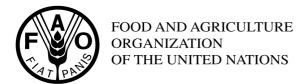
codex alimentarius commission





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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:

1110 0110 01	
a. Screening	Yes
b. Routine	
c. Reference	
d. Confirmatory	Yes
6. Test matrix (e.g. muscle, kidney, urine,	Liver etc)
7. Summary of principal steps Sample preparation:	n Homogenise sample with acetonitrile
8. Summary of principal steps extraction procedure:	n Acetonitrile extract is evaporated to dryness under vacuum residue dissolved in hexane/dichloromethane mix.
9. Summary of principal steps	
Analyte clean-up procedure	•
	imidazole/dimethyl formamide and derivatised mixture cleaned up by C-18 sep-pak
	C 10 sep pan
10. Measurement procedure:	
a. Chemical	HPLC
1. Instrumentation	
2. Detector system	Fluorescence wavelength – 360nm excitation and 468nm emission
3. Chromatographic column	Reverse phase C18 column
(if applicable)	
b. Immunochemical/Immuno	assay N/A
1. Technique:	
(e.g. Elisa, RIA,	
Immunochromatog, etc)	NT/A
2. Critical reagents:(e.g. antibody specificity and	N/A
Availability)	1
3. Special equipment require	ed: N/A
-	
c. Microbiological	N/A
 Technique: Organism: 	
2. Organism: 3. Media:	
4. Special equipment require	ed:
1 1 1 1	1

11. Sample/Analyte Stability Warning (if applicable):		
10 T: D 6	·	
12. Literature References Available:		
13. Contact for Information:		
a. Name	Terry Spencer	
b. Country	Australia	
c. Affiliation	AGAL ACT	
d. Address	Level 10, Allai ACT 2600	ra Street, Canberra,
e. Telephone	(+61) 2 6213 6	5102
f. FAX	(+61) 2 6213 6	
g. Email	Terry.spencer@a	gal.gov.au
B. Method Performance		
1. a. Limit of Detection (LOD) How was LOD determined		0.001mg/kg (Avermectin B1a)
b. Limit of Quantification (I How was LOQ determined?	~	0.005mg/kg (Avermectin B1a)
c. Method sensitivity (The smallest difference in concentration that can be measured)		N/A
	Fat (cattle): 0.1m ttle): 0.05mg/kg	
3. Is analytical data corrected f	For recovery?	Yes
4. How is recovery estimated (e.g. external standard; inter	rnal standard etc	External standard
5. Accuracy (Avermectin B1a)		
a. Concentration(s) tested		.002, Ovine: 0.01, Porcine: 0.005 mg/kg
b. Concentration(s) measure	ed	
c. Recovery (%)	Bovine: 8	2%, Ovine: 86%, Porcine: 73%

Bovine: 0.002, Ovine: 0.01, Porcine: 0.005 mg/kg
Bovine: 9.8%, Ovine: 3.5%, Porcine: 2%
0.005, 0.012, 0.034
19.6%, 10.8%, 11.2%
s "Specificity". Selectivity refers to the ability of the method to analyte of interest when other chemicals or drugs are also resident st in this regard are the effects of:
lass or used
present
]

B. Information relevant to laboratory implementation

1. Training and experience recommended for analytes		
2. Critical steps in the method		
3. Information on availability of Equipment	of unusual reagents or	
4. Special reagent or sample sta	ability concerns	
5. Reagent handling and safety	concerns (if any)	
6. Literature references or othe	r useful information	
C. Descriptive Information		
1. Name of drug or chemical:	Albendazole	
2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agent (Bo	enzimidazoles)
3. Veterinary Use:	Control of mature and lung worms, tape worm	immature gastrointestinal roundworms, large ns and liver flukes.
4. Analyte(s) measured:	Albendazole	
(specify if metabolite)	Alochdazoic	
5. Intended use of the method:		
a. Screening	Yes	
b. Routine		
c. Reference		
d. Confirmatory	Yes	
	T •	
6. Test matrix (e.g. muscle, kidney, urine,	etc) Liver	
		1 01 10 11 1
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/Immunoassa 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ay N/A	
Critical reagents:(e.g. antibody specificity and Availability)	N/A	
3. Special equipment required:	N/A	
c. Microbiological1. 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 Di	r Robert Symons	
· · ·	ustralia	
	MDEL 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	g/kg)	0.01
How was LOD determined?		
b. Limit of Quantification (LOQ) (mg/l	(g) 0.05
How was LOQ determined?		
c. Method sensitivity		
(The smallest difference in conc	entratio	on
that can be measured)		
2. JECFA MRL Muscle, fat and		
Liver and kidne	ey: 5.0r	ng/kg
3. Is analytical data corrected for re	covery	? Yes
4. How is recovery estimated		External standard
(e.g. external standard; internal s	standar	d etc.)
5 A a a a a a a a a a a a a a a a a a a		
5. Accuracy	0.05	0.1.05 - 2/1/2
a. Concentration(s) tested	0.05,	0.1, 0.5mg/kg
b. Concentration(s) measured		
b. Concentration(s) measured		
c. Recovery (%)	62%	76%, 80%
c. Recovery (70)	0270,	7070, 8070
	1	
6. Precision using fortified		
Control tissue		
		0.05, 0.1, 0.5mg/kg
3511511111111(5) (5)1515		, , 6 6
b. Repeatability (within lab CV) 14%		14%, 10%, 4%
, , , , , , , , , , , , , , , , , , , ,		
c. Reproducibility (between lab	CV)	
1	′	

7. Precision using tissue containing Incurred drug residues

a. Concentration(s) tested		
b. Repeatability (within lab CV)		
c. Reproducibility (between lab CV)		
	as "Specificity". Selectivity refers to the ability of the method to analyte of interest when other chemicals or drugs are also resident est in this regard are the effects of:	
a. Drugs of similar structure or drug cl veterinary drugs that may also be us along with the analyte of interest		
b. Contaminants that are likely to be p in the sample	present	
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 analytes	d for	
2. Critical steps in the method		
3. Information on availability of unusual Equipment	reagents or	
4. Special reagent or sample stability con	ncerns	

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		
1. Ivanie of drug of chemical.	тирии сурстисии и		
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 sample preparation:	in Subsample of fat rendered in microwave		
8. Summary of principal steps extraction procedure:	in 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. Chemical1. Instrumentation	GC
2. Detector system	ECD, NPD and MS
3. Chromatographic column (if applicable)	DB-1 or DB-5
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
Critical reagents: (e.g. antibody specificity and Availability)	N/A
3. Special equipment required:	N/A
c. Microbiological1. 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:

15. 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?			2			
c. Method sensitivity (The smallest difference in concentration that can be measured)						
2. JECFA MRL Muscle, liver and kidney (control of the state of the sta				kens): 0.0	01mg/kg	
3. Is analytical data corrected for re	covery?	Yes	}			
4. How is recovery estimated (e.g. external standard; internal s	tandard e		External standar	rd		
5. Accuracy	0.1 //					
a. Concentration(s) tested	0.1mg/k	g				
b. Concentration(s) measured						
c. Recovery (%)	68%					
6. Precision using fortified Control tissue						
a. Concentration(s) tested	0.	l mg/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 implemen	tation
1. Training and experience recommended for analytes	
2. Critical steps in the method	
3. Information on availability of unusual reagents of Equipment	or
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			
	1				
		Antibacterial agent (Beta-lactams)			
(e.g. antimicrobial,					
Anthelmintic, etc)					
3. Veterinary Use:	Bacte	rial antibiotic chiefly active against Gram-positive microorganisms.			
3. Vetermary esc.		May be used to treat respiratory tract, urinary tract and wound infections,			
		tis and streptococcal mastitis.			
	•	•			
4. Analyte(s) measured:	Penic	illin			
(specify if metabolite)					
5. Intended use of the					
method:					
a. Screening	Yes (5 plate MIT)			
u. Sereening	105 (.	s place MIT)			
b. Routine					
c. Reference					
d. Confirmatory	Yes (s (HPLC)			
6. Test matrix		Kidney			
(e.g. muscle, kidney, urine, etc)		Egg			
7. Summary of principal steps	in				
sample preparation:					
	.				
8. Summary of principal steps	in	Antimicrobials differentially extracted into three separate solutions			
extraction procedure:					
9. Summary of principal steps in		Cleaned up concentrated using SPE and solvent removed under			
Analyte clean-up procedure:		Vacuum. For confirmation derivitsed with 1,2,4-triazole-mercuric			
T P		chloride			
10. Measurement procedure:					
a. Chemical		Confirmation			
1. Instrumentation		HPLC			
		LW 205			
2. Detector system		UV at 325nm			

