codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00100 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

Agenda Item 12(b) **CX/RVDF 01/13-Add. 1**
November 2001
ENGLISH ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS
Thirteenth Session
Charleston, South Carolina, USA, 4-7 December 2001

COMMENTS ON THE CONSIDERATION OF THE IDENTIFICATION OF ROUTINE METHODS OF ANALYSIS FOR VETERINARY DRUG RESIDUES IN FOODS

AUSTRALIA

Analytical Method Information Summary

ABAMECTIN

A. Descriptive Information

1. Name of drug or chemical:	Abamectin
2. Drug or chemical class: (e.g. antimicrobial, Anthelminthic, etc)	Anthelmintic agent (Macrocyclic lactones)
3. Veterinary Use:	Control of internal and external parasites, parasitic worms, cattle tics, lungworms and sucking lice.
4. Analyte(s) measured: (specify if metabolite)	Avermectin B1a and Avermectin B1b

5. Intended use of the

method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver

7. Summary of principal steps in Sample preparation:	Homogenise sample with acetonitrile

8. Summary of principal steps in extraction procedure:	Acetonitrile extract is evaporated to dryness under vacuum residue dissolved in hexane/dichloromethane mix.

9. Summary of principal steps in Analyte clean-up procedure:	a) Silica sep-pak cleanup, eluted with ethyl acetate b) Then derivatised with acetic anhydride/1-methyl imidazole/dimethyl formamide and derivatised mixture cleaned up by C-18 sep-pak

10. Measurement procedure:

a. Chemical 1. Instrumentation	HPLC
2. Detector system	Fluorescence wavelength – 360nm excitation and 468nm emission
3. Chromatographic column (if applicable)	Reverse phase C18 column
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Terry Spencer
b. Country	Australia
c. Affiliation	AGAL ACT
d. Address	Level 10, Allara Street, Canberra, ACT 2600
e. Telephone	(+61) 2 6213 6102
f. FAX	(+61) 2 6213 6815
g. Email	Terry.spencer@agal.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.001mg/kg (Avermectin B1a)
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.005mg/kg (Avermectin B1a)
c. Method sensitivity (The smallest difference in concentration that can be measured)	N/A

2. JECFA MRL	Liver and fat (cattle): 0.1mg/kg Kidney (cattle): 0.05mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy (Avermectin B1a)

a. Concentration(s) tested	Bovine: 0.002, Ovine: 0.01, Porcine: 0.005 mg/kg
b. Concentration(s) measured	
c. Recovery (%)	Bovine: 82%, Ovine: 86%, Porcine: 73%

6. Precision using fortified
Control tissue (Avermectin B1a)

a. Concentration(s) tested	Bovine: 0.002, Ovine: 0.01, Porcine: 0.005 mg/kg
b. Repeatability (within lab CV)	Bovine: 9.8%, Ovine: 3.5%, Porcine: 2%
c. Reproducibility (between lab CV)	

7. Precision using tissue containing
Incurred drug residues (bovine liver)

a. Concentration(s) tested	0.005, 0.012, 0.034
b. Repeatability (within lab CV)	19.6%, 10.8%, 11.2%
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 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	

B. Information relevant to laboratory implementation

1. Training and experience recommended for analytes	
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	

C. Descriptive Information

1. Name of drug or chemical:	Albendazole
2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Benzimidazoles)
3. Veterinary Use:	Control of mature and immature gastrointestinal roundworms, large lung worms, tape worms and liver flukes.
4. Analyte(s) measured: (specify if metabolite)	Albendazole

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver
7. Summary of principal steps in	Tissumize 5g of sample with sodium sulphate and potassium

sample preparation:	carbonate, extracting into ethyl acetate.

8. Summary of principal steps in extraction procedure:	Evaporated residue dissolved in acetonitrile and partitioned with hexane. Hexane discarded and sample made up to volume with 0.02M ammonium acetate

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

10. Measurement procedure:

a. Chemical 1. Instrumentation	LC-MS (screen and confirmation)
2. Detector system	MS-SIM (screen and confirmation)
3. Chromatographic column (if applicable)	Zorbax Phenyl SB 5um, 150 x 4.6mm (screen) C18 (confirmation)
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Dr Robert Symons
b. Country	Australia
c. Affiliation	AMDEL Lilyfield
d. Address	36-40 Halloran St. Lilyfield 2040

e. Telephone	(+61) 2 9818 1033
f. FAX	(+61) 2 9810 8771
g. Email	robert_symons@amdel.com

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.01
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.05
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, fat and milk: 0.1mg/kg Liver and kidney: 5.0mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.05, 0.1, 0.5mg/kg
b. Concentration(s) measured	
c. Recovery (%)	62%, 76%, 80%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.05, 0.1, 0.5mg/kg
b. Repeatability (within lab CV)	14%, 10%, 4%
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 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 analytes	
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	

ALPHA CYPERMETHRIN

D. Descriptive Information

1. Name of drug or chemical:	Alpha Cypermethrin
2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Carbamates, Pyrethroids and other insecticides (Synthetic Pyrethroids)
3. Veterinary Use:	Highly active broad spectrum insecticide effective by contact and ingestion against target pests, in particular, ticks and lice.
4. Analyte(s) measured: (specify if metabolite)	Alpha Cypermethrin

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Fat
7. Summary of principal steps in sample preparation:	Subsample of fat rendered in microwave
8. Summary of principal steps in extraction procedure:	Molten fat dissolved in hexane then extracted with acetonitrile
9. Summary of principal steps in	10% Forisil trap, elute with acetone:diethyl ether:hexane (10:40:50)

10. Measurement procedure:

a. Chemical 1. Instrumentation	GC
2. Detector system	ECD, NPD and MS
3. Chromatographic column (if applicable)	DB-1 or DB-5
b. Immunochemical/lmmunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Mr. Phil Williams
b. Country	Australia
c. Affiliation	Symbio
e. Telephone	(+61) 7 3391 7558
f. FAX	(+61) 7 33916673
g. Email	Symbio@powerup.com.au

B. Method Performance

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

How was LOD determined?	
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.02
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, liver and kidney (cattle, sheep and chickens): 0.01mg/kg Fat (cattle, sheep and chickens): 0.05mg/kg Eggs (chickens): 0.05mg/kg Milk (cattle): 0.05mg/kg

3. Is analytical data corrected for recovery?	Yes	

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

5. Accuracy

a. Concentration(s) tested	0.1mg/kg
b. Concentration(s) measured	
c. Recovery (%)	68%

6. Precision using fortified Control tissue

a. Concentration(s) tested	0.1mg/kg
b. Repeatability (within lab CV)	12%
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 veterinary drugs that may also be used along with the analyte of interest	
b. Contaminants that are likely to be present in the sample	

c. AOAC or other official procedure	

C. **Information relevant to laboratory implementation**

1. Training and experience recommended for analytes	
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	

BENZYL PENICILLIN

E. Descriptive Information

1. Name of drug or chemical:	Benzyl Penicillin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Beta-lactams)

3. Veterinary Use:	Bacterial antibiotic chiefly active against Gram-positive microorganisms. May be used to treat respiratory tract, urinary tract and wound infections, Metritis and streptococcal mastitis.

4. Analyte(s) measured: (specify if metabolite)	Penicillin

5. Intended use of the method:

a. Screening	Yes (5 plate MIT)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney Egg

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions
9. Summary of principal steps in Analyte clean-up procedure:	Cleaned up concentrated using SPE and solvent removed under Vacuum. For confirmation derivitsed with 1,2,4-triazole-mercuric chloride

10. Measurement procedure:

a. Chemical 1. Instrumentation	Confirmation HPLC
2. Detector system	UV at 325nm

3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	5 plate MIT (screening)

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Mr Dennis Hamilton
b. Country	Australia
c. Affiliation	ARI (Chemical Residue Laboratory)
d. Address	665 Fairfield Road Yeerongapilly QLD 4105
e. Telephone	(+61) 7 3362 9415
f. FAX	(+61) 7 3362 9460
g. Email	Hamiltondj@prose.dpi.qld.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.01mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.02mg/kg
c. Method sensitivity	

(The smallest difference in concentration that can be measured)	

2. JECFA MRL	Liver, kidney and muscle (all species): 0.05mg/kg Milk: 0.004mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.08mg/kg
b. Concentration(s) measured	
c. Recovery (%)	86%

6. Precision using fortified Control tissue

a. Concentration(s) tested	0.08mg/kg
b. Repeatability (within lab CV)	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 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 analyte	
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	

CHLORAMPHENICOL

F. Descriptive Information

1. Name of drug or chemical:	Chloramphenicol

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anabolic agent (Unauthorised compound)

3. Veterinary Use:	Chloramphenical is broad spectrum antibiotic, effective against

	Gram-positive and Gram-negative bacteria and certain Rickettsiae and large Viruses.

4. Analyte(s) measured: (specify if metabolite)	Chloramphenicol

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Muscle

7. Summary of principal steps in sample preparation:	Fat removed and muscle homogenised

8. Summary of principal steps in extraction procedure:	Ethyl acetate extraction
9. Summary of principal steps in Analyte clean-up procedure:	Solid phase extraction (C18)

10. Measurement procedure:

a. Chemical 1. Instrumentation	GC
2. Detector system	ECD
3. Chromatographic column (if applicable)	SGE BP1 (screen) HP – 5MS (confirmation)
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and availability)	N/A

3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Terry Spencer
b. Country	Australia
c. Affiliation	AGAL ACT
d. Address	Level 10, Allara Street, Canberra, ACT, 2600
e. Telephone	(+61) 2 6213 6102
f. FAX	(+61) 2 6213 6815
g. Email	Terry.spencer@agal.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.0005 mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.001 mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	No MRLS set.

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.5mg/kg 2.5mg/kg
b. Concentration(s) measured	
c. Recovery (%)	90% 85%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.5, 2.5mg/kg
b. Repeatability (within lab CV)	8%, 10%
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 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 analytes	
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	

CHLORTETRACYCLINE

G. Descriptive Information

1. Name of drug or chemical:	Chlortetracycline

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Tetracyclines)

3. Veterinary Use:	Broad spectrum antibiotic with bacteriostatic action. Highly effective Against both respiratory and gastrointestinal infections. Promotes growth and improves feed efficiency, in particular with poultry and swine.

4. Analyte(s) measured: (specify if metabolite)	Chlortetracycline

5. Intended use of the method:

a. Screening	Yes (5 plate MIT)
b. Routine	

c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions
9. Summary of principal steps in Analyte clean-up procedure:	Cleaned up and concentrated using SPE and solvent removal under Vacuum.

10. Measurement procedure:

a. Chemical 1. Instrumentation	Confirmation HPLC
2. Detector system	Fluorescence
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	5 plate MIT (initial screening)

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Ms Heather Lindsay
b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
d. Address	Cnr Sneydes and South Roads Werribee VIC 3030
e. Telephone	(+61) 3 9742 8779
f. FAX	(+61) 3 9742 8700
g. Email	Heather.Lindsay@nre.vic.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.05mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.05mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle (cattle, pigs and poultry):0.1mg/kg Liver (cattle, pigs, sheep and poultry): 0.3mg/kg Kidney (cattle, pigs, sheep and poultry: 0.6mg/kg Eggs (poultry): 0.2mg/kg Milk (cattle and sheep): 0.1mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.05mg/kg
b. Concentration(s) measured	
c. Recovery (%)	65%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.05mg/kg
b. Repeatability (within lab CV)	10%
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 veterinary drugs that may also be used along with the analyse 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 analyte	
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	

CLOSANTEL
H. Descriptive Information

1. Name of drug or chemical:	Closantel

2. Drug or chemical class: (e.g. antimicrobial, Anthelminthic, etc)	Anthelmintic agent (Salicylanilide)

3. Veterinary Use:	Control of mature and immature gastrointestinal roundworms, large lungworms, liver fluke and nasal bots; reduces output of viable worm and fluke eggs.

4. Analyte(s) measured: (specify if metabolite)	Closantel

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix	Liver

(e.g. muscle, kidney, urine, etc)	

7. Summary of principal steps in sample preparation:	Homogenise 5g sample in acetonitrile

8. Summary of principal steps in extraction procedure:	Extract into acetonitrile

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

10. Measurement procedure:

a. Chemical 1. Instrumentation	LCMS and LC/Diode array
2. Detector system	MS – SIM and diode array
3. Chromatographic column (if applicable)	Zorbax phenyl SB 5um, 150 x 4.6mm
b. Immunochemical/lmmunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Dr Robert Symons

b. Country	Australia
c. Affiliation	AMDEL Lilyfield
d. Address	36-40 Halloran St. Lilyfield 2040
e. Telephone	(+61) 2 9818 1033
f. FAX	(+61) 2 9810 8771
g. Email	Robert_symons@amdel.com

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.01mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.1
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle and liver (sheep): 1.5kg/mg Kidney (sheep): 5mg/kg Fat (sheep): 2mg/kg Muscle and liver (cattle): 1mg/kg Kidney and fat (cattle): 3mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.1 mg/kg
b. Concentration(s) measured	
c. Recovery (%)	88%

6. Precision using fortified Control tissue

a. Concentration(s) tested	
b. Repeatability (within lab CV)	

c. Reproducibility (between lab CV)	

7. Precision using tissue containing
Incurred drug residues

a. Concentration(s) tested	0.1 mg/kg
b. Repeatability (within lab CV)	13%
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 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 analyte(s)	
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	

CYFLUTHRIN

I. Descriptive Information

1. Name of drug or chemical:	Cyfluthrin

2. Drug or chemical class: (e.g. antimicrobial, Anthelminthic, etc)	Carbamates, Pyrethroids and other insecticides (Synthetic pyrethroids)

3. Veterinary Use:	Broadspectrum synthetic type 2 pyrethroid insecticide and acaricide used to control infestations of flies and tabanids.

4. Analyte(s) measured: (specify if metabolite)	Cyfluthrin

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Fat

7. Summary of principal steps in sample preparation:	Subsample of fat rendered in microwave

8. Summary of principal steps in extraction procedure:	Molten fat dissolved in hexane then extracted with acetonitrile

9. Summary of principal steps in Analyte clean-up procedure:	10% Forisil trap, elute with acetone:diethyl ether:hexane (10:40:50)

10. Measurement procedure:

a. Chemical 1. Instrumentation	GC
2. Detector system	ECD, NPD and MS
3. Chromatographic column (if applicable)	DB-1 or DB-5
b. Immunochemical/lmmunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Mr. Phil Williams
b. Country	Australia
c. Affiliation	Symbio
d. Address	47 Manilla St. East Brisbane 4169
e. Telephone	(+61) 7 3391 7558
f. FAX	(+61) 7 33916673
g. Email	Symbio@powerup.com.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.01mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.02mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, liver and kidney (cattle): 0.02mg/kg Fat (cattle): 0.2mg/kg Milk (cattle): 0.04mg/kg

3. Is analytical data corrected for recovery?	Yes	

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

5. Accuracy

a. Concentration(s) tested	0.01 mg/kg 1.0 mg/kg
b. Concentration(s) measured	
c. Recovery (%)	93% 98%

6. Precision using fortified Control tissue

a. Concentration(s) tested	0.1 mg/kg 1.0 mg/kg
b. Repeatability (within lab CV)	7.9% 7.7%
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 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 analyte(s)	
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	

DIHYDRO-STREPTOMYCIN

J. Descriptive Information

1. Name of drug or chemical:	Dihydro-streptomycin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Aminoglycoside)

3. Veterinary Use:	Control of gastrointestinal bacteria, antispasmodic effect. Useful against meningococcal, pneumococcal and haemolytic streptococcal Infections. Not readily absorbed, has a local bactericidal and bacteriostatic action against Gram negative bacteria.

4. Analyte(s) measured: (specify if metabolite)	Dihydro-steptomycin

5. Intended use of the method:

a. Screening	Yes (5 plate MIT and if positive then ELISA)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney Egg

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions

9. Summary of principal steps in	Cleaned up and concentrated using SPE and solvent removal under vacuum. Confirmation: fluorescent derivative formed using 1,2-naphthoquinone-4-sulphone acid
Analyte clean-up procedure:	

10. Measurement procedure:

a. Chemical	Confirmation

1. Instrumentation	HPLC
2. Detector system	Fluorescence
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ELISA (secondary screening test)
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:	5 plate MIT (initial screening)

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Ms Heather Lindsay
b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
d. Address	Cnr Sneydes and South Roads Werribee VIC 3030
e. Telephone	(+61) 3 9742 8779
f. FAX	(+61) 3 9742 8700
g. Email	Heather.Lindsay@nre.vic.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.1mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.1mg/kg

c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, liver and fat (cattle, sheep, pigs and poultry): 0.5mg/kg Kidney (cattle, sheep, pigs and poultry): 1mg/kg Milk (cattle): 0.2mg/kg

3. Is analytical data corrected for recovery?	Yes

4. How is recovery estimated (e.g. external standard; internal standard etc.)	Spiked matrix calibration curve

5. Accuracy

a. Concentration(s) tested	0.2mg/kg
b. Concentration(s) measured	
c. Recovery (%)	100%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	
b. Repeatability (within lab CV)	
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 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 analyte	
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	

DORAMECTIN

K. Descriptive Information

1. Name of drug or chemical:	Doramectin

2. Drug or chemical class: (e.g. antimicrobial,	Anthelmintic agent (Macrocyclic lactones)

Anthelmintic, etc)	

3. Veterinary Use:	Control of mature and immature gastrointestinal roundworms, lungworm and eyeworm. Also controls sucking and biting lice, buffalo fly, cattle tic and mange mite.

