 3 SD ; Analyse 6 times of sample spiked at low concentration (near the lowest of calibration curve) and calculate SD (concentration of analyte)

b. Limit of Quantification (LOQ) (mg/kg) 0.1

How was LOQ determined? LOD = 10 SD ; Analyse 6 times of sample spiked at estimated LOQ and calculate recovery and precision.

c. Method sensitivity : 0.005 µg/ml. (The smallest difference in concentration that can be measured)

2. JECFA MRL: 0.1 mg/kg

3. Are analytical data corrected for recovery? Yes ___ No √_

4. How is recovery estimated? external standard (spike standard in blank sample and analyse with standard curve).(e.g. external standard; internal standard. Etc)

5. Accuracy

a. Concentration(s) tested: (mg/kg)	0.10	0.20	0.40
b. Concentration(s) measured: (mg/kg)	0.095	0.181	0.384
c. Recovery (%)	95	91	97

6. Precision using fortified Control tissue

a. Concentration(s) tested: (mg/kg)	0.10	0.20	0.40
b. Repeatability (within lab CV)	1.76	3.51	1.94
c. Repeatability (between lab CV)	-		

7. Precision using tissue containing incurred drug residues

a. Concentration(s) tested

b. Repeatability (within lab CV)

c. Reproducibility (between lab CV)

8. Selectivity of the method This information is often referenced as “Specificity”. Selectivity refers to the ability of the method to provide accurate measurement of the analyte of interest when other chemicals or drugs are also resident in the laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure or drug class or other veterinary drugs that may also be used along with the analyte of interest

b. Contaminants that are likely to be present in the sample

9. Type of validation studies

a. Single laboratory ✓

b. Multi-laboratory

c. AOAC or other official procedure

C. Information relevant to laboratory implementation

1. Training and experience recommended for analyst: Testing method training; Instrument training – HPLC; Software training - Data processing; Proficiency Test

2. Critical steps in the method : Clean-up the sample extraced on SPE C-18 column

3. Information on availability of unusual reagents or equipment : Control chart of standard, Check chromatogram of blank reagents compare with the old chromatogram when use reagents from different suppliers, Check %recovery when change the lot number of SPE cartridges, Criteria for system suitability test

4. Special reagent or sample stability concerns : Certrified reference standard store at 2-5 °C, stock standard solution prepare every 3 months and store in freezer. Sample should be kept frozen until analyzed.

5. Reagent handling and safety concerns (if any) : Preparation of tetracycline standard solution and mobile phase should be done in a chemical fume hood.

6. Literature references or other useful information:

6.1 AOAC Official Method 995.09, Chlortetracycline, Oxytetracycline, and Tetracycline in Edible Animal Tissues; Liquid Chromatographic Method. AOAC International, 2000: Chapter 23 p.20-23.

6.2 Standard operation procedure: Validation of Methods, FACR Selection, Health of animals laborartory, Saskatoon, SK S7N 5E3.

6.3 The URACHEM Measurement Uncertainty Working Group in collaboration with members of CITAC and AOAC International: Draft EURACHEM/CITAC Guide, Quantifying Uncertainty in Analytical Measurement, Second Edition, Draft: June 1999.

6.4 Taylor, John K. By Lewis Publisher, INC. Michigan: Quality Assurance of Chemical Measurements 1989.

Venezuela

SUBJECT: METHODS OF ANALYSIS FOR VETERINARY DRUG RESIDUES

Venezuela appreciates the opportunity to submit the following comments made on the above document.

1. In the list of chemicals presented we found Chloramphenicol. The use of this substance in animal products intended for human consumption has been banned in Venezuela since 1988. Therefore, we believe that it should not be included in this document to be subjected to the comments' process.

2. Also, Chloramphenicol should solely be included with the condition that it only be used in equines.

3. While "Liquid Chromatography" has been the term used in the context of analytical methods, "HPLC" is the term that should be used.

4. The ELISA method can be used as the technique of choice for Chloramphenicol and Clenbuterol.