3. Chromatographic column		
(if applicable)		
1.7		
b. Immunochemical/Immuno	passay N/A	
1. Technique: (e.g. Elisa, RIA,	IN/A	
Immunochromatog, etc)		
2. Critical reagents:	N/A	
(e.g. antibody specificity ar	nd	
availability)		
3. Special equipment requir	red: N/A	
26: 1: 1: 1		
c. Microbiological	5 mloto	MIT (companing)
 Technique: Organism: 	3 plate	MIT (screening)
3. Media:		
4. Special equipment requir	red:	
11. Sample/Analyte Stability		
Warning (if applicable):		
	•	
12. Literature References		
Available:		
13. Contact for Information:		
a. Name	Mr Dennis Ha	milton
b. Country	Australia	
c. Affiliation	ARI (Chemica	al Residue Laboratory)
d. Address	665 Fairfield l	
Yeerongapilly		`
e. Telephone	(+61) 7 3362 9	
f. FAX	(+61) 7 3362 9 Hamiltondj@pro	
g. Email	нашпонијерго	sse.apr.qia.au
B. Method Performance		
b. Method I crioi mance		
1. a. Limit of Detection (LOD) (mg/kg)		0.01mg/kg
How was LOD determined?		
b. Limit of Quantification (LOQ) (mg/kg)		0.02mg/kg
How was LOQ determined	!	

c. Method sensitivity

i				
(The smallest difference in concentration that can be measured)		n e e e e e e e e e e e e e e e e e e e		
2. JECFA MRL Liver, kidney an Milk: 0.004mg/k		ele (all species): 0.05mg/kg		
3. Is analytical data corrected for rec	covery?	Yes Yes		
4. How is recovery estimated (e.g. external standard; internal st	tandard	External standard letc.)		
5. Accuracy				
a. Concentration(s) tested	0.08mg	g/kg		
b. Concentration(s) measured				
c. Recovery (%)	86%			
6. Precision using fortified Control tissue				
a. Concentration(s) tested	0	0.08mg/kg		
b. Repeatability (within lab CV)	8	8%		
c. Reproducibility (between lab C	CV)			
7. Precision using tissue containing Incurred drug residues				
a. Concentration(s) tested				
b. Repeatability (within lab CV)				
c. Reproducibility (between lab C	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 dr veterinary drugs that may also along with the analyte of inter-	be used			

b. Contaminants that are lik in the sample	ely to be present				
9. Type of Validation studies a. Single laboratory					
b. Multi-laboratory					
c. AOAC or other official p	rocedure				
C. Information relevant to la	boratory implementa	tion			
1. Training and experience rec	ommended for analyte				
2. Critical steps in the method	2. Critical steps in the method				
3. Information on availability of Equipment	of unusual reagents or				
4. Special reagent or sample st	ability concerns				
5. Reagent handling and safety	concerns (if any)				
6. Literature references or other useful information					
CHLORAMPHENICOL F. Descriptive Information	4				
1. Name of drug or chemical:	Chloramphenicol				
2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anabolic agent (Unau	thorised compound)			
3. Veterinary Use:	Chloramphenical is b	road 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				
b. Routine				
c. Reference				
d. Confirmatory	Yes			
6. Test matrix (e.g. muscle, kidney, urine	etc) Muscle			
7. Summary of principal step sample preparation:	in Fat removed and muscle homogenised			
8. Summary of principal step extraction procedure:	in Ethyl acetate extraction			
9. Summary of principal step Analyte clean-up procedur				
10. Measurement procedure:				
a. Chemical 1. Instrumentation	GC			
2. Detector system	ECD			
3. Chromatographic colun (if applicable)	SGE BP1 (screen) HP – 5MS (confirmation)			
b. Immunochemical/Immun 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	passay N/A			
2. Critical reagents: (e.g. antibody specificity a availability)	nd N/A			

3. Special equipment require	ed: N/A				
c. Microbiological N/A 1. Technique: 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	Terry Spencer				
b. Country	Australia				
c. Affiliation	AGAL ACT				
d. Address	Level 10, Allan	ra Street, Canberra,			
	ACT, 2600	,			
e. Telephone	(+61) 2 6213 6	5102			
f. FAX	(+61) 2 6213 6				
g. Email	Terry.spencer@ag				
B. Method Performance	5. 2				
1. a. Limit of Detection (LOD) How was LOD determined		0.0005 mg/kg			
b. Limit of Quantification (I How was LOQ determined?	OQ) (mg/kg)	0.001 mg/kg			
c. Method sensitivity (The smallest difference in c that can be measured)	oncentration				
2. JECFA MRL No MRLS set.					
3. Is analytical data corrected for	or recovery?	Yes			
4. How is recovery estimated (e.g. external standard; internal standard;	nal standard etc	Internal standard			

		19
5. Accuracy		
a. Concentration(s) tested		ng/kg ng/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 containin Incurred drug residues	g	
a. Concentration(s) tested		
b. Repeatability (within lab CV	()	
c. Reproducibility (between lab	CV)	
Provide accurate measurement in	of the	s "Specificity". Selectivity refers to the ability of the method to analyte of interest when other chemicals or drugs are also resident at in this regard are the effects of:
a. Drugs of similar structure or veterinary drugs that may all along with the analyte of into	so be u	
b. Contaminants that are likely in the sample	to be p	resent

9. Type of Validation studies a. Single laboratory

b. Multi-laboratory	
c. AOAC or other official procedure	
C Information relevant to laboratory i	mplementation

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:	Antibacterial agent (Tetracyclines)
(e.g. antimicrobial,	
Anthelmintic, etc)	
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:	Chlortetracycline
(specify if metabolite)	
5. Intended use of the	
method:	
	Yes (5 plate MIT)
a. Screening	1 es (3 piate WIII)
b. Routine	

c. Reference		
d Confirmatory	Yes (H	DI C'
d. Confirmatory	res (m	FLC)
6. Test matrix		idney
(e.g. muscle, kidney, urine,	etc)	
7 Cymmany of minainal stans	in	
7. Summary of principal steps sample preparation:	III	
sample preparation.		
	•	
8. Summary of principal steps	in A	ntimicrobials differentially extracted into three separate solutions
extraction procedure:		
9. Summary of principal steps	in C	leaned up and concentrated using SPE and solvent removal under
Analyte clean-up procedure		acuum.
	·	
10. Measurement procedure: a. Chemical	Ι.	Confirmation
a. Chemical 1. Instrumentation		Confirmation HPLC
1. Instrumentation		HIFEC
2. Detector system		Fluorescence
3. Chromatographic column		
(if applicable)		
h Immun sahami sal/Immun s		
b. Immunochemical/Immuno		N/A
1. Technique: (e.g. Elisa, RIA,		IV/A
Immunochromatog, etc)		
2. Critical reagents:		N/A
(e.g. antibody specificity an	d	
availability)		
3. Special equipment require	ed:	N/A
c. Microbiological		
1. Technique:		5 plate MIT (initial screening)
2. Organism:		
3. Media:		
4. Special equipment require	ed:	
11. Sample/Analyte Stability	<u> </u>	
Warning (if applicable):		
maring (ii applicable).		