4. Analyte(s) measured: (specify if metabolite)	Doramectin

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver

7. Summary of principal steps in sample preparation:	Homogenise sample with acetonitrile

8. Summary of principal steps in extraction procedure:	Acetonitrile extract is evaporated to dryness under vacuum and the residue dissolved in hexane/dichloromethane mix

9. Summary of principal steps in Analyte clean-up procedure:	a) Silica sep-pak cleanup, eluted with ethyl acetate b) Then derivatised with acetic anhydride/1-methyl imidazole/dimethyl formamide and derivatised mixture cleaned up by C-18 sep-pak

10. Measurement procedure:

a. Chemical 1. Instrumentation	HPLC
2. Detector system	Fluorescence wavelength – 360nm excitation and 468nm emission
3. Chromatographic column (if applicable)	Reverse phase C18 column
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA,	N/A

Immunochromatog, etc)	
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Terry Spencer
b. Country	Australia
c. Affiliation	AGAL ACT
d. Address	Level 10, Allara Street, Canberra, ACT, 2600
e. Telephone	(+61) 2 6213 6102
f. FAX	(+61) 2 6213 6815
g. Email	terry.spencer@agal.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.001mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.005mg/kg
c. Method sensitivit (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle (cattle): 0.01mg/kg Liver (cattle): 0.1mg/kg Kidney (cattle): 0.03mg/kg Fat (cattle): 0.15mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	Bovine: 0.002, Ovine: 0.01, Porcine: 0.005 mg/kg
b. Concentration(s) measured	
c. Recovery (%)	Bovine: 84%, Ovine: 93%, Porcine: 84%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.002, 0.010, 0.005 mg/kg
b. Repeatability (within lab CV)	4.8%, 1.5%, 3.1%
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 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 analytes	
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	

EPRINOMECTIN

L. Descriptive Information

1. Name of drug or chemical:	Eprinomectin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Macrocyclic lactone)

3. Veterinary Use:	Control of gastro-intestinal worms, lungworm; sucking and biting lice, mites, buffalo fly and aids in the control of cattle tick.

4. Analyte(s) measured: (specify if metabolite)	Eprinomectin

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver

7. Summary of principal steps in sample preparation:	Homogenise sample with acetonitrile

8. Summary of principal steps in extraction procedure:	Acetonitrile extract is evaporated to dryness under vacuum, and the residue dissolved in hexane/dichloromethane mix

9. Summary of principal steps in Analyte clean-up procedure:	a) Silica sep-pak cleanup, eluted with ethyl acetate b) Then derivatised with acetic anhydride/1-methyl imidazole/dimethyl formamide and derivatised mixture cleaned up by C-18 sep-pak

10. Measurement procedure:

a. Chemical 1. Instrumentation	HPLC
2. Detector system	Fluorescence wavelength – 360nm excitation and 468nm emission
3. Chromatographic column (if applicable)	Reverse phase C18 column
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism:	N/A

3. Media: 4. Special equipment required:	

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Terry Spencer
b. Country	Australia
c. Affiliation	AGAL ACT
d. Address	Level 10, Allara Street, Canberra, ACT 2600
e. Telephone	(+61) 2 6213 6102
f. FAX	(+61) 2 6213 6815
g. Email	Terry.spencer@agal.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.001
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.005
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	(NOT SET)

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.005, 0.010 mg/kg
b. Concentration(s) measured	
c. Recovery (%)	89%, 87%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.005, 0.010 mg/kg
b. Repeatability (within lab CV)	6%, 3%
c. Reproducibility (between lab CV)	

7. Precision using tissue containing
Incurred drug residues

a. Concentration(s) tested	0.013mg/kg
b. Repeatability (within lab CV)	3%
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 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. Mullet- laboratory	
c. AOAC or other official procedure	

C. **Information relevant to laboratory implementation**

1. Training and experience recommended for analytes	
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	

OXYTETRACYCLINE

M. Descriptive Information

1. Name of drug or chemical:	Oxytetracycline

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antimicrobial agent (Tetracyclines)

3. Veterinary Use:	Provides broad spectrum antibiotic coverage against susceptible organisms in alimentary tract infections, respiratory, genitourinary, septicaemia and Superficial bacterial infections.

4. Analyte(s) measured: (specify if metabolite)	Oxytetracycline

5. Intended use of the method:

a. Screening	Yes (5 plate MIT)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions

9. Summary of principal steps in Analyte clean-up procedure:	Cleaned up and concentrated using SPE and solvent removal under Vacuum.

10. Measurement procedure:

a. Chemical 1. Instrumentation	Confirmation HPLC
2. Detector system	UV
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	5 plate MIT (initial screening)

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Ms Heather Lindsay

b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
d. Address	Cnr Sneydes and South Roads Werribee VIC 3030
e. Telephone	(+61) 3 9742 8779
f. FAX	(+61) 3 9742 8700
g. Email	Heather.Lindsay@nre.vic.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.05mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.05mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle (cattle, sheep, pigs, poultry, fish and giant tiger prawn): 0.1mg/kg
	Liver (cattle, sheep, pigs and poultry): 0.3mg/kg
	Kidney (cattle, sheep, pigs and poultry): 0.6mg/kg
	Eggs (poultry): 0.2mg/kg
	Milk (cattle and sheep): 0.1mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.1mg/kg
b. Concentration(s) measured	
c. Recovery (%)	65%

6. Precision using fortified Control tissue

a. Concentration(s) tested	0.1mg/kg
b. Repeatability (within lab CV)	6%
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 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 analyte	
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	

IVERMECTIN
N. Descriptive Information

1. Name of drug or chemical:	Ivermectin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Macrocyclic lactones)

3. Veterinary Use:	Broad spectrum Control agent of mature and immature gastrointestinal roundworms, lungworm and eyeworm. Also controls sucking lice, cattle tick and mites.

4. Analyte(s) measured: (specify if metabolite)	Ivermectin B1a and Ivermectin B1b

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver

7. Summary of principal steps in sample preparation:	Homogenise sample with acetonitrile

8. Summary of principal steps in extraction procedure:	Acetonitrile extract is evaporated to dryness under vacuum and the residue dissolved in hexane/dichloromethane mix

9. Summary of principal steps in Analyte clean-up procedure:	a) Silica sep-pak cleanup, eluted with ethyl acetate b) Then derivatised with acetic anhydride/1-methyl imidazole/dimethyl formamide and derivatised mixture cleaned up by C-18 sep-pak

10. Measurement procedure:

a. Chemical 1. Instrumentation	HPLC
2. Detector system	Fluorescence wavelength – 360nm excitation and 468nm emission
3. Chromatographic column (if applicable)	Reverse phase C18 column
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Terry Spencer
b. Country	Australia
c. Affiliation	AGAL ACT
d. Address	Level 10, Allara Street, Canberra, ACT, 2600
e. Telephone	(+61) 2 6213 6102
f. FAX	(+61) 2 6213 6815
g. Email	terry.spencer@agal.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.001mg/kg (B1a and B1b)
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.005 – B1a 0.001 – B1b
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Liver (cattle): 0.1mg/kg (pigs and sheep): 0.015mg/kg Fat (cattle): 0.04mg/kg (sheep and pigs): 0.02mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy (Ivermectin B1a)

a. Concentration(s) tested	0.005, 0.01 mg/kg
b. Concentration(s) measured	
c. Recovery (%)	93%, 92%

6. Precision using fortified
Control tissue (Ivermectin B1a)

a. Concentration(s) tested	0.005, 0.01mg/kg
b. Repeatability (within lab CV)	5%, 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 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 analytes	
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	

LEVAMISOLE

O. Descriptive Information

1. Name of drug or chemical:	Levamisole

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Imidazothiazole)

3. Veterinary Use:	Control of gastrointestinal roundworms, lungworms and other parasites.

4. Analyte(s) measured: (specify if metabolite)	Levamisole

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Macerated liver extracted into acidified acetonitrile

9. Summary of principal steps in Analyte clean-up procedure:	Silica gel column cleanup, eluted with ammonia in methanol

10. Measurement procedure:

a. Chemical 1. Instrumentation	HPLC
2. Detector system	UV – 210 nm and 235 nm
3. Chromatographic column (if applicable)	Phenyl column and C18 column

b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Terry Spencer
b. Country	Australia
c. Affiliation	AGAL ACT
d. Address	Level 10, Allara Street, Canberra, ACT, 2600
e. Telephone	(+61) 2 6213 6102
f. FAX	(+61) 2 6213 6815
g. Email	terry.spencer@agal.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.005mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.02mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, kidney and fat (cattle, sheep and pigs): 0.01mg/kg Liver (cattle, sheep and pigs): 0.1mg/kg Milk: Previous MRL withdrawn Eggs: No MRL allocated

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.2 mg/kg
b. Concentration(s) measured	Typical recoveries 60 – 100%
c. Recovery (%)	

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.2mg/kg
b. Repeatability (within lab CV)	15%
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 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 analytes	
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	

MOXIDECTIN

P. Descriptive Information

1. Name of drug or chemical:	Moxidectin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Macrocyclic lactones)

3. Veterinary Use:	Control of gastrointestinal roundworms, lungworms, sucking and biting lice, mange mites and cattle ticks. Also used for itchmite, nasal bot and prevention of five clostridial diseases and cheesy gland in sheep.

4. Analyte(s) measured: (specify if metabolite)	Moxidectin

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver

7. Summary of principal steps in sample preparation:	Homogenise sample with acetonitrile

8. Summary of principal steps in extraction procedure:	Acetonitrile extract is evaporated to dryness under vacuum and the residue dissolved in hexane/dichloromethane mix

9. Summary of principal steps in Analyte clean-up procedure:	a) Silica sep-pak cleanup, eluted with ethyl acetate b) Then derivatised with acetic anhydride/1-methyl imidazole/dimethyl formamide and derivatised mixture cleaned up by C-18 sep-pak

10. Measurement procedure:

a. Chemical 1. Instrumentation	HPLC
2. Detector system	Fluorescence wavelength – 360nm excitation and 468nm emission
3. Chromatographic column (if applicable)	Reverse phase C18 column
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A

c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Terry Spencer
b. Country	Australia
c. Affiliation	AGAL ACT
d. Address	Level 10, Allara Street, Canberra, ACT, 2600
e. Telephone	(+61) 2 6213 6102
f. FAX	(+61) 2 6213 6815
g. Email	terry.spencer@agal.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.001mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.005mg/kg
c. Method sensitivit (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle (cattle): 0.02mg/kg; (sheep): 0.05mg/kg Liver (cattle and sheep): 0.1mg/kg Kidney (cattle and sheep): 0.05mg/kg Fat (cattle and sheep): 0.5mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.005, 0.01mg/kg
b. Concentration(s) measured	
c. Recovery (%)	97%, 101%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.005, 0.01mg/kg
b. Repeatability (within lab CV)	6%, 1%
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 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 analytes	
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	

NEOMYCIN
Q. Descriptive Information

1. Name of drug or chemical:	Neomycin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Aminoglycosides)

3. Veterinary Use:	Wide scope of application from antifungal, anti-inflammatory and antibiotic both topical and injection application. Used against neomycin sensitive organisms in particular Gram positive and negative organisms and a number of actinomycetes.

4. Analyte(s) measured: (specify if metabolite)	Neomycin

5. Intended use of the method:

a. Screening	Yes (5 plate MIT and if positive then ELISA)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions
9. Summary of principal steps in Analyse clean-up procedure:	Cleaned up and concentrated using SPE and solvent removal under Vacuum. Confirmation: fluorescence derivative formed using 9-fluorenylmethyl chloroformate

10. Measurement procedure:

a. Chemical 1. Instrumentation	Confirmation HPLC
2. Detector system	Fluorescence
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ELISA (secondary screening test)
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:	5 plate MIT (initial screening)

11. Sample/Analyte Stability	

Warning (if applicable):	

12. Literature References Available:	

13. Contact for Information:

a. Name	Mr Dennis Hamilton
b. Country	Australia
c. Affiliation	ARI (Chemical Residue Laboratory)
d. Address	665 Fairfield Road Yeerongapilly QLD 4105
e. Telephone	(+61) 7 3362 9415
f. FAX	(+61) 7 3362 9460
g. Email	Hamiltondj@prose.dpi.qld.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.1mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.1mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, liver and fat (cattle, sheep, pigs, goats, turkeys, ducks and chickens)
	Eggs (chickens) and milk (cattle): 0.5mg/kg
	Kidney (cattle, sheep, pigs, goats, turkeys, ducks and chickens): 10mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.25mg/kg
b. Concentration(s) measured	

c. Recovery (%)	90%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.25mg/kg
b. Repeatability (within lab CV)	10%
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 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 analyte	
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	

OXFENDAZOLE

R. Descriptive Information

1. Name of drug or chemical:	Oxfendazole

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Benzimidazole)

3. Veterinary Use:	Broad spectrum anthelmintic. Controls gastrointestinal roundworms, tapeworms and lungworms. Sterilises roundworm eggs.

4. Analyte(s) measured: (specify if metabolite)	Oxfendazole

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver

7. Summary of principal steps in sample preparation:	Tissumize 5g of sample with sodium sulphate and potassium carbonate, extracting into ethyl acetate.

8. Summary of principal steps in extraction procedure:	Evaporated residue dissolved in acetonitrile and partitioned with hexane. Hexane discarded and sample made up to volume with 0.02M. ammonium acetate

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

10. Measurement procedure:

a. Chemical 1. Instrumentation	LC-MS (screen and confirmation)
2. Detector system	MS-SIM (screen and confirmation)
3. Chromatographic column (if applicable)	Zorbax Phenyl SB 5um, 150 x 4.6mm (screen) C18 (confirmation)
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Dr Robert Symons
b. Country	Australia

c. Affiliation	AMDEL Lilyfield
d. Address	36-40 Halloran St. Lilyfield 2040
e. Telephone	(+61) 2 9818 1033
f. FAX	(+61) 2 9810 8771
g. Email	Robert_symons@amdel.com

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.01
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.05
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, kidney and fat (cattle, sheep and pigs): 0.1mg/kg
	Liver (cattle, sheep and pigs): 0.5mg/kg
	Milk (cattle and sheep): 0.1mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.05, 0.1, 0.5 mg/kg
b. Concentration(s) measured	
c. Recovery (%)	112%, 91%, 102%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.05, 0.1, 0.5 mg/kg
b. Repeatability (within lab CV)	10%, 3%, 40%
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 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 analytes	
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	

TILMICOSIN

S. Descriptive Information

1. Name of drug or chemical:	Tilmicosin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Macrolides)

3. Veterinary Use:	Macrolide antibiotic active against Mycoplasma sp. and mainly Gram positive bacteria. Can also be used in the prevention and treatment of chronic respiratory diseases. Growth stimulant; improves feed conversion efficiency in pigs, reduction of incidence of liver abscess in beef cattle.

4. Analyte(s) measured: (specify if metabolite)	Tilmicosin

5. Intended use of the method:

a. Screening	Yes (5plate MIT and if positive followed by ELISA screen)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions
9. Summary of principal steps in Analyte clean-up procedure:	Cleaned up and concentrated using SPE and solvent removal under Vacuum.

10. Measurement procedure:

a. Chemical 1. Instrumentation	Confirmation HPLC
2. Detector system	UV-287nm
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ELISA (secondary screening test)
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:	5 plate MIT (initial screening)

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Ms Heather Lindsay
b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
d. Address	Cnr Sneydes and South Roads Werribee VIC 3030
e. Telephone	(+61) 3 9742 8779
f. FAX	(+61) 3 9742 8700
g. Email	Heather.Lindsay@nre.vic.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.1mg/kg

b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.1mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle (cattle, sheep and pigs): 0.1mg/kg Liver (cattle and sheep): 1mg/kg (pigs): 1.5mg/kg Kidney (cattle and sheep): 0.3mg/kg (pigs): 1mg/kg Fat (cattle, sheep and pigs): 0.1mg/kg Milk (sheep): 0.05mg/kg (temporary)

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.1mg/kg
b. Concentration(s) measured	
c. Recovery (%)	85%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.1mg/kg
b. Repeatability (within lab CV)	20%
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 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 analyte	
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	

PROCAINE PENICILLIN

T. Descriptive Information

1. Name of drug or chemical:	Procaine penicillin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Beta-lactams)

3. Veterinary Use:	Bacterial antibiotic chiefly active against Gram-positive microorganisms. May be used to treat respiratory tract, urinary tract and wound infections, metritis and streptococcal mastitis.

4. Analyte(s) measured: (specify if metabolite)	Penicillin

5. Intended use of the method:

a. Screening	Yes (5 plate MIT)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney Egg

7. Summary of principal steps in Sample preparation:	

8. Summary of principal steps in Extraction procedure:	Antimicrobials differentially extracted into three separate solutions
9. Summary of principal steps in Analyte clean-up procedure:	Cleaned up concentrated using SPE and solvent removed under Vacuum. For confirmation derivitsed with 1,2,4-triazole-mercuric chloride

10. Measurement procedure:

a. Chemical 1. Instrumentation	HPLC
2. Detector system	UV at 325nm
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay	

1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	5 plate MIT (screening)

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Mr Dennis Hamilton
b. Country	Australia
c. Affiliation	ARI (Chemical Residue Laboratory)
d. Address	665 Fairfield Road Yeerongapilly QLD 4105
e. Telephone	(+61) 7 3362 9415
f. FAX	(+61) 7 3362 9460
g. Email	Hamiltondj@prose.dpi.qld.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.01mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.02mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Liver, kidney and muscle (all species): 0.05mg/kg

	Milk: 0.004mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.08mg/kg
b. Concentration(s) measured	
c. Recovery (%)	86%

6. Precision using fortified Control tissue

a. Concentration(s) tested	0.08mg/kg
b. Repeatability (within lab CV)	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 veterinary drugs that may also be used along with the analyse 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 analyte	
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	

STREPTOMYCIN

U. Descriptive Information

1. Name of drug or chemical:	Streptomycin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Aminoglycoside)

3. Veterinary Use:	Control of gastrointestinal bacteria, antispasmodic effect. Useful against meningococcal, pneumococcal and haemolytic streptococcal infections. Not readily absorbed, has a local bactericidal and bacteriostatic action against Gram negative bacteria.

4. Analyte(s) measured: (specify if metabolite)	Streptomycin

5. Intended use of the method:

a. Screening	Yes (5 plate MIT and if positive then ELISA)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney Egg

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions Including confirmation

9. Summary of principal steps in Analyte clean-up procedure:	Cleaned up and concentrated using SPE and solvent removal under Vacuum. Confirmation: fluorescent derivative formed using 1,2-naphthoquinone-4-sulphonic acid

10. Measurement procedure:

a. Chemical 1. Instrumentation	Confirmation HPLC
2. Detector system	Fluorescence
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ELISA (secondary screening test)
2. Critical reagents: (e.g. antibody specificity and availability)	
3. Special equipment required:	
c. Microbiological 1. Technique:	5 plate MIT (initial screening)

2. Organism: 3. Media: 4. Special equipment required:	

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Ms Heather Lindsay
b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
d. Address	Cnr Sneydes and South Roads Werribee VIC 3030
e. Telephone	(+61) 3 9742 8779
f. FAX	(+61) 3 9742 8700
g. Email	Heather.Lindsay@nre.vic.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.1mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.1mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, liver and fat (cattle, sheep, pigs and poultry): 0.5mg/kg Kidney (cattle, sheep, pigs and poultry): 1mg/kg Milk (cattle): 0.2mg/kg

3. Is analytical data corrected for recovery?	Yes

4. How is recovery estimated (e.g. external standard; internal standard etc.)	Spiked matrix calibration curve

5. Accuracy

a. Concentration(s) tested	0.2mg/kg

b. Concentration(s) measured	
c. Recovery (%)	100%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	
b. Repeatability (within lab CV)	
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 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 analyte	
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	

SULPHADIMIDINE

V. Descriptive Information

1. Name of drug or chemical:	Sulphadimidine (Sulfamethazine)

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Sulphonamides)

3. Veterinary Use:	Control of gastrointestinal bacteria through bacteriostatic effect over wide range. Useful against meningococcal, pneumococcal and haemolytic streptococcal infections, swine dysentery. Also used for respiratory infection such as pneumonia.

4. Analyte(s) measured: (specify if metabolite)	Sulphadimidine

5. Intended use of the method:

a. Screening	Yes (HPLC)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	
9. Summary of principal steps in Analyte clean-up procedure:	Extract derivatised

10. Measurement procedure:

a. Chemical 1. Instrumentation	HPLC
2. Detector system	Fluorescence
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	 N/A N/A N/A N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Ms Heather Lindsay
b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
d. Address	Cnr Sneydes and South Roads Werribee VIC 3030
e. Telephone	(+61) 3 9742 8779
f. FAX	(+61) 3 9742 8700
g. Email	Heather.Lindsay@nre.vic.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.02mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.05mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, liver, kidney and fat (cattle, sheep, pigs and poultry): 0.1mg/kg
	Eggs (poultry): No MRL should not be used in laying hens
	Milk (cattle): 0.025mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.1mg/kg
b. Concentration(s) measured	
c. Recovery (%)	91%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.1mg/kg
b. Repeatability (within lab CV)	7%

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 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 analyte	
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	

TETRACYCLINE
W. Descriptive Information

1. Name of drug or chemical:	Tetracycline

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antimicrobial agent (Tetracyclines)

3. Veterinary Use:	Provides broad spectrum antibiotic coverage against susceptible organisms in alimentary tract infections, respiratory, genitourinary, septicaemia and superficial bacterial infections.

4. Analyte(s) measured: (specify if metabolite)	Tetracycline

5. Intended use of the method:

a. Screening	Yes (5 plate MIT)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney Egg

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions

9. Summary of principal steps in Analyte clean-up procedure:	Cleaned up and concentrated using SPE and solvent removal under Vacuum.

10. Measurement procedure:

a. Chemical 1. Instrumentation	Confirmation HPLC
2. Detector system	UV
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	5 plate MIT (screening)

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Ms Heather Lindsay
b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
d. Address	Cnr Sneydes and South Roads Werribee VIC 3030
e. Telephone	(+61) 3 9742 8779
f. FAX	(+61) 3 9742 8700
g. Email	Heather.Lindsay@nre.vic.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.05mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.05mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle (cattle, sheep, pigs, poultry, fish and giant tiger prawn): 0.1mg/kg
	Liver (cattle, sheep, pigs and poultry): 0.3mg/kg
	Kidney (cattle, sheep, pigs and poultry): 0.6mg/kg
	Eggs (poultry): 0.2mg/kg
	Milk (cattle and sheep): 0.1mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.1mg/kg
b. Concentration(s) measured	
c. Recovery (%)	60%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.1mg/kg
b. Repeatability (within lab CV)	9%
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 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 analyte	
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	

THIABENDAZOLE

X. Descriptive Information

1. Name of drug or chemical:	Thiabendazole
2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Benzimidazoles)
3. Veterinary Use:	Orally administered, broad spectrum anthelmintic used against gastrointestinal parasites. May be used in controlling fungal diseases.
4. Analyte(s) measured: (specify if metabolite)	Thiabendazole

5. Intended use of the method:

a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver
7. Summary of principal steps in sample preparation:	Tissumize 5g of sample with sodium sulphate and potassium Carbonate, extracting into ethyl acetate.
8. Summary of principal steps in extraction procedure:	Evaporated residue dissolved in acetonitrile and partitioned with hexane. Hexane discarded and sample made up to volume with 0.02M Ammonium acetate
9. Summary of principal steps in Analyte clean-up procedure:	

10. Measurement procedure:

a. Chemical 1. Instrumentation	LC-MS (screen and confirmation)
2. Detector system	MS-SIM (screen and confirmation)

3. Chromatographic column (if applicable)	Zorbax Phenyl SB 5um, 150 x 4.6mm (screen) C18 (confirmation)
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Dr Robert Symons
b. Country	Australia
c. Affiliation	AMDEL Lilyfield
d. Address	36-40 Halloran St. Lilyfield 2040
e. Telephone	(+61) 2 9818 1033
f. FAX	(+61) 2 9810 8771
g. Email	Robert_Symons@amdel.com

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.01mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.05mg/kg

c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle, kidney, liver and fat (cattle, sheep, pigs and goats): 0.1mg/kg
	Milk (cattle and goats): 0.1mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	0.05mg/kg	0.1mg/kg	0.5mg/kg
b. Concentration(s) measured			
c. Recovery (%)	80%	93%	93%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.05-0.5mg/kg
b. Repeatability (within lab CV)	7-12%
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 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 analyte	
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	

TILMICOSIN

Y. Descriptive Information

1. Name of drug or chemical:	Tilmicosin

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Antibacterial agent (Macrolides)

3. Veterinary Use:	Macrolide antibiotic active against Mycoplasma sp. and mainly Gram positive bacteria. Can also be used in the prevention and treatment of

	chronic respiratory diseases. Growth stimulant; improves feed conversion efficiency in pigs, reduction of incidence of liver abscess in beef cattle.

4. Analyte(s) measured: (specify if metabolite)	Tilmicosin

5. Intended use of the method:

a. Screening	Yes (5plate MIT and if positive followed by ELISA screen)
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney

7. Summary of principal steps in sample preparation:	

8. Summary of principal steps in extraction procedure:	Antimicrobials differentially extracted into three separate solutions
9. Summary of principal steps in Analyte clean-up procedure:	Cleaned up and concentrated using SPE and solvent removal under Vacuum.

10. Measurement procedure:

a. Chemical 1. Instrumentation	Confirmation HPLC
2. Detector system	UV-287nm
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ELISA (secondary screening test)
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:	5 plate MIT (initial screening)

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

12. Literature References Available:	

13. Contact for Information:

a. Name	Ms Heather Lindsay
b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
d. Address	Cnr Sneydes and South Roads Werribee VIC 3030
e. Telephone	(+61) 3 9742 8779
f. FAX	(+61) 3 9742 8700
g. Email	Heather.Lindsay@nre.vic.gov.au

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.1mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.1mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle (cattle, sheep and pigs): 0.1mg/kg Liver (cattle and sheep): 1mg/kg (pigs): 1.5mg/kg Kidney (cattle and sheep): 0.3mg/kg (pigs): 1mg/kg Fat (cattle, sheep and pigs): 0.1mg/kg Milk (sheep): 0.05mg/kg (temporary)

3. Is analytical data corrected for recovery?	Yes

4. How is recovery estimated	External standard

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

5. Accuracy

a. Concentration(s) tested	0.1mg/kg
b. Concentration(s) measured	
c. Recovery (%)	85%

6. Precision using fortified
Control tissue

a. Concentration(s) tested	0.1mg/kg
b. Repeatability (within lab CV)	20%
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 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 analyte	
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	

TRICLABENDAZOLE

Z. Descriptive Information

1. Name of drug or chemical:	Triclabendazole

2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Benzimidazoles)

3. Veterinary Use:	Flukicide for the treatment of early immature, immature and mature liver fluke in cattle, buffalo, goats and sheep.

4. Analyte(s) measured: (specify if metabolite)	Triclabendazole, Triclabendazole sulphone, Triclabendazole sulphoxide

5. Intended use of the method:

a. Screening	Yes
b. Routine	

c. Reference	
d. Confirmatory	Yes

6. Test matrix (e.g. muscle, kidney, urine, etc)	Liver

7. Summary of principal steps in sample preparation:	Tissumize 5g of sample with sodium sulphate and potassium carbonate, extracting into ethyl acetate.

8. Summary of principal steps in Extraction procedure:	Evaporated residue dissolved in acetonitrile and partitioned with hexane. Hexane discarded and sample made up to volume with 0.02M ammonium acetate

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

10. Measurement procedure:

a. Chemical 1. Instrumentation	LC-MS (screen and confirmation)
2. Detector system	MS-SIM (screen and confirmation)
3. Chromatographic column (if applicable)	Zorbax Phenyl SB 5um, 150 x 4.6mm (screen) C18 (confirmation)
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required:	N/A

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

12. Literature References	

Available:	

13. Contact for Information:

a. Name	Dr Robert Symons
b. Country	Australia
c. Affiliation	AMDEL Lilyfield
d. Address	36-40 Halloran St. Lilyfield 2040
e. Telephone	(+61) 2 9818 1033
f. FAX	(+61) 2 9810 8771
g. Email	Robert_Symons@amdel.com

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?	0.01mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?	0.05mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)	

2. JECFA MRL	Muscle (cattle): 0.2mg/kg (sheep): 0.1mg/kg
	Liver and kidney (cattle): 0.3mg/kg (sheep): 0.1mg/kg
	Fat (cattle and sheep): 0.1mg/kg

3. Is analytical data corrected for recovery?	Yes

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

5. Accuracy

a. Concentration(s) tested	Triclabendazole		Sulphoxide			Sulphone		
	0.05mg/kg	0.1	0.05	0.1	0.5	0.05	0.1	0.5
b. Concentration(s) measured								
c. Recovery (%)	69%	78%	70%	69%	110%	69%	71%	83%

6. Precision using fortified

Control tissue

a. Concentration(s) tested	0.05-0.5mg/kg
b. Repeatability (within lab CV)	5-10%
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 analyse 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 Veterinary drugs that may also be used along with the analyse of interest	
b. Contaminants that are likely to be present in the sample	

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

C. **Information relevant to laboratory implementation**

1. Training and experience recommended for analyte	
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	

FRANCE

Please find join the list of methods available at the french national reference laboratory for the control of veterinary drugs. We hope this contribution will be helpful for the work of the Codex Alimentarius and offer our services to send to the expert complementary
information under request.

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **ABAMECTIN**
2. Drug or chemical class: Avermectins
3. Veterinary use: Anthelmintics
4. Analyte(s) measured (specified if metabolite): Abamectin
5. Intended use of the method: Confirmatory
6. Test matrix: liver
7. Summary of principal steps in sample preparation:

Thawing//Weighing of 20 grams of liver//Homogenization//Weighing of 1 gram of homogenized liver

8. Summary of principal steps in extraction procedure:

Extraction with methanol/acetonitrile//Ultrasonication//Centrifugation//Transfer the supernatant//Evaporation under nitrogen stream at 60°C

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

Adjusting with acetonitrile and addition of ultra-pure water//Purification on C18 SPE cartridge 100mg by eluting with acetonitrile/water (90/10;v/v)//Centrifugation of the eluate//Evaporation under nitrogen stream at 60°C//For

derivatization
recover the dried residue with N-methylimidazole//Addition of trifluoroacetic acid before injection (caution: derivative is light
sensitive)

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : TSP Pump P4000//Autosampler model AS300 with 20µl loop//Data station TSP
PC1000

2. DetectorSystem/Reagents/Organism:
Fluorescence detector model Jasco 821-FP set at exc 361 nm and em 465 nm

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

3. Column/Special equipment:
Licrospher 100, RP18-e (125x4mm;5µm) with guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
anhydric conditions for derivatization//caution: fluorescent derivatives of avermectins and moxidectin are
light sensitive - Take care avoiding light before injecting within 8 hours after derivatization

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments
vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite:
http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 1.5 µg/kg

1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 4 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy

a. Concentration(s) tested: 20 µg/kg (n=6)
b. Concentration(s) measured:

c. Recovery (%): 79.3 +/- 7.7 % (n=6)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 20 µg/kg (n=6)
b.RepeatabilityWithinlabCV: 8.1 %

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

c. RepeatabilityBetweenlabCV: 8.6 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **ALBENDAZOLE**
2. Drug or chemical class: Benzimidazoles and pro-benzimidazoles
3. Veterinary use: Anthelmintics
4. Analyte(s) measured (specified if metabolite): Albendazole and its metabolites : albendazole sulfoxyde, albendazole sulfone and albendazole 2-aminosulfone
5. Intended use of the method: Screening
6. Test matrix: milk
7. Summary of principal steps in sample preparation:

Thawing//Weighing of 1 mL of milk

8. Summary of principal steps in extraction procedure:

samples pH adjustment at pH 10.0 with sodium hydroxide//Extraction wit ethyl acetate//Centrifugation//Transfer of a fraction of the supernatant

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

Addition of ultrapure water//Centrifugation//Transfer of the organic phase//Evaporation under nitrogen stream at 50°C//Recover with a solution of 0.017M orthophosphoric acid / acetonitrile (85/15;v/v)//Ultrasonicate before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : TSP Pump P4000//TSP Autosampler AS300

2. DetectorSystem/Reagents/Organism:

UV detector set at 287 nm

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

3. Column/Special equipment:
Inertsil ODS3 desactivated (150x4.6mm;5µm) and a guard column Inertsil ODS3 (10x3mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Take care of possible oxydation of the benzimidazoles when not sufficiently controlling ther extraction-purification steps

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 10 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg):
1.c. Method sensitivity:
2. JECFA MRL:
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy
a. Concentration(s) tested: 100 µg/kg (n=14)
b. Concentration(s) measured:
c. Recovery (%): 82.1 +/- 5.6 % (n=14)

6. Precision using fortified control tissue:
a. Concentration(s) tested: 100 µg/kg (n=14)
b.RepeatabilityWithinlabCV: 6.75 %

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

c. RepeatabilityBetweenlabCV: 6.75 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure:

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for azaperone and azaperol in porcine muscle by LC/MS**

A. Descriptive information

1. Name of drug or chemical: **AZAPERONE**
2. Drug or chemical class: Butyrophenones
3. Veterinary use: Tranquillizing agent
4. Analyte(s) measured (specified if metabolite): Azaperone and its metabolite azaperol
5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Acetonitrile//Homogenization//Centrifugation//Transfer of the supernatant in ultra-pure water

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

SPE clean-up on Bond-Elut C18 cartridge with LC mobile phase elution and ultra-pure water dilution before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MS : HP1050 and Finnigan SSQ7000

2. DetectorSystem/Reagents/Organism:
esi MS with 4 ions monitored (positive mode)

Name of the method: **Confirmatory method for azaperone and azaperol in porcine muscle by LC/MS**

3. Column/Special equipment:
RP18e (125x4mm; 5µm) + Guard column RP18e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): <12.5 ug/kg
1.b. Limit of quantification (LOQ) (mg/kg): 12.5 µg/kg

1.c. Method sensitivity:

2. JECFA MRL: 60 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery?
4. How is recoverey estimated?

at 4 levels calibrated curve form fortified muscle samples

5. Accuracy

   a. Concentration(s) tested: 12.5//25//50//100 µg/kg
   b. Concentration(s) measured:
   c. Recovery (%):

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 12.5//25//50//100 µg/kg
   b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for azaperone and azaperol in porcine muscle by LC/MS**

   c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure: Good selectivity towards azaperol

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of 7 tranquillizers and 1 beta-agonist (carazolol) in porcine kidney by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **AZAPERONE**
2. Drug or chemical class: Butyrophenones
3. Veterinary use: Tranquillizing agent
4. Analyte(s) measured (specified if metabolite): Azaperone and its metabolite azaperol
5. Intended use of the method: Screening
6. Test matrix: kidney
7. Summary of principal steps in sample preparation:

Thawing//Weighing of 30 grams of tissue//Grinding//Weighing of 10 grams of ground tissue

8. Summary of principal steps in extraction procedure:

Addition of sodium hydroxide//Incubation at 95°C for 60 min//Extraction by ether

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

Purification on diol-coated silica cartridge

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : SpectraPhysics pump model SP8700 with manual injection on a 50µL loop and

Integration on a SpectraPhysics model SP4290

2. DetectorSystem/Reagents/Organism:

UV detector Kratos model Spectroflow 773 set at 245 nm

Name of the method: **Determination of 7 tranquillizers and 1 beta-agonist (carazolol) in porcine kidney by HPLC/UV**

3. Column/Special equipment:
SAS Hypersil H5C1-15F coated C1 (150x4.6mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 1 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 10 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 4 level external standard calibration and fortified muscle samples for recovery calculation

5. Accuracy

a. Concentration(s) tested: 10//50//100//200 µg/kg
b. Concentration(s) measured:
c. Recovery (%): 59 %

6. Precision using fortified control tissue:

a. Concentration(s) tested: azaperone: 100 ug/kg (n=6); azaperol: 100 ug/kg (n=6)
b.RepeatabilityWithinlabCV: azaperone:6.3%; azaperol:5.5%

Name of the method: **Determination of 7 tranquillizers and 1 beta-agonist (carazolol) in porcine kidney by HPLC/UV**

c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

    a. Drugs of similar structure:

    b. Contaminants:

    c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of penicillin G, nafcillin, oxacillin, cloxacillin and dicloxacillin residues in pig muscle by LC/MS/MS (ESI)**

A. Descriptive information

1. Name of drug or chemical: **BENZYLPENICILLIN**
2. Drug or chemical class: Penicillins
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Benzylpenicillin

5. Intended use of the method: Confirmatory

6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water and Internal Standard (Penicillin-V)

8. Summary of principal steps in extraction procedure:

Sodium Phosphate buffer and 2% Sodium Chloride pH 8.2//Centrifugation//Transfer of the supernatant//Addition of ultra-pure water

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

SPE clean-up on Bond-Elut C18 cartridge with ACN elution and ultra-pure water addition before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MSMS : HP1100 and Quattro LCZ

2. DetectorSystem/Reagents/Organism:
esi MS with 2 transitions (Precursor ion>production) monitored-positive mode

Name of the method: **Determination of penicillin G, nafcillin, oxacillin, cloxacillin and dicloxacillin residues in pig muscle by LC/MS/MS (ESI)**

3. Column/Special equipment:
RP18e (125x4mm;5µm) with guard column RP18e(4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored for 1 month at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Hurtaud-Pessel, Dominique
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES

e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: d.pessel@fougeres.afssa.fr WebSite:
http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 3 mg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 12.5 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 50 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?