12. Literature References		
Available:		
Tivanaoie.		
13. Contact for Information:		
a. Name	Ms Heather Li	ndsay
b. Country	Australia	
c. Affiliation	SCL (State Ch	emistry Lab)
d. Address	Cnr Sneydes an	nd South Roads
	Werribee VIC	3030
e. Telephone	(+61) 3 9742 8	779
f. FAX	(+61) 3 9742 8	
g. Email	Heather.Lindsay(
g. zmm		
B. Method Performance		
D. Method I errormance		
1. a. Limit of Detection (LOD)	(ma/ka)	0.05mg/kg
How was LOD determined		0.03mg/kg
now was LOD determined	1.	
	1.00) (/1)	0.05
b. Limit of Quantification (1		0.05mg/kg
How was LOQ determined?	,	
c. Method sensitivity		
(The smallest difference in concentration		
that can be measured)		
2. JECFA MRL Muscle (ca	ttle, pigs and po	ultry):0.1mg/kg
Liver (cattl	le, pigs, sheep ar	nd poultry): 0.3mg/kg
Kidney (ca	ttle, pigs, sheep	and poultry: 0.6mg/kg
Eggs (poul	try): 0.2mg/kg	
Milk (cattle	e and sheep): 0.1	mg/kg
	1/	
3. Is analytical data corrected f	for recovery?	Yes
or an arrangement of the second of the secon		
4. How is recovery estimated		External standard
(e.g. external standard; internal standard etc.)		
(e.g. external standard, inter	mar standard etc	·/
5. Accuracy		
	0.05mg/lz	·
a. Concentration(s) tested	0.05mg/kg	
h Consentration(s)		
b. Concentration(s) measure	eu	
D (0/)	CE0/	
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)	
veterinary drugs that may also be u along with the analyse of interest	
b. Contaminants that are likely to be p in the sample	resent
9. Type of Validation studies a. Single laboratory	
b. Multi-laboratory	
c. AOAC or other official procedure	

\sim	Information	rolovent to	labaratar	ı, imn	lomontation
C .	mormanon	reievani u	iaborator (у шпр	iementanon

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
r i i i i	
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/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. Microbiological1. 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 F	Robert Symons
1 4. 1 14HC	\$\/1/\$\frac{1}{2} \$\frac{1}{2}

b. Country	Australia
c. Affiliation	AMDEL Lilyfield
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e. Telephone	(+61) 2 9818 1033
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g. Email	Robert_symons@amdel.com

B. Method Performance

b. Repeatability (within lab CV)

B. Method Performance			
1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?		g/kg)	0.01mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?) (mg/kg)	0.1
(The smallest of	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 dat	a corrected for re	ecovery?	Yes
4. How is recovery estimated (e.g. external standard; internal standard etc.		standard etc	External standard .)
5. Accuracy			
		0.1 mg/kg	
b. Concentration(s) measured			
c. Recovery (%) 88%		88%	
6. Precision using Control tissue	fortified	1	
a. Concentration(s) tested			

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)	
	s "Specificity". Selectivity refers to the ability of the method to analyte of interest when other chemicals or drugs are also resident st in this regard are the effects of:
a. Drugs of similar structure or drug conveterinary drugs that may also be unalong with the analyte of interest	
b. Contaminants that are likely to be p in the sample	resent
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 analyte(s)	l for
2. Critical steps in the method	

3. Information on availability of Equipment	of unusual reagents or	
4. Special reagent or sample st	ability concerns	
5. Reagent handling and safety	concerns (if any)	
6. Literature references or other	er useful information	
CYFLUTHRIN I. Descriptive Information		
1. Name of drug or chemical:	Cyfluthrin	
2. Drug or chemical class: (e.g. antimicrobial, Anthelminthic, etc)	Carbamates, Pyrethroi	ds and other insecticides (Synthetic pyrethroids)
3. Veterinary Use:	Broadspectrum synthe to control infestations	tic type 2 pyrethroid insecticide and acaricide used 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 Cummony of main aims 1 -4	in Cubcomala of Ca	wondowed in microsylves
7. Summary of principal steps sample preparation:	iii Suosampie of fat	rendered in microwave

8. Summary of principal steps in	Molten fat dissolved in hexane then extracted with acetonitrile		
extraction procedure:			
O. Communication of maintain all attentions	100/ E- i-il (10-40-50)		
9. Summary of principal steps in	10% Forisil trap, elute with acetone:diethyl ether:hexane (10:40:50)		
Analyte clean-up procedure:			
10. Measurement procedure:			
a. Chemical	GC		
1. Instrumentation			
2. Detector system	ECD, NPD and MS		
3. Chromatographic column	DB-1 or DB-5		
(if applicable)			
1. Tanana and 1. 17	NI/A		
b. Immunochemical/Immunoassa	y N/A		
1. Technique: (e.g. Elisa, RIA,			
Immunochromatog, etc)			
2. Critical reagents:	N/A		
(e.g. antibody specificity and			
Availability)			
3. Special equipment required:	N/A		
c. Microbiological	N/A		
1. Technique:			
2. Organism:			
3. Media:			
4. Special equipment required:			
11. Sample/Analyte Stability			
Warning (if applicable):			
10 L' , D C			
12. Literature References Available:			
Avanable.			
13. Contact for Information:			
a. Name M	r. Phil Williams		
b. Country Australia			
	ymbio		
d. Address 47	Manilla St. East Brisbane 4169		
e. Telephone (+	61) 7 3391 7558		
-	7 33916673		
`	/mbio@powerup.com.au		
<u> </u>			

B. Method Performance

1. a. Limit of Detection (LOD) (mg/kg) How was LOD determined?			0.0	01mg/kg
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?			0.0	02mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)				
2. JECFA MRL Muscle, liver and kidney (carrier fat (cattle): 0.2mg/kg Milk (cattle): 0.04mg/kg			attle	e): 0.02mg/kg
3. Is analytical data	corrected for red	covery?	Υe	es
4. How is recovery e (e.g. external star		tandard etc.	.)	External standard
5. Accuracy				
a. Concentration((s) tested	0.01 mg/kg 1.0 mg/kg	_	
b. Concentration(s) measured		<u> </u>		
c. Recovery (%) 93% 98%				
6. Precision using for Control tissue	ortified			
a. Concentration(s) tested 0.1 r 1.0 r				
b. Repeatability (within lab CV) 7.9%				
c. Reproducibility (between lab CV)			U .	
7. Precision using tis		1		
Incurred drug residues a. Concentration(s) tested				
b. Repeatability (within lab CV)				

c. Reproducibility (between lab CV)		
	analyte of int	y". Selectivity refers to the ability of the method to terest when other chemicals or drugs are also resident ard are the effects of:
a. Drugs of similar structure or drug cl veterinary drugs that may also be us along with the analyte of interest		
b. Contaminants that are likely to be pain the sample	resent	
9. Type of Validation studies a. Single laboratory		
b. Multi-laboratory		
c. AOAC or other official procedure		
C. Information relevant to laboratory i	mplementa	tion
1. Training and experience recommended analyte(s)	for	
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 in	formation	