A 3 to 6 levels calibration curve from fortified muscle samples with internal standard correction (Penicillin-V)

5. Accuracy

a. Concentration(s) tested: 25//50//75//100/ µg/kg
b. Concentration(s) measured: 25.1//52.0//72.5//95.7 (for n=5)
c. Recovery (%):

6. Precision using fortified control tissue:

a. Concentration(s) tested: 25//50//75//100 µg/kg
b.RepeatabilityWithinlabCV: 7//4.5//11.8//6.9 (for n=5)

Name of the method: **Determination of penicillin G, nafcillin, oxacillin, cloxacillin and dicloxacillin residues in pig muscle by LC/MS/MS (ESI)**

c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:

2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Quantitative determination of 8 penicillins in pig muscle by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **BENZYLPENICILLIN**
2. Drug or chemical class: Penicillins
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Benzylpenicillin
5. Intended use of the method: Confirmatory
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Extraction by centrifugation after homogenization of minced muscle samples with phosphate buffer pH 9

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

SPE Purification and reconcentration on 500mg C18 cartridges//Derivatization of the penicillin residues by chemical reaction with benzoic anhydride at 50°C to prepare aminopenicillins (ampicillin and amoxicillin) and then with 1,2,4-triazole and mercuric chloride at 65°C for the 8 penicillin compounds to obtain the N-penicillenic acid mercury(II) mercaptide conjuguates

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : TSP pump model P1000XR and autosampler model AS100

2. DetectorSystem/Reagents/Organism:

Name of the method: **Quantitative determination of 8 penicillins in pig muscle by HPLC/UV**

3. Column/Special equipment:
Symmetry C8 (150x3.9mm;5µm) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in ultrapure water stored 1 week at +4°C and residues of penicillins in muscle are
stable stored at -80°C one year at least and stored at -20°C 3 months at the most

12. Literature references available:

13. Contact for information:

a. Name: Verdon, Eric
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: e.verdon@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 3 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 25 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 50 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration with 2 muscle samples fortified at MRL level

5. Accuracy

a. Concentration(s) tested: 25//50//100//200 µg/kg (n=4x12)
b. Concentration(s) measured: 24.7//49.9//99.1//203.1 µg/kg (n=4x12)

c. Recovery (%): 74 +/- 6 % (n=48)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 25//50 µg/kg (n=12)

b.RepeatabilityWithinlabCV: 7.9 %//5.6 % (n=12)

Name of the method: **Quantitative determination of 8 penicillins in pig muscle by HPLC/UV**

c. RepeatabilityBetweenlabCV: 7.9 %//8.4 % (n= 3 x 4 days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other pencillins (ampicillin, amoxicillin, penicillin-V, oxacillin, nafcillin, cloxacillin, dicloxacillin

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Quantitative determination of penicillin-G and penicillin-V in milk by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **BENZYLPENICILLIN**
2. Drug or chemical class: Penicillins
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Benzylpenicillin

5. Intended use of the method: Confirmatory
6. Test matrix: milk

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 mL of raw milk//Penicillin V may be added as quality control for extraction and chromatography

8. Summary of principal steps in extraction procedure:

Extraction by centrifugation at 0°C-5°C after acidification (phosphate buffer pH8, 2N sulfuric acid//Transfer the supernatant//Adjust its pH to 8.0//Centrifuge at 0°C-5°C

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

SPE Purification and reconcentration of the supernatant on 500mg C18 cartridges by eluting with ultrapure water/acetonitrile (60/40;v/v)//Derivatization of the benzylpenicillin (Pen-G) and phenoxymethylpenicillin (Pen-V) residues by chemical reaction with 1,2,4-triazole and mercuric chloride at 65°C to obtain the N-penicillenic acid mercury(II) mercaptide conjuguates

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : SpectraPhysics pump 8800 and Autosampler SpectraPhysics model 8775

2. DetectorSystem/Reagents/Organism:
UV detector Kratos model Spectroflow 773 set at 325 nm

Name of the method: **Quantitative determination of penicillin-G and penicillin-V in milk by HPLC/UV**

3. Column/Special equipment:
Symmetry C8 (150x3.9mm;5µm) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

Stocked standard solutions in ultrapure water stored 1 week at +4°C and residues of penicillins in muscle are stable stored at -80°C for one year at least and stored at -20°C for 3 months at the most

12. Literature references available:

13. Contact for information:

a. Name: Verdon, Eric
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: e.verdon@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 2.5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 4.0 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 4 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?

A 5 level external standard calibration with 2 muscle samples fortified at MRL level

5. Accuracy

a. Concentration(s) tested: 4//8//16//32//64 µg/kg (n=4)
b. Concentration(s) measured: 4.16//7.77//15.34//29.69//62.13 µg/kg (n=4)
c. Recovery (%): 89.7//83.7//82.6//79.9//83.6 % (n=4)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 4//16 µg/kg (n=16)
b.RepeatabilityWithinlabCV: 7.9 %//3.5 % (n=16)

Name of the method: **Quantitative determination of penicillin-G and penicillin-V in milk by HPLC/UV**

c. RepeatabilityBetweenlabCV: 12.6 %//3.7 % (n=4 x 4 days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other pencillins (ampicillin, amoxicillin, penicillin-V, oxacillin, nafcillin, cloxacillin, dicloxacillin

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of 7 tranquillizers and 1 beta-agonist (carazolol) in porcine kidney by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **CARAZOLOL**
2. Drug or chemical class: Antiadrenergics
3. Veterinary use: Tranquilizing agent
4. Analyte(s) measured (specified if metabolite):

5. Intended use of the method: Screening

6. Test matrix: kidney

7. Summary of principal steps in sample preparation:

Thawing//Weighing of 30 grams of tissue//Grinding//Weighing of 10 grams of ground tissue

8. Summary of principal steps in extraction procedure:

Addition of sodium hydroxide//Incubation at 95°C for 60 min//Extraction by ether

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

Purification on diol-coated silica cartridge

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : SpectraPhysics pump model SP8700 with manual injection on a 50µL loop and

Integration on a SpectraPhysics model SP4290

2. DetectorSystem/Reagents/Organism:

UV detector Kratos model Spectroflow 773 set at 245 nm

Name of the method: **Determination of 7 tranquillizers and 1 beta-agonist (carazolol) in porcine kidney by HPLC/UV**

3. Column/Special equipment:

SAS Hypersil H5C1-15F coated C1 (150x4.6mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 1 µg/kg

1.b. Limit of quantification (LOQ) (mg/kg): 10 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 25 µg/kg (52nd meeting - Feb 1999)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 4 level external standard calibration and fortified muscle samples for recovery calculation

5. Accuracy

   a. Concentration(s) tested: 10//50//100//200 µg/kg
   b. Concentration(s) measured:

   c. Recovery (%): 51 %

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 50 ug/kg (n=6)
   b.RepeatabilityWithinlabCV: 5.2%

Name of the method: **Determination of 7 tranquillizers and 1 beta-agonist (carazolol) in porcine kidney by HPLC/UV**

   c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure:

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of ceftiofur in muscle and milk by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **CEFTIOFUR**
2. Drug or chemical class: Cephalosporins
3. Veterinary use: Antimicrobials
4. Analyte(s) measured (specified if metabolite): Ceftiofur and desfuroylceftiofur

5. Intended use of the method: Confirmatory
6. Test matrix: muscle//milk
7. Summary of principal steps in sample preparation:

Muscle: Thawing//Grinding//Weighing of 3 grams of tissue
Milk: Thawing//Homogenizing//Pipeting of 6 ml of milk

8. Summary of principal steps in extraction procedure:

Extraction of ceftiofur and its metabolite desfuroylceftiofur at 50°C with pH 9 dithioerytritol buffer for converting ceftiofur into desfuroylceftiofur//
Derivatization with iodoacetamide solution at room temperature for stabilizing the desfuroylceftiofur by converting it into desfuroylceftiofur acetamide

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

Purification no1 onto a 500mg C18 Sep-Pak cartridge//Purification no2 onto a 500mg SAX Bond-elut cartridge//Purification no3 onto a 100mg SCX Bond-elut cartridge

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : TSP pump model P1000XR and autosampler model AS100

2. DetectorSystem/Reagents/Organism:

UV detector TSP UV3000 set at 266 nm

Name of the method: **Determination of ceftiofur in muscle and milk by HPLC/UV**

3. Column/Special equipment:
Symmetry C8 (150x3.9mm;5µm) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in pH 7 phosphate buffer stored 1 month at +4°C and residues of ceftiofur in
muscle and in milk are stable stored at -20°C at least one month

12. Literature references available:

13. Contact for information:

a. Name: Verdon, Eric
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: e.verdon@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 70 µg/kg in muscle and 13 µg/kg in milk
1.b. Limit of quantification (LOQ) (mg/kg): 250 µg/kg in muscle and 50 µg/kg in milk
1.c. Method sensitivity:
2. JECFA MRL: 1000 µg/kg in muscle and 100 µg/kg in milk
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration with 2 muscle samples fortified at MRL level

5. Accuracy
a. Concentration(s) tested: 1000 µg/kg (n=8 muscle)//100µg/kg (n=8 milk)
b. Concentration(s) measured: 950 µg/kg (n=8 in muscle)//98.4 µg/kg (n=8 in milk)
c. Recovery (%): 81 +/- 6 % (n=8 in muscle)//82 +/- 5 % (n=8 in milk)
6. Precision using fortified control tissue:
a. Concentration(s) tested: 1000 µg/kg (n=8 in muscle)//100 µg/kg (n=8 in milk)

b.RepeatabilityWithinlabCV: 5.0 % (n=8 in muscle)//5.6 % (n=8 in milk)

Name of the method: **Determination of ceftiofur in muscle and milk by HPLC/UV**

c. RepeatabilityBetweenlabCV: 5.0 % (n=2 x 4 days)//5.8 % (n=8 milk)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: selectivity checked versus other veterinary-used cephalosporins and versus penicillins (cephapirine, desacetylcephapirin, cefacetrile,

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Detection and confirmation of residues of chloramphenicol in biological matrices by GC/MS nci**

A. Descriptive information

1. Name of drug or chemical: **CHLORAMPHENICOL**
2. Drug or chemical class: Phenicolated compounds

3. Veterinary use: Antimicrobial

4. Analyte(s) measured (specified if metabolite): Chloramphenicol

5. Intended use of the method: Confirmatory

6. Test matrix: muscle//egg//milk//urine

7. Summary of principal steps in sample preparation:

Eggs : yolk separated and mixed with ultra-pure water (v/v)//stored frozen

Urine: pH of urine adjusted to 4.8//urine mixed with sodium acetate pH 4.8 buffer//glucuronidase added//incubation 1 night at 37°C//stored frozen

Milk and muscle: No specific preparation unlike stored frozen and grinded

8. Summary of principal steps in extraction procedure:

Eggs: Thawing//Weighing of 4 grams of prepared yolk//Addition of IS CAP-D5//Addition of ethyl acetate//Addition of ACN//Centrifugation//Organic phase recovered//Second addition of ethyl acetate//Centrifugation//Both organic phases recovered and mixed together//ACN evaporation under nitrogen stream//Residue recovered with ACN and washed with hexane//Evaporation with ACN phase//Centrifugation//Combination of both ACN phases//Evaporation of ACN under nitrogen stream;Urine and Milk: Thawing//Weighing of 2.4 grams of milk or prepared urine//Addition of IS CAP-D5//Transfer to Extrelut 3cc cartridge//Addition of ethyl acetate//Organic phase recovered//Evaporation under nitrogen stream;Muscle: Thawing//Weighing of 2 grams of grinded muscle//Addition of IS CAP-D5//Addition of ethyl acetate//Centrifugation//Organic phase recovered //Second addition of ethyl acetate//Centrifugation//Both organic phases recovered and mixed together//ACN evaporation under nitrogen stream//Residue recovered with ACN and hexane//Centrifugation//Separation of both organic phases//Addition of ACN in the hexane phase and of hexane in the ACN phase//Centrifugation//Combination of both ACN phases//Evaporation of ACN under nitrogen stream

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

All matrices: Recovering of the residue with toluene//SPE on Silica 500mg cartridge//Elution with a toluene-acetone (60/40;v/v) solution//Evaporation//Residue recovered with diethylic ether//Transfer in derivation flask//Evaporation Derivatization step:Addition of hexamethyldisilazane/chlorotrimethylsilane/pyridine//3/1/9//Reacting for 1 hour at 60°C//Evaporation//Residue recovered with hexane for injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
GC/MS : HP 6973 and HPMSD5973

2. DetectorSystem/Reagents/Organism:
nci MSD with 4 ions monitored + 2 ions from Int Std CAP D5

Name of the method: **Detection and confirmation of residues of chloramphenicol in biological matrices by GC/MS nci**

3. Column/Special equipment:
capillary column 5% phenyl 95%methylsiloxane (30m;0.25mm;0.25µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Abjean, Jean-Pierre
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: jp.abjean@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 0.16 µg/kg (CCalpha)
1.b. Limit of quantification (LOQ) (mg/kg): 0.25 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: banned substance
3. Is analytical data corrected for recovery? No
4. How is recoverey estimated?
Internal standard (deuterated chloramphenicol D5)

5. Accuracy

a. Concentration(s) tested: 0.25//0.5//1.0//2.0 µg/kg
b. Concentration(s) measured:
c. Recovery (%):

6. Precision using fortified control tissue:

a. Concentration(s) tested: 0.25//0.5//1.0//2.0 µg/kg
b.RepeatabilityWithinlabCV:

Name of the method: **Detection and confirmation of residues of chloramphenicol in biological matrices by GC/MS nci**

c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Method for the identification of chloramphenicol in milk by LC/MSMS**

A. Descriptive information

1. Name of drug or chemical: **CHLORAMPHENICOL**
2. Drug or chemical class: Phenicolated compounds
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Chloramphenicol
5. Intended use of the method: Confirmatory
6. Test matrix: milk

7. Summary of principal steps in sample preparation:

acidification by hcl

8. Summary of principal steps in extraction procedure:

ethyl acetate/homogeneisation/centrifugation/transfer of the organic supermutant

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

evaporation of ethylacetate/recover with a mixture of carbone tetrachloride and hexane and water/centrifugation transfer the aqueous phase for injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MSMS : HP1100 and PE-SCIEX API2000

2. DetectorSystem/Reagents/Organism:
apci MSMS in MRM mode with 3 transitions (one precurseur with three products) monitored (negative mode)

Name of the method: **Method for the identification of chloramphenicol in milk by LC/MSMS**

3. Column/Special equipment:
RP18e (125x4mm; 5µm) + Guard column RP18e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 0.5 ug/kg
1.b. Limit of quantification (LOQ) (mg/kg): 0.5 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: banned substance
3. Is analytical data corrected for recovery?
4. How is recoverey estimated?

5. Accuracy
    a. Concentration(s) tested:
    b. Concentration(s) measured:
    c. Recovery (%):
6. Precision using fortified control tissue:
    a. Concentration(s) tested:
    b.RepeatabilityWithinlabCV:

Name of the method: **Method for the identification of chloramphenicol in milk by LC/MSMS**

    c. RepeatabilityBetweenlabCV:
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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

    a. Drugs of similar structure:

    b. Contaminants:

    c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):

6. Literature references or other useful

Name of the method: **Method for the identification of chloramphenicol in muscle by LC/MSMS**

A. Descriptive information

1. Name of drug or chemical: **CHLORAMPHENICOL**
2. Drug or chemical class: Phenicolated compounds
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Chloramphenicol

5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Ethyl acetate//Homogenization//Centrifugation//Transfer of the organic supernatant

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

Evaporation of ethyl acetate//Recover with a mixture of Carbon tetrachloride and hexane (v/v)//Addition of ultra-pure water//Centrifugation//Transfer of the aqueous phase for injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MSMS : HP1100 and PE-SCIEX API2000

2. DetectorSystem/Reagents/Organism:
apci MSMS in MRM mode with 3 transitions (one precurseur with three products) monitored
(negative mode)

Name of the method: **Method for the identification of chloramphenicol in muscle by LC/MSMS**

3. Column/Special equipment:
RP18e (125x4mm; 5µm) + Guard column RP18e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): <1 ug/kg
1.b. Limit of quantification (LOQ) (mg/kg): 1 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: banned substance
3. Is analytical data corrected for recovery?
4. How is recoverey estimated?

5. Accuracy
   a. Concentration(s) tested: 1.0//2.5//5.0//10.0 µg/kg
   b. Concentration(s) measured:
   c. Recovery (%):
6. Precision using fortified control tissue:
   a. Concentration(s) tested:
   b.RepeatabilityWithinlabCV:

Name of the method: **Method for the identification of chloramphenicol in muscle by LC/MSMS**

c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **CHLORTETRACYCLINE**
2. Drug or chemical class: Tetracyclines
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Chlortetracycline//4-epichlortetracycline
5. Intended use of the method: Confirmatory

6. Test matrix: muscle//kidney

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Mac Ilvaine/EDTA buffer//Homogenization//Centrifugation//Transfer of the supernatant for clean-up step

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

Deproteinization with Trichloroacetic acid//SPE clean-up on Bond-Elut C18 cartridge eluting with a 0.01M oxalic acid methanolic solution followed by ultra-pure water//Ultraspeed centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : HP pump series 1050 and autosampler series 1100

2. DetectorSystem/Reagents/Organism:

UV detector HP1050 set at 355 nm

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

3. Column/Special equipment:

Purospher RP18-e (125x4mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

Stocked standard solutions in methanol stored 1 month at -20°C and Residues of tetracyclines in muscle and kidney are stable stored at -20°C. Tissues must be thawed just before the analysis

12. Literature references available:

13. Contact for information:

a. Name: Gaugain-Juhel, Murielle
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: m.juhel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 15 µg/kg in muscle//170 µg/kg in kidney
1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg in muscle//300 µg/kg in kidney
1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg in M//600 µg/kg in K (47th meeting)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration and 1 MRL level fortified muscle or kidney sample

5. Accuracy

a. Concentration(s) tested: 100 µg/kg//600 µg/kg (n=12)
b. Concentration(s) measured:
c. Recovery (%): 50.1 +/- 4.5 % in M//63.5 +/- 4.7 % in K (n=12)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 100 µg/kg in M//600 µg/kg in K (n=12)
b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

c. RepeatabilityBetweenlabCV: 9.04 % in M//7.42 % in K

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other tetracyclines and their 4-epimer for oxytetracycline, chlortetracycline and tetracycline

b. Contaminants: Caution: chlortetracycline standards may contain tetracycline as well. Take care avoiding preparation of CTC and TC standards in the same standard solution.

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of tetracycline residues in pork muscle by LC/MS (ESI)**

A. Descriptive information

1. Name of drug or chemical: **CHLORTETRACYCLINE**
2. Drug or chemical class: Tetracyclines
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Chlortetracycline//4-epichlortetracycline
5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Mac Ilvaine/EDTA buffer//Centrifugation//Transfer of the supernatant

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

Protein precipitation with TCA//SPE clean-up on Bond-Elut C18 cartridge eluting with a mixture of methanol-2% oxalic acid followed by ultra-pure water before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MS : HP1050 and Finnigan SSQ7000

2. DetectorSystem/Reagents/Organism:
esi MS with 4 ions monitored-positive mode

Name of the method: **Determination of tetracycline residues in pork muscle by LC/MS (ESI)**

3. Column/Special equipment:
Symmetry C18 (150x3.9mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored for 1 month at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Hurtaud-Pessel, Dominique
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: d.pessel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 20 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 100 µg/kg (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 levels calibration curve from fortified muscle samples

5. Accuracy

a. Concentration(s) tested: 100 µg/kg (n=5)

b. Concentration(s) measured: 91.50 µg/kg (n=5)

c. Recovery (%): 56.1 +/- 6.7 % (n=8)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 50//100//150//200 µg/kg (n=8)

b.RepeatabilityWithinlabCV: 10.8 % for n=5 samples of 100 µg/kg

Name of the method: **Determination of tetracycline residues in pork muscle by LC/MS (ESI)**

c. RepeatabilityBetweenlabCV: 7.2//7.5//5.6//2.4 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Good selectivity checked towards oxytetracycline, 4 epitetracycline, chlortetracycline, 4 epitetracycline and doxycycline

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

A. Descriptive information

1. Name of drug or chemical: **CIPROFLOXACIN**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Enrofloxacin and ciprofloxacin

5. Intended use of the method: Confirmatory

6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Homogenization//Weighing 0.5 grams of tissue as a test portion

8. Summary of principal steps in extraction procedure:

Extraction of the quinolone residues in poultry meat by addition of a solution containing acetonitrile-pH 9.18 Tetraborate buffer//Ultrasonic pulverisation//Centrifugation// Transfer of the supernatant

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

Filtration before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : Alliance pump autosampler device

2. DetectorSystem/Reagents/Organism:

TSP Fluorimetric Detector FL3000 set at exc 280 nm and em 450 nm

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

3. Column/Special equipment:

PLRP-S (150x4.6mm;5µm;100A) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

Avoid contact with glassware at neutral pH as quinolones are chelating agents to divalent ions//Quinolones

are light sensitive compounds

12. Literature references available:

13. Contact for information:

a. Name: Yorke, Jean Christophe
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: jc.yorke@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 2 µg/kg

1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg

1.c. Method sensitivity:

2. JECFA MRL:

3. Is analytical data corrected for recovery? yes

4. How is recoverey estimated?

A 5 level external standard calibration and 1 MRL level fortified muscle sample

5. Accuracy

a. Concentration(s) tested: 15 µg/kg (n=58)

b. Concentration(s) measured:

c. Recovery (%): 67 +/- 15 % (n=58)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 15 µg/kg (n=12)

b.RepeatabilityWithinlabCV: 8.4 %

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

c. RepeatabilityBetweenlabCV: 12.4 % (n=3 x 4 days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus enrofloxacin, sarafloxacin and difloxacin

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

A. Descriptive information

1. Name of drug or chemical: **DANOFLOXACIN**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Danofloxacin
5. Intended use of the method: Confirmatory
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Phosphate buffer pH 7.4//Homogenization//Centrifugation//Filtration of the supernatant

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

SPE clean-up on Bond-Elut C18 cartridge eluting with trifluoroacetic acid 1% in ACN followed by pure ACN//Dry under nitrogen flow//Recover with ACN and ultra-pure water for injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MSMS : HP1100 and PE-SCIEX API2000

2. DetectorSystem/Reagents/Organism:
apci MSMS with 2 transitions (one precurseur with two products) monitored (positive mode)

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

3. Column/Special equipment:
Symmetry C18 (150x3.9mm; 5µm) + Guard column Waters (20x3.9mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 200 µg/kg (48th meeting - Feb 1997)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?

A 4 levels calibration curve from fortified muscle samples

5. Accuracy

   a. Concentration(s) tested: 100//200//300//600 µg/kg
   b. Concentration(s) measured: 99.5//201.1//299.3//600.1 µg/kg (n=5)
   c. Recovery (%):

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 100//200//300//600 µg/kg
   b.RepeatabilityWithinlabCV: 8.2//11.4//9.3//6.4 % (n=5)

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

   c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure: Good selectivity towards other quinolones

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Quantitative determination of 2 quinolones (marbofloxacin-danofloxacin) in chicken muscle by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **DANOFLOXACIN**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Danofloxacin

5. Intended use of the method: Confirmatory

6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing 0.5 grams of tissue as a test portion

8. Summary of principal steps in extraction procedure:

Extraction of the quinolone residues in poultry meat by addition of a solution containing acetonitrile-pH 9.18 Tetraborate buffer//Ultrasonic pulverisation//Centrifugation// Transfer of the supernatant

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

Filtration before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : Alliance pump autosampler device

2. DetectorSystem/Reagents/Organism:

TSP Fluorimetric Detector FL3000 set at exc 294 nm and em 514 nm

Name of the method: **Quantitative determination of 2 quinolones (marbofloxacin-danofloxacin) in chicken muscle by HPLC/FLD**

3. Column/Special equipment:

PLRP-S (150x4.6mm;5µm;100A) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

Avoid contact with glassware at neutral pH as quinolones are chelating agents to divalent ions//Quinolones

are light sensitive compounds

12. Literature references available:

13. Contact for information:

a. Name: Yorke, Jean Christophe
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: jc.yorke@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 7 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 150 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 200 µg/kg in chicken muscle (48th meeting-Feb1997)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?

A 5 level external standard calibration and 1 MRL level fortified muscle sample

5. Accuracy

a. Concentration(s) tested: 300 µg/kg (n=28)
b. Concentration(s) measured:
c. Recovery (%): 67 +/- 12 % (n=28)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 300 µg/kg (n=6)
b.RepeatabilityWithinlabCV: 4.7 %

Name of the method: **Quantitative determination of 2 quinolones (marbofloxacin-danofloxacin) in chicken muscle by HPLC/FLD**

c. RepeatabilityBetweenlabCV: 9.9 % (n=2x3days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

    a. Drugs of similar structure: Selectivity checked versus marbofloxacin

    b. Contaminants:

    c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for streptomycin and dihydrostreptomycin in bovine muscle by LC/MS**

A. Descriptive information

1. Name of drug or chemical: **DIHYDROSTREPTOMYCINE**
2. Drug or chemical class: Aminoglycosides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Dihydrostreptomycin and streptomycin

5. Intended use of the method: Confirmatory
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

5% Trichloroacetic acid / EDTA//Homogenization//Centrifugation//Transfer of the supernatant in 2M ammonium acetate for injection

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

No clean-up

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

LC/MS : HP1050 and Finnigan SSQ7000

2. DetectorSystem/Reagents/Organism:

esi MS with 4 ions monitored (positive mode)

Name of the method: **Confirmatory method for streptomycin and dihydrostreptomycin in bovine muscle by LC/MS**

3. Column/Special equipment:

RP18e (125x4mm; 5µm) + Guard column RP18e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 65 µg/kg (n=5)
1.b. Limit of quantification (LOQ) (mg/kg): 250 µg/kg

1.c. Method sensitivity:

2. JECFA MRL: 600 µg/kg (52nd meeting - Feb 1999)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?

A 4 levels calibration curve from fortified muscle samples

5. Accuracy

   a. Concentration(s) tested: 250//500//750//1000 µg/kg
   b. Concentration(s) measured:
   c. Recovery (%): 43.2 +/- 5.3 (n=5)

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 250//500//750//1000 µg/kg
   b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for streptomycin and dihydrostreptomycin in bovine muscle by LC/MS**

   c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure: Good selectivity towards streptomycin

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **DORAMECTIN**
2. Drug or chemical class: Avermectins
3. Veterinary use: Anthelmintics
4. Analyte(s) measured (specified if metabolite): Doramectin
5. Intended use of the method: Confirmatory
6. Test matrix: liver
7. Summary of principal steps in sample preparation:

Thawing//Weighing of 20 grams of liver//Homogenization//Weighing of 1 gram of homogenized liver

8. Summary of principal steps in extraction procedure:

Extraction with methanol/acetonitrile//Ultrasonication//Centrifugation//Transfer the supernatant//Evaporation under nitrogen stream at 60°C

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

Adjusting with acetonitrile and addition of ultra-pure water//Purification on C18 SPE cartridge 100mg by eluting with acetonitrile/water (90/10;v/v)//Centrifugation of the eluate//Evaporation under nitrogen stream at 60°C//For derivatization recover the dried residue with N-methylimidazole//Addition of trifluoroacetic acid before injection (caution: derivative is light sensitive)

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : TSP Pump P4000//Autosampler model AS300 with 20µl loop//Data station TSP PC1000

2. DetectorSystem/Reagents/Organism:

Fluorescence detector model Jasco 821-FP set at exc 361 nm and em 465 nm

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

3. Column/Special equipment:
Licrospher 100, RP18-e (125x4mm;5µm) with guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
anhydric conditions for derivatization//caution: fluorescent derivatives of avermectins and moxidectin are
light sensitive - Take care avoiding light before injecting within 8 hours after derivatization

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 2.5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg (52nd meeting - Feb 1999)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 4 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy

a. Concentration(s) tested: 100 µg/kg (n=6)
b. Concentration(s) measured:
c. Recovery (%): 77.5 +/- 2.7 % (n=6)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 100 µg/kg (n=6)
b.RepeatabilityWithinlabCV: 4.3 %

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

c. RepeatabilityBetweenlabCV: 6.3 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **DOXYCYCLINE**
2. Drug or chemical class: Tetracyclines
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Doxycycline
5. Intended use of the method: Confirmatory

6. Test matrix: muscle//kidney

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Mac Ilvaine/EDTA buffer//Homogenization//Centrifugation//Transfer of the supernatant for clean-up step

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

Deproteinization with Trichloroacetic acid//SPE clean-up on Bond-Elut C18 cartridge eluting with a 0.01M oxalic acid methanolic solution followed by ultra-pure water//Ultraspeed centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : HP pump series 1050 and autosampler series 1100

2. DetectorSystem/Reagents/Organism:

UV detector HP1050 set at 355 nm

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

3. Column/Special equipment:

Purospher RP18-e (125x4mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

Stocked standard solutions in methanol stored 1 month at -20°C and Residues of tetracyclines in muscle and kidney are stable stored at -20°C. Tissues must be thawed just before the analysis

12. Literature references available:

13. Contact for information:

a. Name: Gaugain-Juhel, Murielle
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: m.juhel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 12 µg/kg in muscle//160 µg/kg in kidney
1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg in muscle//300 µg/kg in kidney
1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg in M//600 µg/kg in K (47th meeting)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration and 1 MRL level fortified muscle or kidney sample

5. Accuracy

a. Concentration(s) tested: 100 µg/kg//600 µg/kg (n=12)
b. Concentration(s) measured:
c. Recovery (%): 41.9 +/- 3.5 % in M//41.6 +/- 1.8 % in K (n=12)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 100 µg/kg in M//600 µg/kg in K (n=12)
b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

c. RepeatabilityBetweenlabCV: 8.33 % in M//4.31 % in K

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other tetracyclines and their 4-epimer for oxytetracycline, chlortetracycline and tetracycline

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

A. Descriptive information

1. Name of drug or chemical: **ENROFLOXACIN**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Enrofloxacin + Ciprofloxacin
5. Intended use of the method: Confirmatory
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Phosphate buffer pH 7.4//Homogenization//Centrifugation//Filtration of the supernatant

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

SPE clean-up on Bond-Elut C18 cartridge eluting with trifluoroacetic acid 1% in ACN followed by pure ACN//Dry under

nitrogen
flow//Recover with ACN and ultra-pure water for injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MSMS : HP1100 and PE-SCIEX API2000

2. DetectorSystem/Reagents/Organism:
apci MSMS with 2 transitions (one precurseur with two products) monitored (positive mode)

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

3. Column/Special equipment:
Symmetry C18 (150x3.9mm; 5µm) + Guard column Waters (20x3.9mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg

1.c. Method sensitivity:

2. JECFA MRL: Evaluation of residues postponed (48th meeting - Feb 1997)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?

A 4 levels calibration curve from fortified muscle samples

5. Accuracy

   a. Concentration(s) tested: 30//50//100//200 µg/kg
   b. Concentration(s) measured: 29.4//48.7//103.0//198.9 µg/kg
   c. Recovery (%):

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 30//50//100//200 µg/kg
   b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

   c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure: Good selectivity towards other quinolones

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

A. Descriptive information

1. Name of drug or chemical: **ENROFLOXACIN**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Enrofloxacin + Ciprofloxacin

5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Homogenization//Weighing 0.5 grams of tissue as a test portion

8. Summary of principal steps in extraction procedure:

Extraction of the quinolone residues in poultry meat by addition of a solution containing acetonitrile-pH 9.18 Tetraborate buffer//Ultrasonic pulverisation//Centrifugation// Transfer of the supernatant

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

Filtration before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : Alliance pump autosampler device

2. DetectorSystem/Reagents/Organism:

TSP Fluorimetric Detector FL3000 set at exc 280 nm and em 450 nm

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

3. Column/Special equipment:
PLRP-S (150x4.6mm;5µm;100A) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Avoid contact with glassware at neutral pH as quinolones are chelating agents to divalent ions//Quinolones
are light sensitive compounds

12. Literature references available:

13. Contact for information:

a. Name: Yorke, Jean Christophe
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: jc.yorke@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 1 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: Evaluation of residues postponed (48th meeting)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 5 level external standard calibration and 1 MRL level fortified muscle sample

5. Accuracy
a. Concentration(s) tested: 15 µg/kg (n=58)
b. Concentration(s) measured:
c. Recovery (%): 77 +/- 11 % (n=58)
6. Precision using fortified control tissue:
a. Concentration(s) tested: 15 µg/kg (n=12)

b.RepeatabilityWithinlabCV: 10.7 %

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

c. RepeatabilityBetweenlabCV: 12.7 % (n=3x4days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus sarafloxacin, ciprofloxacin and difloxacin

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **FENBENDAZOLE**
2. Drug or chemical class: Benzimidazoles and pro-benzimidazoles
3. Veterinary use:
4. Analyte(s) measured (specified if metabolite):
5. Intended use of the method: Screening
6. Test matrix: milk
7. Summary of principal steps in sample preparation:

Thawing//Weighing of 1 mL of milk

8. Summary of principal steps in extraction procedure:

samples pH adjustment at pH 10.0 with sodium hydroxide//Extraction wit ethyl acetate//Centrifugation//Transfer of a fraction of the supernatant

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

Addition of ultrapure water//Centrifugation//Transfer of the organic phase//Evaporation under nitrogen stream at 50°C//Recover with a solution of 0.017M orthophosphoric acid / acetonitrile (85/15;v/v)//Ultrasonicate before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : TSP Pump P4000//TSP Autosampler AS300

2. DetectorSystem/Reagents/Organism:

UV detector set at 287 nm

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

3. Column/Special equipment:

Inertsil ODS3 desactivated (150x4.6mm;5µm) and a guard column Inertsil ODS3 (10x3mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Take care of possible oxydation of the benzimidazoles when not sufficiently controlling ther extraction-purification steps

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg):
1.b. Limit of quantification (LOQ) (mg/kg):
1.c. Method sensitivity:
2. JECFA MRL: 100 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy

a. Concentration(s) tested: 100 µg/kg (n=14)
b. Concentration(s) measured:
c. Recovery (%): 42.3 +/- 6.2 % (n=14)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 100 µg/kg (n=14)
b.RepeatabilityWithinlabCV:

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

c. RepeatabilityBetweenlabCV: 14.6 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

A. Descriptive information

1. Name of drug or chemical: **FLUMEQUINE**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Flumequine
5. Intended use of the method: Confirmatory
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Phosphate buffer pH 7.4//Homogenization//Centrifugation//Filtration of the supernatant

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

SPE clean-up on Bond-Elut C18 cartridge eluting with trifluoroacetic acid 1% in ACN followed by pure ACN//Dry under nitrogen flow//Recover with ACN and ultra-pure water for injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MSMS : HP1100 and PE-SCIEX API2000

2. DetectorSystem/Reagents/Organism:
apci MSMS with 2 transitions (one precurseur with two products) monitored (positive mode)

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

3. Column/Special equipment:
Symmetry C18 (150x3.9mm; 5µm) + Guard column Waters (20x3.9mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 500 µg/kg (54th meeting - Feb 2000)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 levels calibration curve from fortified muscle samples