DIHYDRO-STREPTOMYCIN

J. Descriptive Information

1 Name of dwg or shamisal.	Dihydro strontomyoin
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 sample preparation:	in
8. Summary of principal steps extraction procedure:	in Antimicrobials differentially extracted into three separate solutions
9. Summary of principal steps	
Analyte clean-up procedure:	vacuum. Confirmation: fluorescent derivative formed using 1,2-naphthoquinone-4-sulphone acid
10. Measurement procedure:	
a. Chemical	Confirmation

1. Instrumentation	HPLC	
2. Detector system	Fluorescence	
3. Chromatographic column (if applicable)		
b. Immunochemical/Immunoa 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	1:	
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment required	5 plate MIT (initial screening) d:	
11. Sample/Analyte Stability		
Warning (if applicable):		
12. Literature References Available:		
13. Contact for Information:		
	Ms Heather Lindsay	
1	Australia	
	State Chemistry Lab)	
	Cnr Sneydes and South Roads Werribee VIC 3030	
e. Telephone	(+61) 3 9742 8779	
	(+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		heep, pigs a	e, sheep, pigs and poultry): 0.5mg/kg and poultry): 1mg/kg		
	1.0				
3. Is analytical data	a corrected for red	covery?	Yes		
4. How is recovery (e.g. external sta	estimated andard; internal s	tandard etc.	Spiked matrix calibration curve		
5. Accuracy					
a. Concentration	n(s) tested	0.2mg/kg			
b. Concentration	n(s) measured				
c. Recovery (%))	100%	%		
6. Precision using Control tissue	fortified				
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. Reproducibili	ity (between lab (CV)			
0.01.41.4.64		ı			

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 of veterinary drugs that may along with the analyte of its	also be used	
b. Contaminants that are likel in the sample	y to be present	
		<u>l</u>
9. Type of Validation studies a. Single laboratory		
b. Multi-laboratory		
c. AOAC or other official pro	cedure	
	<u>I</u>	
C. Information relevant to lab	oratory impleme	ntation
1. Training and experience recor	nmended for anal	yte
2. Critical steps in the method		
3. Information on availability of Equipment	unusual reagents	or
4. Special reagent or sample stab	oility concerns	
5. Reagent handling and safety of	oncerns (if any)	
6. Literature references or other	useful informatio	n
		,
DORAMECTIN K. Descriptive Information		
1. Name of drug or chemical:	Doramectin	
2. Drug or chemical class: (e.g. antimicrobial,	Anthelmintic age	nt (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,	Liver etc)
7. Summary of principal steps sample preparation:	in Homogenise sample with acetonitrile
8. Summary of principal steps extraction procedure:	in Acetonitrile extract is evaporated to dryness under vacuum and the residue dissolved in hexane/dichloromethane mix
9. Summary of principal steps Analyte clean-up procedure:	in 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/Imm	assay N/A

Immunochromatog, etc)	I	
2. Critical reagents:	N/A	
(e.g. antibody specificity		
Availability)		
3. Special equipment requ	ired: N/A	
	- "	
c. Microbiological	N/A	
1. Technique:		
2. Organism:		
3. Media:		
4. Special equipment requ	iired:	
	1	
11. Sample/Analyte Stability	7	
Warning (if applicable):		
12. Literature References		
Available:		
a a a a a a a a a a a a a a a a a a a		
13. Contact for Information:		
a. Name	Terry Spencer	
b. Country	Australia	
c. Affiliation	AGAL ACT	
d. Address	Level 10, Alla	ra Street, Canberra,
	ACT, 2600	
e. Telephone	(+61) 2 6213	6102
f. FAX	(+61) 2 6213	6815
g. Email	terry.spencer@ag	gal.gov.au
B. Method Performance		
1. a. Limit of Detection (LO)		0.001mg/kg
How was LOD determin	ed?	
b. Limit of Quantification		0.005mg/kg
How was LOQ determine	d?	
c. Method sensitivit		
(The smallest difference in concentration		
that can be measured)		
	cattle): 0.01mg/kg	
Liver (ca	ttle): 0.1mg/kg	
I '	, ,	
Kidney (cattle): 0.03mg/kg e): 0.15mg/kg	

3. Is analytical data corrected for recovery? Yes		
4. How is recovery estimated (e.g. external standard; internal s	tandard 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)	
Provide accurate measurement of in	ced as "Specificity". Selectivity refers to the ability of the method to f the analyte of interest when other chemicals or drugs are also resident nterest in this regard are the effects of:	
veterinary drugs that may also along with the analyte of inter	be used	

b. Contaminants that are like in the sample	ely to be present	
9. Type of Validation studies a. Single laboratory		
b. Multi-laboratory		
c. AOAC or other official pr	rocedure	
C. Information relevant to lal 1. Training and experience reco		ntation
analytes		
2. Critical steps in the method		
3. Information on availability of Equipment	f unusual reagents	or
4. Special reagent or sample sta	ability concerns	
5. Reagent handling and safety	concerns (if any)	
6. Literature references or other	r useful information	
EPRINOMECTIN L. Descriptive Information		
1. Name of drug or chemical:	Eprinomectin	
2. Drug or chemical class: (e.g. antimicrobial, Anthelmintic, etc)	Anthelmintic agen	t (Macrocyclic lactone)
3. Veterinary Use:		ntestinal worms, lungworm; sucking and biting 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	Liver
(e.g. muscle, kidney, urine, e	tc)
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	a) Silian can pak alaanun alutad with athyd acetata
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. Chemical1. Instrumentation	HPLC
2. Detector system	Fluorescence wavelength – 360nm excitation and 468nm emission
3. Chromatographic column (if applicable)	Reverse phase C18 column
b. Immunochemical/Immunoa: 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ssay N/A
 Critical reagents: (e.g. antibody specificity and Availability) 	N/A
3. Special equipment required	d: N/A
c. Microbiological1. Technique:2. Organism:	N/A

3. Media:4. Special equipment require	ed:
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,
a Talankana	ACT 2600
e. Telephone f. FAX	(+61) 2 6213 6102 (+61) 2 6213 6815
g. Email	Terry.spencer@agal.gov.au
g. Eman	Torry is ported Cagarigoviau
B. Method Performance 1. a. Limit of Detection (LOD) How was LOD determined	
b. Limit of Quantification (I How was LOQ determined?	
c. Method sensitivity (The smallest difference in contract that can be measured)	concentration
2. JECFA MRL (NOT SET)	
3. Is analytical data corrected for	for recovery? Yes
4. How is recovery estimated (e.g. external standard; internal standard;	External standard rnal standard etc.)
5. Accuracy	
a. Concentration(s) tested	0.005, 0.010 mg/kg
b. Concentration(s) measure	ed ed
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)	
	as "Specificity". Selectivity refers to the ability of the method to analyte of interest when other chemicals or drugs are also resident st in this regard are the effects of:
a. Drugs of similar structure or drug of veterinary drugs that may also be unalong with the analyte of interest	
b. Contaminants that are likely to be print the sample	present
O. T	
9. Type of Validation studies a. Single laboratory	
b. Mullet- laboratory	
c. AOAC or other official procedure	

$C. \ \textbf{Information relevant to laboratory implementation} \\$

1. Training and experience recommended for analytes	
anarytes	
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:	Antimicrobial agent (Tetracyclines)
(e.g. antimicrobial,	
Anthelmintic, etc)	
, ,	
3. Veterinary Use:	Provides broad spectrum antibiotic coverage against susceptible organisms
	in alimentary tract infections, respiratory, genitourinary, septicaemia and
	Superficial bacterial infections.
	Superioral Succession infections.
4. Analyte(s) measured:	Oxytetracycline
(specify if metabolite)	Oxytetrae jenne
(speeny if metaconte)	
5. Intended use of the	
method:	
a. Screening	Yes (5 plate MIT)
b. Routine	
b. Routine	
c. Reference	
d. Confirmatory	Yes (HPLC)

6. Test matrix (e.g. muscle, kidney, urine, etc)	Kidney
(org. masses, maney, arms, over)	
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	Confirmation
1. Instrumentation	HPLC
2. Detector system	UV
3. Chromatographic column (if applicable)	
b. Immunochemical/Immunoassay 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	N/A
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):	
L	
12. Literature References Available:	
13. Contact for Information:	
	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	External standard
(e.g. external standard; internal standard etc.)	