5. Accuracy

   a. Concentration(s) tested: 15//30//50//100 µg/kg
   b. Concentration(s) measured: 15.6//29.7//49.4//100.3 µg/kg
   c. Recovery (%):

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 15//30//50//100 µg/kg
   b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

   c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure:

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of 3 quinolones in fish muscle by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **FLUMEQUINE**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Flumequine

5. Intended use of the method: Screening

6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of ca 20 grams of tissue//Homogenization//Weighing 0.5 grams of tissue as a test portion

8. Summary of principal steps in extraction procedure:

Extraction of the quinolone residues in fish meat by acetonitrile-pH 9.1 Tris buffer mixing solution

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

Double purification with hexane before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : TSP Pump P4000//TSP Autosampler AS300

2. DetectorSystem/Reagents/Organism:
Fluorimeter set at exc320 nm and em 380 nm for oxolinic acid

Name of the method: **Determination of 3 quinolones in fish muscle by HPLC/FLD**

3. Column/Special equipment:

PLRP-S (150x4.6mm;5µm;100A) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Avoid contact with glassware at neutral pH//Light sensitive analytes

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 7 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 75 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 500 µg/kg in Trout (54th meeting - Feb 2000)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
4 level external standard calibration and 1 MRL level fortified muscle sample

5. Accuracy

a. Concentration(s) tested: 600 µg/kg (n=12)
b. Concentration(s) measured:
c. Recovery (%): 66.4 +/- 6.6 % (n=12)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 600 µg/kg (n=12)
b.RepeatabilityWithinlabCV: 5.5 %

Name of the method: **Determination of 3 quinolones in fish muscle by HPLC/FLD**

c. RepeatabilityBetweenlabCV: 10.7 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 3 aminoglycosides in porcine kidney by LC/MS**

A. Descriptive information

1. Name of drug or chemical: **GENTAMICINE**
2. Drug or chemical class: Aminoglycosides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Gentamicin C1, C1a, C2
5. Intended use of the method: Confirmatory
6. Test matrix: kidney

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

5% Trichloroacetic acid//Homogenization//Centrifugation//Filtration//Transfer of the supernatant for clean-up

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

SPE on 15 mg SCX cartridge//Elution with 0.05M sodium hydroxide solution//Transfer in Trichloroacetic acid 50% solution//Ultra-speed Centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MS : HP1050 and Finnigan SSQ7000

2. DetectorSystem/Reagents/Organism:
esi MS with 4 ions monitored (positive mode)

Name of the method: **Confirmatory method for 3 aminoglycosides in porcine kidney by LC/MS**

3. Column/Special equipment:
RP18e (125x4mm; 5µm) + Guard column RP18e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): <500 ug/kg

1.b. Limit of quantification (LOQ) (mg/kg): 500 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 5000 µg/kg (52nd meeting - Feb 1999)
3. Is analytical data corrected for recovery?
4. How is recoverey estimated?

5. Accuracy

a. Concentration(s) tested: 500//1000//1500//2000 µg/kg
b. Concentration(s) measured:
c. Recovery (%):

6. Precision using fortified control tissue:

a. Concentration(s) tested: 500//1000//1500//2000 µg/kg
b.RepeatabilityWithinlabCV:

Name of the method by: **Confirmatory method for 3 aminoglycosides in porcine kidney LC/MS**

c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Good selectivity towards neomycin and paromomycin

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):

6. Literature references or other useful

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **IVERMECTIN**
2. Drug or chemical class: Avermectines
3. Veterinary use: Anthelmintics
4. Analyte(s) measured (specified if metabolite): Ivermectin H2B1a
5. Intended use of the method: Confirmatory
6. Test matrix: liver
7. Summary of principal steps in sample preparation:

Thawing//Weighing of 20 grams of liver//Homogenization//Weighing of 1 gram of homogenized liver

8. Summary of principal steps in extraction procedure:

Extraction with methanol/acetonitrile//Ultrasonication//Centrifugation//Transfer the supernatant//Evaporation under nitrogen stream at 60°C

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

Adjusting with acetonitrile and addition of ultra-pure water//Purification on C18 SPE cartridge 100mg by eluting with acetonitrile/water (90/10;v/v)//Centrifugation of the eluate//Evaporation under nitrogen stream at 60°C//For derivatization recover the dried residue with N-methylimidazole//Addition of trifluoroacetic acid before injection (caution: derivative is light sensitive)

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : TSP Pump P4000//Autosampler model AS300 with 20µl loop//Data station TSP PC1000

2. DetectorSystem/Reagents/Organism:

Fluorescence detector model Jasco 821-FP set at exc 361 nm and em 465 nm

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

3. Column/Special equipment:
Licrospher 100, RP18-e (125x4mm;5µm) with guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
anhydric conditions for derivatization//caution: fluorescent derivatives of avermectins and moxidectin are
light sensitive - Take care avoiding light before injecting within 8 hours after derivatization

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 2.5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg
1.c. Method sensitivity:
2. JECFA MRL:
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 4 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy
a. Concentration(s) tested: 100 µg/kg (n=6)
b. Concentration(s) measured:
c. Recovery (%): 78.8 +/- 3.3 % (n=6)

6. Precision using fortified control tissue:
a. Concentration(s) tested: 100 µg/kg (n=6)
b.RepeatabilityWithinlabCV: 4.8 %

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

c. RepeatabilityBetweenlabCV: 4.8 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of ivermectin residues in milk by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **IVERMECTIN**
2. Drug or chemical class: Avermectines
3. Veterinary use: Anthelmintics
4. Analyte(s) measured (specified if metabolite): Ivermectin H2B1a

5. Intended use of the method: Screening
6. Test matrix: milk

7. Summary of principal steps in sample preparation:

Thawing//Weighing of 1 mL of milk

8. Summary of principal steps in extraction procedure:

Extraction with methanol/water/acetonitrile//Centrifugation//Transfer the supernatant avoiding pieces of fat

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

Purification on SPE cartridge C18 100mg by eluting with methanol//Evaporation of methanol at 60°C//Recover the dried residue

with N-methylimidazole//Addition of trifluoroacetic acid for derivatization before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : TSP Pump P4000//manual injection on a 50µL loop//Data station TSP PC1000

2. DetectorSystem/Reagents/Organism:

Fluorescence detector model Jasco 820-FP set at exc 365 nm and em 475 nm

Name of the method: **Determination of ivermectin residues in milk by HPLC/FLD**

3. Column/Special equipment:

Licrospher 100, RP18-e (125x4mm;5µm) with guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

anhydric conditions for derivatization//caution: fluorescent derivatives of avermectins and moxidectin are

light sensitive - Take care avoiding light before injecting within 8 hours after derivatization

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 0.5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 1 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 10 µg/kg (54th meeting - Feb 2000)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 5 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy
   a. Concentration(s) tested: 4 µg/kg (n=6)
   b. Concentration(s) measured:
   c. Recovery (%): 77.7 +/- 6.5 % (n=6)
6. Precision using fortified control tissue:
   a. Concentration(s) tested: 4 µg/kg (n=6)
   b.RepeatabilityWithinlabCV: 5.7 %

Name of the method: **Determination of ivermectin residues in milk by HPLC/FLD**

   c. RepeatabilityBetweenlabCV: 11.5 %
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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure:

   b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **MOXIDECTIN**
2. Drug or chemical class: Avermectines
3. Veterinary use: Anthelmintics
4. Analyte(s) measured (specified if metabolite): Moxidectin
5. Intended use of the method: Confirmatory
6. Test matrix: liver

7. Summary of principal steps in sample preparation:

Thawing//Weighing of 20 grams of liver//Homogenization//Weighing of 1 gram of homogenized liver

8. Summary of principal steps in extraction procedure:

Extraction with methanol/acetonitrile//Ultrasonication//Centrifugation//Transfer the supernatant//Evaporation under nitrogen stream at 60°C

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

Adjusting with acetonitrile and addition of ultra-pure water//Purification on C18 SPE cartridge 100mg by eluting with acetonitrile/water (90/10;v/v)//Centrifugation of the eluate//Evaporation under nitrogen stream at 60°C//For derivatization recover the dried residue with N-methylimidazole//Addition of trifluoroacetic acid before injection (caution: derivative is light sensitive)

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : TSP Pump P4000//Autosampler model AS300 with 20µl loop//Data station TSP
PC1000

2. DetectorSystem/Reagents/Organism:
Fluorescence detector model Jasco 821-FP set at exc 361 nm and em 465 nm

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

3. Column/Special equipment:
Licrospher 100, RP18-e (125x4mm;5µm) with guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
anhydric conditions for derivatization//caution: fluorescent derivatives of avermectins and moxidectin are
light sensitive - Take care avoiding light before injecting within 8 hours after derivatization

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 0.8 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 100 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?

A 4 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy

a. Concentration(s) tested: 100 µg/kg (n=6)
b. Concentration(s) measured:

c. Recovery (%): 79.7 +/- 6.5 % (n=6)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 100 µg/kg (n=6)
b.RepeatabilityWithinlabCV: 4.1 %

Name of the method: **Determination of avermectin and moxidectin residues in liver by HPLC/FLD**

c. RepeatabilityBetweenlabCV: 4.1%

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 3 aminoglycosides in porcine kidney by LC/MS**

A. Descriptive information

1. Name of drug or chemical: **NEOMYCINE**
2. Drug or chemical class: Aminoglycosides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Neomycin B (Framycetin)

5. Intended use of the method: Confirmatory
6. Test matrix: kidney
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

5% Trichloroacetic acid//Homogenization//Centrifugation//Filtration//Transfer of the supernatant for clean-up

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

SPE on 15 mg SCX cartridge//Elution with 0.05M sodium hydroxide solution//Transfer in Trichloroacetic acid 50% solution//Ultra-speed Centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MS : HP1050 and Finnigan SSQ7000

2. DetectorSystem/Reagents/Organism:
esi MS with 4 ions monitored (positive mode)

Name of the method by: **Confirmatory method for 3 aminoglycosides in porcine kidney**
LC/MS

3. Column/Special equipment:
RP18e (125x4mm; 5µm) + Guard column RP18e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): <500 ug/kg
1.b. Limit of quantification (LOQ) (mg/kg): 500 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 20000 µg/kg (52nd meeting - Feb 1999)
3. Is analytical data corrected for recovery?
4. How is recoverey estimated?

5. Accuracy

a. Concentration(s) tested: 500//1000//1500//2000 µg/kg
b. Concentration(s) measured:
c. Recovery (%):

6. Precision using fortified control tissue:

a. Concentration(s) tested: 500//1000//1500//2000 µg/kg
b.RepeatabilityWithinlabCV:

Name of the method by: **Confirmatory method for 3 aminoglycosides in porcine kidney LC/MS**

c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Good selectivity towards gentamicin and paromomycin

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of neomycin in milk by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **NEOMYCINE**
2. Drug or chemical class: Aminoglycosides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Neomycin B (Framycetin)
5. Intended use of the method: Confirmatory
6. Test matrix: milk
7. Summary of principal steps in sample preparation:

Thawing//Homogenizing//Pipeting of 1 ml of milk

8. Summary of principal steps in extraction procedure:

Precipitation of proteins with 20% trichloroacetic acid//Centrifugation//Transfer of the supernatant//Addition of the couter-ion solution (0.2M sodium pentanesulfonate in 1% acetic acid)//Injection on a silica coated column C18 and ion-pairing system and post-column derivatization with derivatizing reagent : orthophtalaldehyde(OPA), 2-mercaptoethanol and Brij-35

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

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : SpectraPhysics pump model P4000 with manual injection on a 50µL loop and
Integration on a SpectraPhysics model Chromjet

2. DetectorSystem/Reagents/Organism:
Fluorescence detector model Jasco 821-FP set at exc 340 nm and em 455 nm

Name of the method: **Determination of neomycin in milk by HPLC/FLD**

3. Column/Special equipment:
Licrospher 100, RP18-e (125x4mm;5µm) with guard column RP18-e (4x4mm)//post column reactor

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in ultra-pure water stored 2 month at +4°C and residues of neomycin in milk stable for 8 months at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 100 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 250 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 500 µg/kg (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level calibration with standards of neomycin prepared in milk extracts

5. Accuracy
   a. Concentration(s) tested: 3000 µg/kg (n=8)
   b. Concentration(s) measured:
   c. Recovery (%): 89.8 +/- 4.0 % (n=8)
6. Precision using fortified control tissue:
   a. Concentration(s) tested: 3000 µg/kg
   b.RepeatabilityWithinlabCV: 1.4 %

Name of the method: **Determination of neomycin in milk by HPLC/FLD**

   c. RepeatabilityBetweenlabCV: 4.3 %
7. Precision using tissue containing incurred drug residues:
   a. Concentration(s) tested: 3.170 ug/kg (n=60)
   b. Repeatability (within lab CV): 4.3%
   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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:
   a. Drugs of similar structure:
   b. Contaminants:
   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment: Post-column reactor and pump
4. Special reagent:
5. Reagent handling and safety concerns (if any):

6. Literature references or other useful

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **NEOSPIRAMYCIN**
2. Drug or chemical class: Macrolides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Spiramycin and Neospiramycin

5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Acetonitrile//Homogenization//Hexane//Homogenization//Centrifugation//Transfer of the supernatant for clean-up step

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

SPE clean-up on Bond-Elut C18 cartridge eluting with a 0.1M methanolic ammonium acetate solution into 0.01% trifluoroacetic acid solution//Evaporation of methanol under nitrogen stream at 60°C//Filtration or Ultraspeed centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : HP series 1050

2. DetectorSystem/Reagents/Organism:

UV detector HP1050 set at 232 nm for spira and neospira and at 287 nm for tilmicosin and tylosin

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

3. Column/Special equipment:
Inertsil ODS3, RP18-e (150x4mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored 2 month at -20°C and Residues of macrolides in muscle are
stable stored at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Gaugain-Juhel, Murielle
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: m.juhel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 25 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 100 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 200 µg/kg (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level fortified muscle samples calibration

5. Accuracy
a. Concentration(s) tested: 100//200//400//800 µg/kg (n=72)
b. Concentration(s) measured:
c. Recovery (%): 42 +/- 6 % (n=72)

6. Precision using fortified control tissue:
a. Concentration(s) tested: 200 µg/kg (n=18)
b.RepeatabilityWithinlabCV: 9.4 %

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

c. RepeatabilityBetweenlabCV: 15.7 % (n= 3 x 6 days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other macrolides (spiramycin, tylosin, tilmicosin)

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **OXFENDAZOLE**
2. Drug or chemical class: Benzimidazoles and pro-benzimidazoles
3. Veterinary use: Anthelmintics
4. Analyte(s) measured (specified if metabolite): fenbendazole, oxfendazole and oxfendazole sulfone expressed as oxfendazole sulfone equivalents
5. Intended use of the method: Screening
6. Test matrix: milk

7. Summary of principal steps in sample preparation:

Thawing//Weighing of 1 mL of milk

8. Summary of principal steps in extraction procedure:

samples pH adjustment at pH 10.0 with sodium hydroxide//Extraction wit ethyl acetate//Centrifugation//Transfer of a fraction of
the supernatant

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

Addition of ultrapure water//Centrifugation//Transfer of the organic phase//Evaporation under nitrogen stream at 50°C//Recover with a solution of 0.017M orthophosphoric acid / acetonitrile (85/15;v/v)//Ultrasonicate before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : TSP Pump P4000//TSP Autosampler AS300

2. DetectorSystem/Reagents/Organism:
UV detector set at 287 nm

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

3. Column/Special equipment:
Inertsil ODS3 desactivated (150x4.6mm;5µm) and a guard column Inertsil ODS3 (10x3mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Take care of possible oxydation of the benzimidazoles when not sufficiently controlling ther extraction-purification steps

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte

b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments
vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite:
http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg):

1.b. Limit of quantification (LOQ) (mg/kg):

1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy

a. Concentration(s) tested: 100 µg/kg (n=14)
b. Concentration(s) measured:

c. Recovery (%): 42.3 +/- 6.2 % (n=14)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 100 µg/kg (n=14)
b.RepeatabilityWithinlabCV:

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

c. RepeatabilityBetweenlabCV: 14.6 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of 3 quinolones in fish muscle by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **OXOLINIC ACID**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Oxolinic acid
5. Intended use of the method: Screening
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of ca 20 grams of tissue//Homogenization//Weighing 0.5 grams of tissue as a test portion

8. Summary of principal steps in extraction procedure:

Extraction of the quinolone residues in fish meat by acetonitrile-pH 9.1 Tris buffer mixing solution

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

Double purification with hexane before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : TSP Pump P4000//TSP Autosampler AS300

2. DetectorSystem/Reagents/Organism:
Fluorimeter set at exc320 nm and em 380 nm for oxolinic acid

Name of the method: **Determination of 3 quinolones in fish muscle by HPLC/FLD**

3. Column/Special equipment:
PLRP-S (150x4.6mm;5µm;100A) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Avoid contact with glassware at neutral pH//Light sensitive analytes

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 75 µg/kg
1.c. Method sensitivity:
2. JECFA MRL:
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?