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 print in the sample	resent		
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	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 sample preparation:	in Homogenise sample with acetonitrile		
8. Summary of principal steps extraction procedure:	in 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/Immunoassa 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ny N/A		
2. Critical reagents: (e.g. antibody specificity and Availability)	N/A		
3. Special equipment required:	N/A		
c. Microbiological1. 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:			
	erry Spencer		
	ustralia		
c. Affiliation AG	GAL ACT		
A	evel 10, Allara Street, Canberra, CT, 2600		
-	61) 2 6213 6102		
	61) 2 6213 6815		
a Descrit	ry changer (Alaga) goy au		

terry.spencer@agal.gov.au

B. Method Performance

g. Email

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?		g) 0.005 – B1a 0.001 – B1b
c. Method sensitivity (The smallest difference in concentration that can be measured)		1
		g (pigs and sheep): 0.015mg/kg (sheep and pigs): 0.02mg/kg
3. Is analytical data corrected for re	covery?	Yes
4. How is recovery estimated (e.g. external standard; internal standard)	tandard et	etc.) External standard
5. Accuracy (Ivermectin B1a)		
a. Concentration(s) tested	0.005, 0.	0.01 mg/kg
b. Concentration(s) measured		
c. Recovery (%)	93%, 92)2%
6. Precision using fortified Control tissue (Ivermectin B1a)		
a. Concentration(s) tested 0.00		0.005, 0.01mg/kg
b. Repeatability (within lab CV) 5%,		7%, 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 veterinary drugs that may also be us along with the analyte of interest		
b. Contaminants that are likely to be print in the sample	resent	
O.T. CM I'I d'		
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,	Liver etc)	
7. Summary of principal steps sample preparation:	in	
8. Summary of principal steps extraction procedure:	in Macerated liver extracted into acidified acetonitrile	
9. Summary of principal steps Analyte clean-up procedure		
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/Immunoa 1. Technique:	ssay N/A	
(e.g. Elisa, RIA,		
Immunochromatog, etc) 2. Critical reagents:	N/A	
(e.g. antibody specificity and		
Availability)		
3. Special equipment required	d: N/A	
c. Microbiological	N/A	
1. Technique:		
2. Organism:		
3. Media:		
4. Special equipment required	d:	
11.0 1/4 1 0 1/2		
11. Sample/Analyte Stability		
Warning (if applicable):		
12. Literature References		
Available:		
13. Contact for Information:		
	Terry Spencer	
3	Australia	
	AGAL ACT	
	Level 10, Allara Street, Canberra,	
	ACT, 2600	
•	(+61) 2 6213 6102	
f. FAX	(+61) 2 6213 6815	
g. Email	mail 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)	0.02mg/kg
How was LOQ determined?	
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 dat	3. Is analytical data corrected for recovery? Yes			
4. How is recovery estimated (e.g. external standard; internal standard etc.)				
5. Accuracy				
		0.2 n	ng/kg	
b. Concentratio	on(s) measured	Турі	cal recover	ies 60 – 100%
c. Recovery (%)			
6. Precision using Control tissue				
a. Concentratio	a. Concentration(s) tested		0.2mg/kg	
b. Repeatability (within lab CV) 15%				
c. Reproducibility (between lab CV)				
7. Precision using Incurred drug r				
a. Concentratio	on(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 lik in the sample	ely to be present	
9. Type of Validation studies a. Single laboratory		
b. Multi-laboratory		
c. AOAC or other official p	rocedure	
C. Information relevant to la		tion
Training and experience rec analytes	ommended for	
2. Critical steps in the method		
3. Information on availability of Equipment	of unusual reagents or	
4. Special reagent or sample st	ability concerns	
5. Reagent handling and safety	concerns (if any)	
6. Literature references or other	r 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 (I	Macrocyclic lactones)
3. Veterinary Use:	mange mites and catt	stinal roundworms, lungworms, sucking and biting lice, le ticks. Also used for itchmite, nasal bot and stridial diseases and cheesy gland in sheep.

4. Analyte(s) measured:	Moxid	ectin
(specify if metabolite)	WIOXIG	cetiii
(specif i inconcente)		
5. Intended use of the		
method: a. Screening	Yes	
a. Screening	108	
b. Routine		
c. Reference		
d. Confirmatory	Yes	
	•	
6. Test matrix		iver
(e.g. muscle, kidney, urine,	etc)	
7. Summary of principal steps	in H	Homogenise sample with acetonitrile
sample preparation:		
L		
8. Summary of principal steps		Acetonitrile extract is evaporated to dryness under vacuum and the
extraction procedure:	r	residue dissolved in hexane/dichloromethane mix
9. Summary of principal steps	in a	a) Silica sep-pak cleanup, eluted with ethyl acetate
Analyte clean-up procedure		b) Then derivatised with acetic anhydride/1-methyl
	i	midazole/dimethyl formamide and derivatised mixture cleaned up by
	(C-18 sep-pak
10. Measurement procedure:		
a. Chemical		HPLC
1. Instrumentation		
2. Detector system		Fluorescence wavelength – 360nm excitation and 468nm emission
3. Chromatographic column	n	Reverse phase C18 column
(if applicable)		1
b. Immunochemical/Immuno	assay	N/A
1. Technique: (e.g. Elisa, RIA,		
Immunochromatog, etc)		
2. Critical reagents:		N/A
(e.g. antibody specificity an	d	
Availability)		
3. Special equipment require	ed:	N/A