4 level external standard calibration and 1 MRL level fortified muscle sample

5. Accuracy

a. Concentration(s) tested: 300 µg/kg (n=12)
b. Concentration(s) measured:

c. Recovery (%): 67.7 +/- 5.8 % (n=12)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 300 ug/kg
b.RepeatabilityWithinlabCV: 8,1%

Name of the method: **Determination of 3 quinolones in fish muscle by HPLC/FLD**

c. RepeatabilityBetweenlabCV: 8,7%

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **OXYTETRACYCLINE**
2. Drug or chemical class: Tetracyclines
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Oxytetracycline//4-epitetracycline

5. Intended use of the method: Confirmatory
6. Test matrix: muscle//kidney
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Mac Ilvaine/EDTA buffer//Homogenization//Centrifugation//Transfer of the supernatant for clean-up step

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

Deproteinization with Trichloroacetic acid//SPE clean-up on Bond-Elut C18 cartridge eluting with a 0.01M oxalic acid methanolic solution followed by ultra-pure water//Ultraspeed centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : HP pump series 1050 and autosampler series 1100

2. DetectorSystem/Reagents/Organism:
UV detector HP1050 set at 355 nm

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

3. Column/Special equipment:
Purospher RP18-e (125x4mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

Stocked standard solutions in methanol stored 1 month at -20°C and Residues of tetracyclines in muscle and kidney are stable stored at -20°C. Tissues must be thawed just before the analysis

12. Literature references available:

13. Contact for information:

a. Name: Gaugain-Juhel, Murielle
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: m.juhel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 8 µg/kg in muscle//80 µg/kg in kidney
1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg in muscle//300 µg/kg in kidney
1.c. Method sensitivity:
2. JECFA MRL: 100 µg/kg in Muscle//600 µg/kg in Kidney (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration and 1 MRL level fortified muscle or kidney sample

5. Accuracy
   a. Concentration(s) tested: 100 µg/kg in Muscle (n=12)//600 µg/kg in Kidney (n=12)
   b. Concentration(s) measured:
   c. Recovery (%): 64.3 +/- 3.7 % in Muscle//64.0 +/- 2.2 % in Kidney (n=12)
6. Precision using fortified control tissue:
   a. Concentration(s) tested: 100 µg/kg in Muscle//600 µg/kg in Kidney (n=12)
   b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

   c. RepeatabilityBetweenlabCV: 5.75 % in Muscle//3.50 % in Kidney
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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other tetracyclines and their 4-epimer for oxytetracycline, chlortetracycline and tetracycline

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of tetracycline residues in pork muscle by LC/MS (ESI)**

A. Descriptive information

1. Name of drug or chemical: **OXYTETRACYCLINE**
2. Drug or chemical class: Tetracyclines
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Oxytetracycline//4-epitetracycline
5. Intended use of the method: Confirmatory
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Mac Ilvaine/EDTA buffer//Centrifugation//Transfer of the supernatant

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

Protein precipitation with TCA//SPE clean-up on Bond-Elut C18 cartridge eluting with a mixture of methanol-2% oxalic acid followed by ultra-pure water before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MS : HP1050 and Finnigan SSQ7000

2. DetectorSystem/Reagents/Organism:
esi MS with 4 ions monitored-positive mode

Name of the method: **Determination of tetracycline residues in pork muscle by LC/MS (ESI)**

3. Column/Special equipment:
Symmetry C18 (150x3.9mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored for 1 month at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Hurtaud-Pessel, Dominique
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: d.pessel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 35 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg

1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?

A 4 levels calibration curve from fortified muscle samples

5. Accuracy

   a. Concentration(s) tested: 100 µg/kg (n=5)
   b. Concentration(s) measured: 99.88 µg/kg (n=5)
   c. Recovery (%): 57.1 +/- 6.4 % (n=8)

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 50//100//150//200 µg/kg (n=8)
   b.RepeatabilityWithinlabCV: 8.9 % for n=5 samples of 100 µg/kg

Name of the method: **Determination of tetracycline residues in pork muscle by LC/MS (ESI)**

   c. RepeatabilityBetweenlabCV: 6.0//3.8//6.2//2.9 % (n=8)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure: Good selectivity checked towards tetracycline, 4 epitetracycline, chlortetracycline, 4 epichlortetracycline and doxycycline
   b. Contaminants:
   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

A. Descriptive information

1. Name of drug or chemical: **SARAFLOXACIN**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Sarafloxacin

5. Intended use of the method: Confirmatory

6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Phosphate buffer pH 7.4//Homogenization//Centrifugation//Filtration of the supernatant

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

SPE clean-up on Bond-Elut C18 cartridge eluting with trifluoroacetic acid 1% in ACN followed by pure ACN//Dry under nitrogen flow//Recover with ACN and ultra-pure water for injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MSMS : HP1100 and PE-SCIEX API2000

2. DetectorSystem/Reagents/Organism:
apci MSMS with 2 transitions (one precurseur with two products) monitored (positive mode)

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

3. Column/Special equipment:
Symmetry C18 (150x3.9mm; 5µm) + Guard column Waters (20x3.9mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 5 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 7.5 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 10 µg/kg (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 levels calibration curve from fortified muscle samples

5. Accuracy
   a. Concentration(s) tested:
   b. Concentration(s) measured:
   c. Recovery (%):
6. Precision using fortified control tissue:
   a. Concentration(s) tested:
   b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for 10 quinolones in poultry muscle by LC/MSMS**

c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

    a. Drugs of similar structure: Good seletivity towards other quinolone

    b. Contaminants:

    c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: Determination of 3 quinolones in fish muscle by HPLC/FLD

A. Descriptive information

1. Name of drug or chemical: **SARAFLOXACIN**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Sarafloxacin

5. Intended use of the method: Screening
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of ca 20 grams of tissue//Homogenization//Weighing 0.5 grams of tissue as a test portion

8. Summary of principal steps in extraction procedure:

Extraction of the quinolone residues in fish meat by acetonitrile-pH 9.1 Tris buffer mixing solution

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

Double purification with hexane before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : TSP Pump P4000//TSP Autosampler AS300

2. DetectorSystem/Reagents/Organism:

Fluorimeter set at exc320 nm and em 380 nm for oxolinic acid

Name of the method: **Determination of 3 quinolones in fish muscle by HPLC/FLD**

3. Column/Special equipment:

PLRP-S (150x4.6mm;5µm;100A) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

Avoid contact with glassware at neutral pH//Light sensitive analytes

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 2 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 15 µg/kg

1.c. Method sensitivity:

2. JECFA MRL:

3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?

4 level external standard calibration and 1 MRL level fortified muscle sample

5. Accuracy

   a. Concentration(s) tested: 30 µg/kg (n=12)
   b. Concentration(s) measured:
   c. Recovery (%): 58.2 +/-4.5 % (n=12)

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 30 µg/kg (n=12)
   b.RepeatabilityWithinlabCV: 7.9 %

Name of the method: **Determination of 3 quinolones in fish muscle by HPLC/FLD**

   c. RepeatabilityBetweenlabCV: 7.9 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure:

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

A. Descriptive information

1. Name of drug or chemical: **SARAFLOXACIN**
2. Drug or chemical class: Quinolones
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Sarafloxacin

5. Intended use of the method: Confirmatory

6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Homogenization//Weighing 0.5 grams of tissue as a test portion

8. Summary of principal steps in extraction procedure:

Extraction of the quinolone residues in poultry meat by addition of a solution containing acetonitrile-pH 9.18 Tetraborate buffer//Ultrasonic pulverisation//Centrifugation// Transfer of the supernatant

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

Filtration before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : Alliance pump autosampler device

2. DetectorSystem/Reagents/Organism:

TSP Fluorimetric Detector FL3000 set at exc 280 nm and em 450 nm

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

3. Column/Special equipment:

PLRP-S (150x4.6mm;5µm;100A) and a guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

Avoid contact with glassware at neutral pH as quinolones are chelating agents to divalent ions//Quinolones

are light sensitive compounds

12. Literature references available:

13. Contact for information:

a. Name: Yorke, Jean Christophe
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: jc.yorke@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 1 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 25 µg/kg
1.c. Method sensitivity:
2. JECFA MRL: 10 µg/kg in chicken and (50th meeting - Feb 1998)
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 5 level external standard calibration and 1 MRL level fortified muscle sample

5. Accuracy

a. Concentration(s) tested: 50 µg/kg (n=58)
b. Concentration(s) measured:
c. Recovery (%): 71+/- 10 % (n=58)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 50 µg/kg (n=12)
b.RepeatabilityWithinlabCV: 5.5 %

Name of the method: **Quantitative determination of 4 quinolones (ciprofloxacin-enrofloxacin-sarafloxacin-difloxacin) in chicken**

c. RepeatabilityBetweenlabCV: 9.8 % (n=3x4days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure: Selectivity checked versus enrofloxacin, ciprofloxacin and difloxacin

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **SPIRAMYCIN**
2. Drug or chemical class: Macrolides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Spiramycin and neospiramycin
5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Acetonitrile//Homogenization//Hexane//Homogenization//Centrifugation//Transfer of the supernatant for clean-up step

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

SPE clean-up on Bond-Elut C18 cartridge eluting with a 0.1M methanolic ammonium acetate solution into 0.01% trifluoroacetic acid solution//Evaporation of methanol under nitrogen stream at 60°C//Filtration or Ultraspeed centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : HP series 1050

2. DetectorSystem/Reagents/Organism:
UV detector HP1050 set at 232 nm for spira and neospira and at 287 nm for tilmicosin and tylosin

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

3. Column/Special equipment:
Inertsil ODS3, RP18-e (150x4mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored 2 month at -20°C and Residues of macrolides in muscle are
stable stored at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Gaugain-Juhel, Murielle
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments
vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: m.juhel@fougeres.afssa.fr WebSite:
http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 30 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 100 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 200 µg/kg (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level fortified muscle samples calibration

5. Accuracy

a. Concentration(s) tested: 100//200//400//800 µg/kg (n=72)
b. Concentration(s) measured:

c. Recovery (%): 51 +/- 7 % (n=72)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 200 µg/kg (n=18)
b.RepeatabilityWithinlabCV: 7.6 %

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

c. RepeatabilityBetweenlabCV: 15.1 % (n=3 x 6 days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other macrolides (neospiramycin, tylosin, tilmicosin)
b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):

6. Literature references or other useful

Name of the method: **Confirmatory method for streptomycin and dihydrostreptomycin in bovine muscle by LC/MS**

A. Descriptive information

1. Name of drug or chemical: **STREPTOMYCINE**
2. Drug or chemical class: Aminoglycosides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Streptomycin and Dihydrostreptomycin
5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

5% Trichloroacetic acid / EDTA//Homogenization//Centrifugation//Transfer of the supernatant in 2M ammonium acetate for injection

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

No clean-up

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

LC/MS : HP1050 and Finnigan SSQ7000

2. DetectorSystem/Reagents/Organism:
esi MS with 4 ions monitored (positive mode)

Name of the method: **Confirmatory method for streptomycin and dihydrostreptomycin in bovine muscle by LC/MS**

3. Column/Special equipment:
RP18e (125x4mm; 5µm) + Guard column RP18e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.delepine@fougeres.afssa.fr WebSite: Error! Hyperlink reference not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 117 µg/kg (n=5)
1.b. Limit of quantification (LOQ) (mg/kg): 250 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 600 µg/kg (52nd meeting - Feb 1999)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 levels calibration curve from fortified muscle samples

5. Accuracy

   a. Concentration(s) tested: 250//500//750//1000 µg/kg
   b. Concentration(s) measured:

   c. Recovery (%): 45.7 +/- 6.1 % (n=5)

6. Precision using fortified control tissue:

   a. Concentration(s) tested: 250//500//750//1000 µg/kg
   b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for streptomycin and dihydrostreptomycin in bovine muscle by LC/MS**

   c. RepeatabilityBetweenlabCV:

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure: Good selectivity towards dihydrostreptomycin

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of 5 sulfonamides in milk by HPLC/FLD**

A. Descriptive information

1. Name of drug or chemical: **SULFADIMIDINE (SULFADIMERAZINE-SULFAMETHAZINE)**
2. Drug or chemical class: Sulfonamides
3. Veterinary use: Antiinfectives
4. Analyte(s) measured (specified if metabolite): Sulfadimidine

5. Intended use of the method: Confirmatory

6. Test matrix: milk

7. Summary of principal steps in sample preparation:

Thawing//Weighing of 20 mL of raw milk//Centrifugation//Weighing of 1 mL of decreamed milk

8. Summary of principal steps in extraction procedure:

Extraction with chlorhydric acid//Centrifugation//Transfer 0.5 mL of the supernatant//Addition of Internal Standard (Sulfameter
or Sulfanilamide)

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

Pre-column derivatization with 0.02% fluorescamine in acetone and buffer pH 3 of 3M chlorhydric acid/3M sodium acetate (15/20;v/v) and 1.25 M sodium acetate

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : TSP Pump P4000//TSP Autosampler AS300

2. DetectorSystem/Reagents/Organism:
Fluorescence detector model Jasco 821-FP set at exc 405 nm and em 495 nm

Name of the **Determination of 5 sulfonamides in milk by HPLC/FLD**

method:

3. Column/Special equipment:
Licrospher 100, RP18-e (125x4mm;5µm) with guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 10 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg
1.c. Method sensitivity:
2. JECFA MRL:
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 4 level internal standard calibration and 1 muscle sample fortified with sulfadimidine at the MRL level

5. Accuracy
a. Concentration(s) tested: 100 µg/kg (n=8)
b. Concentration(s) measured:
c. Recovery (%): 93.2 +/- 3.0 (n=8)

6. Precision using fortified control tissue:
a. Concentration(s) tested: 100 ug/kg
b.RepeatabilityWithinlabCV: 2.6%

Name of the method: **Determination of 5 sulfonamides in milk by HPLC/FLD**

c. RepeatabilityBetweenlabCV: 3.3%

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

   a. Drugs of similar structure:

   b. Contaminants:

   c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of sulfonamide residues in muscle by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **SULFADIMIDINE (SULFADIMERAZINE-SULFAMETHAZINE)**
2. Drug or chemical class: Sulfonamides
3. Veterinary use: Antiinfectives

4. Analyte(s) measured (specified if metabolite): Sulfadimidine

5. Intended use of the method: Confirmatory

6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Weighing of 20 grams of tissue//Grinding//Weighing of 5 grams of ground tissue

8. Summary of principal steps in extraction procedure:

Double extraction by dichloromethane//Transfer of the organic phases//Addition of n-hexane

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

SPE on Silica Sep Pak cartridge with elution by 0.05M dipotassium phosphate//Eluate pH adjusted to 7.0 with 0.1M sodium hydroxide//Addition of ethyl acetate//Centrifugation//Transfer of the organic phase for rotary evaporation at 30°C//Recover the residue with a solution of acetonitrile/0.01M ammonium acetate (14/86;v/v) for injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : SpectraPhysics pump model SP8700 with manual injection on a 50µL loop and

Integration on a SpectraPhysics model SP4290

2. DetectorSystem/Reagents/Organism:

UV detector Kratos model Spectroflow 773 set at 254 nm

Name of the method: **Determination of sulfonamide residues in muscle by HPLC/UV**

3. Column/Special equipment:

Licrospher 100, RP18-e (125x4mm;5µm) with guard column RP18-e (4x4mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 10 ug/kg
1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg
1.c. Method sensitivity:
2. JECFA MRL:
3. Is analytical data corrected for recovery? yes
4. How is recoverey estimated?
A 4 level external standard calibration and 4 muscle samples fortified with sulfadimidine

5. Accuracy
   a. Concentration(s) tested: 100//500 µg/kg (n=6)
   b. Concentration(s) measured:
   c. Recovery (%): 81.0 +/- 6.4 %
6. Precision using fortified control tissue:
   a. Concentration(s) tested: 100 ug/kg (n=6)
   b.RepeatabilityWithinlabCV: 7.9%

Name of the method: **Determination of sulfonamide residues in muscle by HPLC/UV**

   c. RepeatabilityBetweenlabCV:
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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **TETRACYCLINE**
2. Drug or chemical class: Tetracyclines
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Tétracycline//4-epitetracycline
5. Intended use of the method: Confirmatory
6. Test matrix: muscle//kidney

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Mac Ilvaine/EDTA buffer//Homogenization//Centrifugation//Transfer of the supernatant for clean-up step

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

Deproteinization with Trichloroacetic acid//SPE clean-up on Bond-Elut C18 cartridge eluting with a 0.01M oxalic acid methanolic solution followed by ultra-pure water//Ultraspeed centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : HP pump series 1050 and autosampler series 1100

2. DetectorSystem/Reagents/Organism:
UV detector HP1050 set at 355 nm

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

3. Column/Special equipment:
Purospher RP18-e (125x4mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored 1 month at -20°C and Residues of tetracyclines in muscle and
kidney are stable stored at -20°C. Tissues must be thawed just before the analysis

12. Literature references available:

13. Contact for information:

a. Name: Gaugain-Juhel, Murielle
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: m.juhel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 9 µg/kg in muscle//60 µg/kg in kidney

1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg in muscle//300 µg/kg in kidney
1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg in M//600 µg/kg in K (47th meeting)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration and 1 MRL level fortified muscle or kidney sample

5. Accuracy

a. Concentration(s) tested: 100 µg/kg//600 µg/kg (n=12)
b. Concentration(s) measured:

c. Recovery (%): 59.7 +/- 3.9 % in M//63.7 +/- 1.8 % in K (n=12)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 100 µg/kg in M//600 µg/kg in K (n=12)
b.RepeatabilityWithinlabCV:

Name of the method: **Confirmatory method for 4 tetracyclines and their 4-epimers in muscle and kidney by HPLC/UV**

c. RepeatabilityBetweenlabCV: 6.54 % in M//2.88 % in K

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other tetracyclines and their 4-epimer for oxytetracycline, chlortetracycline and tetracycline
b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of tetracycline residues in pork muscle by LC/MS (ESI)**

A. Descriptive information

1. Name of drug or chemical: **TETRACYCLINE**
2. Drug or chemical class: Tetracyclines
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Tétracycline//4-epitetracycline
5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 2 grams of muscle tissue//Homogenising with ultra-pure water

8. Summary of principal steps in extraction procedure:

Mac Ilvaine/EDTA buffer//Centrifugation//Transfer of the supernatant

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

Protein precipitation with TCA//SPE clean-up on Bond-Elut C18 cartridge eluting with a mixture of methanol-2% oxalic acid followed by ultra-pure water before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
LC/MS : HP1050 and Finnigan SSQ7000

2. DetectorSystem/Reagents/Organism:
esi MS with 4 ions monitored-positive mode

Name of the method: **Determination of tetracycline residues in pork muscle by LC/MS (ESI)**

3. Column/Special equipment:
Symmetry C18 (150x3.9mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored for 1 month at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Hurtaud-Pessel, Dominique
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: d.pessel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 9 µg/kg (n=8)
1.b. Limit of quantification (LOQ) (mg/kg): 50 µg/kg (n=8)
1.c. Method sensitivity:
2. JECFA MRL: 100 µg/kg (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 levels calibration curve from fortified muscle samples