	N/A	
1. Technique:		
2. Organism:		
3. Media:		
4. Special equipment requir	ed:	
11. Sample/Analyte Stability		
Warning (if applicable):		
warming (in uppriousio).		
12. Literature References		
Available:		
Avanable:		
13. Contact for Information:		
a. Name	Terry Spencer	
b. Country	Australia	
c. Affiliation	AGAL ACT	
d. Address		ra Street, Canberra,
u. Address	ACT, 2600	ia Siloci, Caliberta,
e. Telephone	(+61) 2 6213 6	5102
f. FAX	(+61) 2 6213 6	
g. Email	terry.spencer@ag	
g. Eman	terry.sperieer@ag	an.gov.au
B. Method Performance		
) (mg/kg)	0.001mg/kg
1. a. Limit of Detection (LOD)	, , ,	0.001mg/kg
	, , ,	0.001mg/kg
1. a. Limit of Detection (LOD)	, , ,	0.001mg/kg
1. a. Limit of Detection (LOD) How was LOD determined	1?	
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (2)	LOQ) (mg/kg)	0.001mg/kg 0.005mg/kg
1. a. Limit of Detection (LOD) How was LOD determined	LOQ) (mg/kg)	
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (2)	LOQ) (mg/kg)	
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (How was LOQ determined)	LOQ) (mg/kg)	
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (How was LOQ determined) c. Method sensitivit	LOQ) (mg/kg)	
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (How was LOQ determined) c. Method sensitivit (The smallest difference in	LOQ) (mg/kg)	
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (How was LOQ determined) c. Method sensitivit	LOQ) (mg/kg)	
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (How was LOQ determined) c. Method sensitivit (The smallest difference in that can be measured)	LOQ) (mg/kg) ? concentration	0.005mg/kg
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (How was LOQ determined) c. Method sensitivit (The smallest difference in that can be measured) 2. JECFA MRL Muscle (ca	LOQ) (mg/kg) ? concentration	0.005mg/kg c; (sheep): 0.05mg/kg
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (How was LOQ determined) c. Method sensitivit (The smallest difference in that can be measured) 2. JECFA MRL Muscle (can Liver (catt)	LOQ) (mg/kg) concentration attle): 0.02mg/kg le and sheep): 0.1	0.005mg/kg ;; (sheep): 0.05mg/kg lmg/kg
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (Annual Medical Medi	LOQ) (mg/kg) concentration attle): 0.02mg/kg le and sheep): 0.	0.005mg/kg 0.005mg/kg (; (sheep): 0.05mg/kg 1mg/kg 0.05mg/kg
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (Annual Medical Medi	LOQ) (mg/kg) concentration attle): 0.02mg/kg le and sheep): 0.1	0.005mg/kg 0.005mg/kg (; (sheep): 0.05mg/kg 1mg/kg 0.05mg/kg
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (In the smallest difference in that can be measured) 2. JECFA MRL Muscle (can be	ttle): 0.02mg/kg le and sheep): 0.5m	0.005mg/kg c; (sheep): 0.05mg/kg lmg/kg 0.05mg/kg ng/kg
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (Annual Medical Medi	ttle): 0.02mg/kg le and sheep): 0.5m	0.005mg/kg 0.005mg/kg (; (sheep): 0.05mg/kg 1mg/kg 0.05mg/kg
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (How was LOQ determined) c. Method sensitivit (The smallest difference in that can be measured) 2. JECFA MRL Muscle (can be	ttle): 0.02mg/kg le and sheep): 0.5m	0.005mg/kg c; (sheep): 0.05mg/kg 1mg/kg 0.05mg/kg mg/kg Yes
1. a. Limit of Detection (LOD) How was LOD determined b. Limit of Quantification (In the smallest difference in that can be measured) 2. JECFA MRL Muscle (can be	LOQ) (mg/kg) concentration attle): 0.02mg/kg le and sheep): 0.5 attle and sheep): 0.5 for recovery?	0.005mg/kg g; (sheep): 0.05mg/kg 1mg/kg 0.05mg/kg mg/kg Yes 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	5	
a. Concentration(s) tested		
b. Repeatability (within lab CV))	
c. Reproducibility (between lab	CV)	
Provide accurate measurement of in	of the	as "Specificity". Selectivity refers to the ability of the method to analyte of interest when other chemicals or drugs are also resident est in this regard are the effects of:
a. Drugs of similar structure or veterinary drugs that may als along with the analyte of inte	o be u	
b. Contaminants that are likely to in the sample	to be p	present

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

$C. \ \textbf{Information relevant to laboratory implementation} \\$

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 is sample preparation:	in	
8. Summary of principal steps in extraction procedure:	in 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/Immunoa 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ELISA (secondary screening test)	
Critical reagents: (e.g. antibody specificity and Availability)	d	
3. Special equipment require	ed:	
c. Microbiological 1. Technique: 2. Organism: 3. Media: 4. Special equipment require	5 plate MIT (initial screening) ed:	
11. Sample/Analyte Stability		

Warning (if a	pplicable):		
12. Literature Refe	erences		
Available:			
13. Contact for Inf	formation:		
a. Name	Mr	Dennis Hamilt	ton
b. Country		stralia	
c. Affiliation		,	esidue Laboratory)
d. Address		Fairfield Road	
a Talanhana		erongapilly Q 51) 7 3362 9415	
e. Telephone f. FAX	,	51) 7 3362 941. 51) 7 3362 946(
g. Email		niltondj@prose.dp	
g. Eman			
B. Method Perfor	rmance		
1. a. Limit of Dete	ection (LOD) (mo	·/kg) 0	1mg/kg
	D determined?	(18)	
_	intification (LOQ) (mg/kg) 0.	1mg/kg
How was LOQ	determined?		
c. Method sensitivity			
		entration	
(The smallest difference in concentration that can be measured)			
mat can be incustred;			
2. JECFA MRL	Muscle, liver ar	nd fat (cattle, sh	neep, 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		
2.1 1.: 11.	, 1 C	0 17	
3. Is analytical data corrected for recovery? Yes			
4. How is recovery estimated External standard			External standard
(e.g. external standard; internal standard etc.)			
	•	,	
5. Accuracy		0.05	
a. Concentratio	n(s) tested	0.25mg/kg	
h Concentration	n(a) maggyrad		
b. Concentratio	n(s) measured		

c. Recovery (%)	90%	
6. Precision using fortified Control tissue		
		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)	
Provide accurate measurement of in The laboratory sample. Data of i a. Drugs of similar structure or deveterinary drugs that may also along with the analyte of interview.	of the a	sed
b. Contaminants that are likely to in the sample	be p	resent
9. Type of Validation studies		
a. Single laboratory		
b. Multi-laboratory		
c. AOAC or other official proceed	dure	

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:	g or chemical: Oxfendazole	
2. Drug or chemical class:	Anthelmintic agent (Benzimidazole)	
(e.g. antimicrobial,		
Anthelmintic, etc)		
3. Veterinary Use:	Broad spectrum anthelmintic. Controls gastrointestinal roundworms,	
	tapeworms and lungworms. Sterilises roundworm eggs.	
	tupe werns und tang werns see reason to and we see	
4. Analyte(s) measured:	Oxfendazole	
(specify if metabolite)		
(specify if inctationite)		
5. Intended use of the		
method:		
	Yes	
a. Screening	res	
1.70		
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:	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/Immunoassa 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	ay 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:			
	Dr Robert Symons		
b. Country A	ustralia		

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
3. Is analytical data corrected for recovery:	103

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

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)	

Incurred drug residues		
a. Concentration(s) tested		
b. Repeatability (within lab CV)		
c. Reproducibility (between lab CV)		
	analyte of	city". Selectivity refers to the ability of the method to interest when other chemicals or drugs are also resident egard are the effects of:
a. Drugs of similar structure or drug claveterinary drugs that may also be us along with the analyte of interest		
b. Contaminants that are likely to be print in the sample	resent	
9. Type of Validation studies a. Single laboratory		
b. Multi-laboratory		
c. AOAC or other official procedure		
C. Information relevant to laboratory in	mplemer	ntation
1. Training and experience recommended analytes	for	
2. Critical steps in the method		
3. Information on availability of unusual r Equipment	reagents (or

4. Special reagent or sample st	ability 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: Tilmicosin (specify if metabolite)			
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 extraction procedure: 9. Summary of principal steps Analyte clean-up procedure	in Cleaned up and	differentially extracted into three separate solutions concentrated using SPE and solvent removal under	

10. Measurement procedure:

10. Measurement procedure:		
a. Chemical	Confirmation	
1. Instrumentation	HPLC	
2. Detector system	UV-287nm	
j		
3. Chromatographic colum	n	
(if applicable)		
b. Immunochemical/Immuno	passay	
1. Technique:	ELISA (secondary screening test)	
(e.g. Elisa, RIA,		
Immunochromatog, etc)		
2. Critical reagents:		
(e.g. antibody specificity an	nd	
availability)		
3. Special equipment requi	red:	
c. Microbiological		
1. Technique:	5 plate MIT (initial screening)	
2. Organism:		
3. Media:		
4. Special equipment requi	red:	
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	
	(<1) 0.0740.0770	

B. Method Performance

e. Telephone f. FAX

g. Email

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

(+61) 3 9742 8779 (+61) 3 9742 8700

Heather.Lindsay@nre.vic.gov.au

b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?			(kg) 0.1mg/kg
c. Method sensitivity (The smallest difference in concentration that can be measured)		entratio	on
			p): 1mg/kg (pigs): 1.5mg/kg eep): 0.3mg/kg (pigs): 1mg/kg pigs): 0.1mg/kg
3. Is analytical dat	a corrected for re	covery	y? Yes
4. How is recovery estimated (e.g. external standard; internal standard etc.			External standard rd etc.)
5. Accuracy			
	a. Concentration(s) tested 0.1mg/kg		ng/kg
b. Concentration(s) measured			
c. Recovery (%) 85%		85%	
6. Precision using Control tissue	fortified		
		(0.1mg/kg
b. Repeatability (within lab CV) 20%			20%
c. Reproducibility (between lab CV)		CV)	
7. Precision using Incurred drug r	_	,	
a. Concentration(s) tested			
b. Repeatability (within lab CV)			
c. Reproducibility (between lab CV)		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

The laboratory sample. Data of interest in this regard are the effects of:

in

a. Drugs of similar structure or drug cla	ass or		
veterinary drugs that may also be us			
along with the analyte of interest			
b. Contaminants that are likely to be pr	resent		
in the sample			
	;		
9. Type of Validation studies			
a. Single laboratory			
a. Single laboratory			
1.26.11.1			
b. Multi-laboratory			
c. AOAC or other official procedure			
C. Information relevant to laboratory is	mplementation	n	
•	•		
1. Training and experience recommended	for analyte		
Training and experience recommended	ioi anaiyee		
2. Critical steps in the method			
2. Critical steps in the method			
3. Information on availability of unusual i	eagents or		
Equipment			
4. Special reagent or sample stability cond	erns		
5. Reagent handling and safety concerns (if any)			
or reading and surery concerns (if any)			
6. Literature references or other useful information			
o. Literature references of 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,	Egg Kidney Egg			
7. Summary of principal steps Sample preparation:	in			
8. Summary of principal steps Extraction procedure:	Antimicrobials differentially extracted into three separate solutions			
9. Summary of principal steps Analyte clean-up procedure				
10. Measurement procedure:				
a. Chemical				
1. Instrumentation	HPLC			
2. Detector system	UV at 325nm			
3. Chromatographic column (if applicable)				
b. Immunochemical/Immuno	assay			

(e.g. Elisa, RIA, Immunochromatog, etc) 2. Critical reagents: (e.g. antibody specificity and				
2. Critical reagents: N/A				
1 (c.g. antibody specificity and				
availability)				
3. Special equipment required: N/A				
5. Special equipment required.				
c. Microbiological				
1. Technique: 5 plate MIT (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 Mr Dennis Hamilton				
b. Country Australia				
c. Affiliation ARI (Chemical Residue Laboratory)				
d. Address 665 Fairfield Road				
Yeerongapilly QLD 4105				
	(+61) 7 3362 9415			
	(+61) 7 3362 9460			
g. Email <u>Hamiltondj@prose.dpi.qld.au</u>	Hamiltondj@prose.dpi.qld.au			
B. Method Performance				
1. a. Limit of Detection (LOD) (mg/kg) 0.01mg/kg				
How was LOD determined?				
b. Limit of Quantification (LOQ) (mg/kg) 0.02mg/kg				
b. Limit of Quantification (LOQ) (mg/kg) 0.02mg/kg How was LOQ determined?				
How was Log determined:				
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 re	ecovery'	? Y	res
4. How is recovery estimated (e.g. external standard; internal standard etc.)			External standard
5. Accuracy			
a. Concentration(s) tested	0.08n	ng/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	ţ		
a. Concentration(s) tested			
b. Repeatability (within lab CV)			
c. Reproducibility (between lab CV)			
Provide accurate measurement of in The laboratory sample. Data of its	of the ar	nalyte of	icity". Selectivity refers to the ability of the method to f interest when other chemicals or drugs are also resident 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			

in the sample	ery to be pres	Sent	
9. Type of Validation studies			
a. Single laboratory			
b. Multi-laboratory			
c. AOAC or other official p	rocedure		
C. Information relevant to la 1. Training and experience rec			ion
		or unury to	
2. Critical steps in the method			
3. Information on availability of Equipment	of unusual rea	agents or	
4. Special reagent or sample st	ability concer	rns	
5. Reagent handling and safety	concerns (if	any)	
6. Literature references or other	er useful infor	rmation	
STREPTOMYCIN U. Descriptive Information 1. Name of drug or chemical:	Streptomyc	in	
2. Drug or chemical class:	Antibacteria	al agent (A	Aminoglycoside)
(e.g. antimicrobial, Anthelmintic, etc)		ur ugʻin (r	
3. Veterinary Use:	Useful again infections.	nst mening Not readily	tinal bacteria, antispasmodic effect. gococcal, pneumococcal and haemolytic streptococcal absorbed, has a local bactericidal and bacteriostatic egative bacteria.
4. Analyte(s) measured:	Streptomyc	in	

5. Intended use of the method:		
a. Screening	Yes (5	plate MIT and if positive then ELISA)
b. Routine		
c. Reference		
d. Confirmatory	Yes (F	HPLC)
6. Test matrix	K	Kidney
(e.g. muscle, kidney, urine,		Lgg
7. Summary of principal steps sample preparation:	in	
8. Summary of principal steps extraction procedure:		Antimicrobials differentially extracted into three separate solutions Including confirmation
9. Summary of principal steps 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)	l	
b. Immunochemical/Immunoa 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	assay	ELISA (secondary screening test)
 Critical reagents: (e.g. antibody specificity and availability) 	d	
3. Special equipment require	ed:	
c. Microbiological 1. Technique:		5 plate MIT (initial screening)

2. Organism:3. Media:4. Special equipment requ	nired:	
11. Sample/Analyte Stability Warning (if applicable):		
12. Literature References Available:		
13. Contact for Information:		
a. Name	Ms Heather Li	indsay
b. Country	Australia	
c. Affiliation	SCL (State Ch	
d. Address	Cnr Sneydes a Werribee VIC	nd South Roads C 3030
e. Telephone	(+61) 3 9742 8	3779
f. FAX	(+61) 3 9742 8	3700
g. Email	Heather.Lindsay	@nre.vic.gov.au
How was LOD determined?		0.1mg/kg 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	1	
a. Concentration(s) tested	0.2mg/kg	

	T	
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)	
	f the analyte of	city". Selectivity refers to the ability of the method to interest when other chemicals or drugs are also resident egard are the effects of:
a. Drugs of similar structure or d veterinary drugs that may also along with the analyte of inter	be used	
b. Contaminants that are likely to be present in the sample		
9. Type of Validation studies		
a. Single laboratory		
b. Multi-laboratory		

	77		
c. AOAC or other official p	rocedure		
C. Information relevant to la	boratory implementation		
1. Training and experience rec	ommended for analyte		
2. Critical steps in the method			
3. Information on availability of Equipment	of unusual reagents or		
4. Special reagent or sample st	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		

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
(e.g. muscle, kidney, urme, etc)	
7. Summary of principal steps in	
sample preparation:	
8. Summary of principal steps in	
extraction procedure:	
9. Summary of principal steps in	Extract derivatised
Analyte clean-up procedure:	
10. Measurement procedure:	
a. Chemical	HPLC
1. Instrumentation	
2. Detector existem	Fluorescence
2. Detector system	Fluorescence
3. Chromatographic column	
(if applicable)	
b. Immunochemical/Immunoassay	N/A
1. Technique:	14/71
(e.g. Elisa, RIA,	
Immunochromatog, etc)	
2. Critical reagents:	N/A
(e.g. antibody specificity and availability)	
3. Special equipment required:	N/A
c. Microbiological 1. Technique:	N/A
2. Organism:	N/A N/A
3. Media:	N/A
4. Special equipment required:	N/A
11. Sample/Analyte Stability	
Warning (if applicable):	
10.1%	
12. Literature References Available:	
rivanaoic.	