5. Accuracy
   a. Concentration(s) tested: 100 µg/kg (n=5)
   b. Concentration(s) measured: 93.47 µg/kg (n=5)
   c. Recovery (%): 66.8 +/- 7.4 % (n=8)
6. Precision using fortified control tissue:
   a. Concentration(s) tested: 50//100//150//200 µg/kg (n=8)
   b.RepeatabilityWithinlabCV: 8.5 % for n=5 samples of 100 µg/kg

Name of the **Determination of tetracycline residues in pork muscle by LC/MS**

method: **(ESI)**

c. RepeatabilityBetweenlabCV: 5.8//4.2//4.5//2.1 %

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Good selectivity checked towards oxytetracycline, 4 epitetracycline, chlortetracycline, 4 epitetracycline and doxycycline

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **THIABENDAZOLE**
2. Drug or chemical class: Benzimidazoles and pro-benzimidazoles
3. Veterinary use: Anthelmintic

4. Analyte(s) measured (specified if metabolite): Thiabendazole and its metabolite 5-hydroxythiabendazole

5. Intended use of the method: Screening

6. Test matrix: milk

7. Summary of principal steps in sample preparation:

Thawing//Weighing of 1 mL of milk

8. Summary of principal steps in extraction procedure:

samples pH adjustment at pH 10.0 with sodium hydroxide//Extraction wit ethyl acetate//Centrifugation//Transfer of a fraction of the supernatant

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

Addition of ultrapure water//Centrifugation//Transfer of the organic phase//Evaporation under nitrogen stream at 50°C//Recover with a solution of 0.017M orthophosphoric acid / acetonitrile (85/15;v/v)//Ultrasonicate before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : TSP Pump P4000//TSP Autosampler AS300

2. DetectorSystem/Reagents/Organism:

UV detector set at 287 nm

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

3. Column/Special equipment:

Inertsil ODS3 desactivated (150x4.6mm;5µm) and a guard column Inertsil ODS3 (10x3mm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Take care of possible oxydation of the benzimidazoles when not sufficiently controlling ther extraction-purification steps

12. Literature references available:

13. Contact for information:

a. Name: Roudaut, Brigitte
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: b.roudaut@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 10 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg):
1.c. Method sensitivity:
2. JECFA MRL: 100 µg/kg (48th meeting - Feb 1997)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level external standard calibration with a fortified muscle samples at the MRL level

5. Accuracy
   a. Concentration(s) tested: 100 µg/kg (n=14)
   b. Concentration(s) measured:
   c. Recovery (%): 69.4+/-5.3 % for thia//41.2+/-3.9 % for 5-hydroxy
6. Precision using fortified control tissue:
   a. Concentration(s) tested: 100 µg/kg (n=14)
   b.RepeatabilityWithinlabCV:

Name of the method: **Screening method for benzimidazoles in milk by HPLC/UV**

   c. RepeatabilityBetweenlabCV: 6.4 % for thia// 9.5 % for 5-hydroxy
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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure:

b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **TILMICOSIN**
2. Drug or chemical class: Macrolides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Tlimicosin

5. Intended use of the method: Confirmatory
6. Test matrix: muscle

7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Acetonitrile//Homogenization//Hexane//Homogenization//Centrifugation//Transfer of the supernatant for clean-up step

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

SPE clean-up on Bond-Elut C18 cartridge eluting with a 0.1M methanolic ammonium acetate solution into 0.01% trifluoroacetic acid solution//Evaporation of methanol under nitrogen stream at 60°C//Filtration or Ultraspeed centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:
HPLC apparatus : HP series 1050

2. DetectorSystem/Reagents/Organism:
UV detector HP1050 set at 232 nm for spira and neospira and at 287 nm for tilmicosin and tylosin

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

3. Column/Special equipment:
Inertsil ODS3, RP18-e (150x4mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored 2 month at -20°C and Residues of macrolides in muscle are
stable stored at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Gaugain-Juhel, Murielle
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments
vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: m.juhel@fougeres.afssa.fr WebSite:
http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 15 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 25 µg/kg
1.c. Method sensitivity:

2. JECFA MRL: 100 µg/kg (47th meeting - Jun 1996)
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level fortified muscle samples calibration

5. Accuracy

a. Concentration(s) tested: 25//50//100//200 µg/kg (n=72)
b. Concentration(s) measured:

c. Recovery (%): 59.2 +/- 7.6 % (n=72)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 50 µg/kg (n=18)
b.RepeatabilityWithinlabCV: 12.1 %

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

c. RepeatabilityBetweenlabCV: 15.9 % (n=3 x 6 days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other macrolides (spiramycin, neospiramycin, tylosin)
b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

A. Descriptive information

1. Name of drug or chemical: **TYLOSIN**
2. Drug or chemical class: Macrolides
3. Veterinary use: Antimicrobial
4. Analyte(s) measured (specified if metabolite): Tylosin

5. Intended use of the method: Confirmatory
6. Test matrix: muscle
7. Summary of principal steps in sample preparation:

Thawing//Grinding//Weighing of 5 grams of tissue

8. Summary of principal steps in extraction procedure:

Acetonitrile//Homogenization//Hexane//Homogenization//Centrifugation//Transfer of the supernatant for clean-up step

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

SPE clean-up on Bond-Elut C18 cartridge eluting with a 0.1M methanolic ammonium acetate solution into 0.01% trifluoroacetic acid solution//Evaporation of methanol under nitrogen stream at 60°C//Filtration or Ultraspeed centrifugation before injection

10. Measurement procedure:

Nature: Chimique

1. Instrumentation/Technique:

HPLC apparatus : HP series 1050

2. DetectorSystem/Reagents/Organism:

UV detector HP1050 set at 232 nm for spira and neospira and at 287 nm for tilmicosin and tylosin

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

3. Column/Special equipment:
Inertsil ODS3, RP18-e (150x4mm;5µm)

4. Media:

11. Sample/Analyte stability warning (if applicable):
Stocked standard solutions in methanol stored 2 month at -20°C and Residues of macrolides in muscle are
stable stored at -20°C

12. Literature references available:

13. Contact for information:

a. Name: Gaugain-Juhel, Murielle
b. Country: France
c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
d. Address: Javené - BP90203 - 35302 FOUGERES
e.Telephone: 02 99 94 78 78 f. Fax: 02 99 94 78 80
g. Email: m.juhel@fougeres.afssa.fr WebSite: http://www.fougeres.afssa.fr/

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 15 µg/kg
1.b. Limit of quantification (LOQ) (mg/kg): 25 µg/kg
1.c. Method sensitivity:
2. JECFA MRL:
3. Is analytical data corrected for recovery? Yes
4. How is recoverey estimated?
A 4 level fortified muscle samples calibration

5. Accuracy

a. Concentration(s) tested: 25//50//100//200 µg/kg (n=72)
b. Concentration(s) measured:
c. Recovery (%): 63.2 +/- 5.9 % (n=72)

6. Precision using fortified control tissue:

a. Concentration(s) tested: 50 µg/kg (n=18)
b.RepeatabilityWithinlabCV: 4.8 %

Name of the method: **Determination of 4 macrolide residues (spiramycin, neospiramycin, tylosin and tilmicosin) in muscle by HPLC/UV**

c. RepeatabilityBetweenlabCV: 9.3 % (n=3 x 6 days)

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 measurment of the analyte of interest when other chemicals or drugs are also resident int he laboratory sample. Data of interest in this regard are the effects of:

a. Drugs of similar structure: Selectivity checked versus other macrolides (neospiramycin, spiramycin, tilmicosin)
b. Contaminants:

c. Type of validation studies: Single-laboratory

C. Information relevant to laboratory implementation

1. Training:
2. Critical Steps:
3. Information on Availibility of unusual reagents or equipment:
4. Special reagent:
5. Reagent handling and safety concerns (if any):
6. Literature references or other useful

THAILAND

A. Descriptive Information

1.Name of drug or chemical : Oxytetracycline, Tetracycline, Chlortetracycline
2.Drug or chemical class : Antibiotic

(e.g. antimicrobial, anthelmintic, etc)

3.Veterinary Use: -

4.Analyte(s) measured: Oxytetracycline hydrochloride, Tetracycline hydrochloride, (specify if metabolite) Chlortetracycline hydrochloride

5.Intended use of method

a. Screening -

b. Routine √

c. Reference -

d.Confirmatory -

6. **Test matrix** Shrimp
(e.g. muscle, kidney, urine,etc)

7. Summary of principal steps in sample preparation:

- Weight 5.00 ± 0.05 g of homogenized sample into 50 ml polypropylene centrifuge tube.

8. Summary of principal steps in extraction procedure

-Shake sample with Mc Ilvaine buffer 20 ml. Centrifuge at 3000 rpm 10 min and though filter paper Re-extracted 2 times with Mc Ilvaine buffer 20 and 10 ml.

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

- Condition each SPE-cartridge with 20 ml. methanol follow by water 20 ml. add sample extracted though SPE and then elute with methanolic oxolic acid 6 ml.

10. Measurement procedure

a.Chemical

1. Instrumentation HPLC

2. Detector system UV. 350 nm.

3. Chromatographic column Lichrosorb RP 18 5 micron
(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) Sample should be kept frozen until analyzed

12.Literature References available Cunniff P. Chlortetracycline, Oxytetracycline and Tetracycline in edible animal tissue, Liquid chromatography method AOAC 1995: p. 19-24 (March 1996, supplement)

13.Contact for information

a.Name Ms. Chanchai Jaengsawang

b.Country Thailand

c.Affiliation Department of Medical Sciences

d.Address 88/7 Moo 4 Tiwanon Road. Muang District Nonthaburi 11000

e.Telephone 662 951 1021

f.Fax -

g.E-mail -

B.Method Performance

1. a. Limit of Detection (LOD) (mg/kg) 0.01 mg/kg for Oxytetracycline and Tetracycline
0.02 mg/kg for Chlortetracycline

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

b. Limit of Quantification (LOQ) (mg/kg) 0.10 mg/kg for Oxytetracycline and Tetracycline
0.20 mg/kg for Chlortetracycline

How was LOQ determined? Using 10 x concentration of detection limit

c. Method sensitivity 0.01 mg/kg for Oxytetracycline and Tetracycline
0.02 mg/kg for Chlortetracycline

(The smallest difference in concentration that can be measured)

2. JECFA MRL 0.2 mg/kg

3. Is analytical data corrected for recovery? Yes - No √

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

5. Accuracy

	Oxytetracycline (mg/kg)			Oxytetracyclin(mg/kg)			Oxytetracyclin(mg/kg)		
a. Concentration(s) tested	0.10	0.20	0.40	0.10	0.20	0.40	0.20	0.40	0.60
b. Concentration measured	0.10	0.19	0.39	0.08	0.15	0.30	0.18	0.29	0.43
c. Recovery(%)	102	94	98	83	75	77	92	73	72

6. Precision using fortified control tissue

	Oxytetracycline (mg/kg)	Oxytetracycline(mg/kg)	Oxytetracycline(mg/kg)
a. Concentration(s) tested	0.10	0.10	0.20
b.Repeatability (wihtin lab CV)	7.38	1.80	9.49
c. Reproducibility (between lab CV)	-	-	-

7. Precision using tissue containing incurred drug residue

a.Concentration(s) tested -

b.Repeatability (wihtin 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 procedures √

C. Information relevant to laboratory implementation

1.Training and experience recommended for analyts : Analyst should train using HPLC

2.Critical steps in the method : Extract sample and Clean-up

3.Information on availability of unusual reagents or equipment:

HPLC: UV. Detector; 350 um , 0.005 AUFS : Fluorescence detector ; Ex380,
Em 540 nm.

4.Special reagent or sample stability concerns: Sample should be kept frozen until analyzed.

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

6.Literature reference or other useful information

- Cunniff P. Cholrtetracycline, oxytetracycline and tetracycline in edible animal tissue, Liquid chromatography method In official Methods of Analysis of AOAC International Maryland: AOAC International, 1995: 19-24 (March 1996, supplement)
- Kawata S, Kazuhiko S, Nishikawa Y and Iwama K. Liquid chromatographic Determination of Oxytetracycline in Swine Tissue-J of AOAC International, 1996 79 (No 6):1463-1465

OUTLINE OF SCIENTIFIC ISSUES COMMONLY CONSIDERED IN THE DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS

1. Determinative (Quantitative) Method

A. Purpose of the Method

* Scope of application (intended use)
  This method is applicable to determine Tetracyclines in animal tissue.
* Target tissue
  Shrimp
* Marker residue (analyte)
  Oxytetracycline, Tetracycline and Chlortetracycline
* Limit of quantification (LOQ), Limit of Detection (LOD) or other Lowest Validated Level
  LOQ 0.1 mg/kg for Oxytetracycline and Tetracycline, 0.2 mg/kg for Chlortetracycline.
  LOD 0.01 mg/kg for Oxytetracycline and Tetracycline, 0.02 mg/kg for Chlortetracycline.

B. Experimental data

*Reagents (purity, strength, grade)
Tetracyclines standard – Certified reference standards of chlortetracycline hydrochloride, oxytetracycline
hydrochloride and tetracycline hydrochloride.
Methanol , Acetonitrile and distilled water - HPLC grade.
Oxalic acid, Dibasic sodium phosphate, Citric acid and Disodium ethylenediaminetetraacetic acid – AR grade.

*Apparatus and Equipment
HPLC - UV detector and Fluorescence detector (for confirmation), Column:Lichosorb RP18, Eletronic Balance,
Centrifuge, Mechanical shaker, Vacuum manifold and SPE cartridge C18.

*Analytical Standards (quality, concentration and solvent)
Stock standard solution of oxytetracycline hydrochloride, tetracycline hydrochloride and chlotetracycline
hydrochloride 1000 µg/ml in methanol.

*Tissue Samples (procedure for preparation for analysis)
Weight 5.00 $\pm$ 0.05 g tissue sample into polypropylene centrifuge tube.

*Analyte Extraction Procedures
Shake with Mc Ilvaine buffer.

*Analyte Clean-up
SPE cartridge - C18 extraction

*Instrumental Procedures and Calibrations

HPLC detector: UV 350 nm;

Mobile phase: oxalic acid solution: acetonitrile: methanol (65:25:10)

Flowrate: 1.2 ml/min and Injection volume 60 µl.

System Suitability test by inject std. tetracyclines solution 0.25 µg/ml 5 time; retention time for replicate

injections of TC chromatographic should match within 0.05 min and RSD of peaks area should be less than 2%.

*Calculations

Calulation from standard curve : $y = mx + b$

where y = peak area, x = tetracycline concentration, m = slope of curve and b = intercept of y.

C. Quality Assurance

* Storage Stability of Analyte in Tissue

Tissue should be kept frozen until analyzed.

* Quality Control Samples

Control samples analyse every 5% of samples.

* System Suitability Criteria

SPE cartridges should provide $\geq$ 80% recovery for spiked tissue sample containing Tetracyclines at LOQ

LC system : Inject 60 µl of std. 0.25 µg/ml ,TC chromatographic standard solution produces 3 distinct peaks

and resolution between oxytetracycline and tetracycline or chlortetracycline peaks should $\geq$ 1.5.

* Readiness to perform assessment

Test accracy and precission by test linearity of calibration curve in the range of 0.05 - 1.00 µg/ml for

oxytetracycline and tetracycline, 0.10 - 2.00 µg/ml for chlortetracycline every month ; gave correlation

coefficient$\geq$ 0.995, and make standard control chart from std.Tetracyclines 0.25 µg/ml that inject for test

system, before sample injection, between and after sample injection.

* Data Acceptability Criteria

The data will be accept if recovery in the range of 60-115%.

2. Confirmation Procedure

*Sample preparation

Take 1 ml of sample solution that detected tetracyclines analyte from UV detector, remove methanol by gas

nitrogen and then dilute to 1 ml with mixture of 1M Imidazole 90 ml with Acetonitrile 10 ml.

*Instrumental procedures and calibrations

HPLC detector: Fluorescence Ex 380 Em 540 nm.

Mobile phase: ml 1 M Imidazole solution: acetonitrile (9:1)

Flowrate : 1.2 ml/min and Injection volume 60 µl.

System Suitability test by inject std. tetracyclines solution 0.25 µg/ml ≥ 3 time; retention time for replicate

injections of TC chromatographic should match within 0.05 min and RSD of peaks area should be less than

2%.

*Standards employed

Standards of chlortetracycline hydrochloride, oxytetracycline hydrochloride and tetracycline hydrochloride:

Sigma Chemical.

*Criteria for positive identification

When the retention time of sample peak same tetracyclines peak as detected from UV detector.

3. Validation considerations

*Accuracy

Oxytetracycline at 0.10 mg/kg CV = 7.38%, 0.20 mg/kg CV = 5.12%, 0.40 mg/kg CV = 2.46% (N=12)

Tetracycline at 0.10 mg/kg CV = 1.80%, 0.20 mg/kg CV = 2.84% , 0.40 mg/kg CV = 2.41% (N=12)

Chlortetracycline at 0.20 mg/kg CV = 9.49%, 0.40 mg/kg CV = 1.34%, 0.60 mg/kg CV = 2.91% (N=12)

*Recovery

Oxytetracycline at 0.10 mg/kg = 102%, 0.20 mg/kg = 95%, 0.40 mg/kg = 98% (N=12)

Tetracycline at 0.10 mg/kg = 83%, 0.20 mg/kg =76 %, 0.40 mg/kg = 78% (N=12)

Chlortetracycline at 0.20 mg/kg = 92%, 0.40 mg/kg = 73%, 0.60 mg/kg = 72% (N=12)

*Precision (repeatability and reproducibility)

Oxytetracycline at 0.10 mg/kg SD = 7.54 , 0.20 mg/kg SD = 4.85 , 0.40 mg/kg SD = 2.41 (N=12)

Tetracycline at 0.10 mg/kg SD = 1.51, 0.20 mg/kg SD = 2.15 , 0.40 mg/kg SD = 1.85 (N=12)

Chlortetracycline at 0.20 mg/kg SD = 8.72 , 0.40 mg/kg SD = 0.98, 0.60 mg/kg SD = 2.10 (N=12)

*Sensitivity and LOQ

Sensitivity (LOD) of oxytetracycline and tetracycline are 0.01 mg/kg, chlortetracycline is 0.02 mg/kg

LOQ of oxytetracycline and tetracycline are 0.10 mg/kg, chlortetracycline is 0.20 mg/kg

*Specificity

-