13. Contact for Information:

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b. Country	Australia
c. Affiliation	SCL (State Chemistry Lab)
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	Werribee VIC 3030
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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

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 (%)	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)		
	s "Specificity". Selectivity refers to the ability of the method to analyte of interest when other chemicals or drugs are also resident at in this regard are the effects of:	
a. Drugs of similar structure or drug cl veterinary drugs that may also be us along with the analyte of interest		
b. Contaminants that are likely to be print in the sample	resent	
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	er 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 sample preparation:	in		
8. Summary of principal steps extraction procedure:	in Antimicrobials differentially extracted into three separate solutions		

Cleaned up and concentrated using SPE and solvent removal under

Analyte clean-up procedure:	Vacuum.	
10. Measurement procedure:		
a. Chemical	Confirmation	
1. Instrumentation	HPLC	
2. Detector system	UV	
3. Chromatographic column		
(if applicable)		
b. Immunochemical/Immunoas	sav	
1. Technique:	N/A	
(e.g. Elisa, RIA,		
Immunochromatog, etc)		
2. Critical reagents:	N/A	
(e.g. antibody specificity and		
availability)		
3. Special equipment required	l: N/A	
c. Microbiological		
1. Technique:	5 plate MIT (screening)	
2. Organism:		
3. Media:		
4. Special equipment required	<u>: </u>	
11 Commis/Amslets Ctability		
11. Sample/Analyte Stability		
Warning (if applicable):		
12. Literature References		
Available:		
13. Contact for Information:		
	Ms Heather Lindsay	
	Australia	
,		
	SCL (State Chemistry Lab) Cnr Sneydes and South Roads	
	Werribee VIC 3030	
	(+61) 3 9742 8779	
1	(+61) 3 9742 8779 (+61) 3 9742 8700	
	Heather.Lindsay@nre.vic.gov.au	
5. Dimii =		

B. Method Performance

9. Summary of principal steps in

/kg)	0.05mg/kg
) (mg/kg)	0.05mg/kg
entration	
sheep, pigs,	, poultry, fish and giant tiger prawn): 0.1mg/kg
	nd poultry): 0.3mg/kg
	and poultry): 0.6mg/kg
	1mg/kg
sneep). 0.1	Tillg/kg
covery?	Yes
tandard etc	External standard
0.1mg/kg	
60%	
0.1n	ng/kg
9%	
CV)	
CV)	
	entration sheep, pigs eep, pigs a sheep, pigs 0.2mg/kg sheep): 0. covery? standard etc 0.1mg/kg 60%

8. S	electivity	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 classes veterinary drugs that may also be us along with the analyte of interest		
b. Contaminants that are likely to be print in the sample	resent	
	.	
9. Type of Validation studies a. Single laboratory		
b. Multi-laboratory		
b. Multi-laboratory		
c. AOAC or other official procedure		
C. Information relevant to laboratory implementation		
1. Training and experience recommended	ioi anaiyte	
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 inf	ormation	

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,		Liver		
* * * *		Tissumize 5g of sample with sodium sulphate and potassium Carbonate, extracting into ethyl acetate.		
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 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/Immunoa	ssay	
1. Technique:	N/A	
(e.g. Elisa, RIA,		
Immunochromatog, etc)	27/4	
2. Critical reagents:	N/A	
(e.g. antibody specificity and		
Availability) 3. Special equipment require	d: N/A	
3. Special equipment require	u. IV/A	
c. Microbiological	N/A	
1. Technique:		
2. Organism:		
3. Media:		
4. Special equipment require	d:	
11. Sample/Analyte Stability		
Warning (if applicable):		
warming (ii applicable).		
12. Literature References		
Available:		
13. Contact for Information:		
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b. Country	Australia	
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f. FAX	(+61) 2 9810 8771	
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B. Method Performance

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

c. Method sensi (The smallest dithat can be me	ifference in concentration	
2. JECFA MRL	Muscle, kidney, liver and fa	at (cattle, sheep, pig

that can be n	difference in conc measured)	centration				
2. JECFA MRL	FA MRL Muscle, kidney, liver and fat (cattle, sheep, pigs and goats): 0.1mg/kg Milk (cattle and goats): 0.1mg/kg					
3. Is analytical da	ata corrected for re	ecovery?	Yes			
4. How is recove (e.g. external s	ry estimated standard; internal	standard etc		ternal standard		
5. Accuracy						
a. Concentrati	ion(s) tested	0.05mg/kg	g	0.1mg/kg	0.5mg/kg	
b. Concentrati	ion(s) measured					
c. Recovery (%)	80%		93%	93%	
6. Precision using Control tissue a. Concentrati	ion(s) tested		6-0.5mg/	kg		
	ty (within lab CV)		2%0			
Incurred drug						
a. Concentrati	ion(s) tested					
b. Repeatability (within lab CV)						
c. Reproducib	ility (between lab	CV)				
	ion is often referei				rs to the ability of the method to hemicals or drugs are also resident	

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 like in the sample	ely to be present			
9. Type of Validation studies a. Single laboratory				
b. Multi-laboratory				
c. AOAC or other official pa	rocedure			
C. Information relevant to la				
1. Training and experience reco	ommended for analyte			
2. Critical steps in the method				
3. Information on availability of Equipment	of unusual reagents or			
4. Special reagent or sample sta	ability concerns			
5. Reagent handling and safety	concerns (if any)			
6. Literature references or other	r 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.					
	1					
4. Analyte(s) measured: (specify if metabolite)						
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)				
<u></u>						
6. Test matrix (e.g. muscle, kidney, urine		Kidney				
7. Summary of principal steps sample preparation:	s in					
8. Summary of principal steps extraction procedure:	s in	Antimicrobials differentially extracted into three separate solutions				
9. Summary of principal steps Analyte clean-up procedur		Cleaned up and concentrated using SPE and solvent removal under Vacuum.				
10. Measurement procedure:						
a. Chemical		Confirmation				
1. Instrumentation		HPLC				
2. Detector system		UV-287nm				
3. Chromatographic colum (if applicable)	nn					
b. Immunochemical/Immun 1. Technique: (e.g. Elisa, RIA, Immunochromatog, etc)	oassay	ELISA (secondary screening test)				
 Critical reagents: (e.g. antibody specificity and availability) 						
3. Special equipment requi	ired:					

c. Microbiologica	ıl					
1. Technique:		5 plate	MIT (initial screening)			
2. Organism:						
3. Media:						
4. Special equip	ment required	l:				
	~	T.				
11. Sample/Analyt	•					
Warning (if ap	plicable):					
12. Literature Refe	rences					
Available:						
13. Contact for Info	ormation:					
a. Name		Ms Heather Li	ndsav			
b. Country		Australia	inusuy			
c. Affiliation		SCL (State Ch	emistry Lab)			
d. Address			nd South Roads			
		Werribee VIC				
e. Telephone	((+61) 3 9742 8	3779			
f. FAX (+61) 3 9742 87			3700			
g. Email]	Heather.Lindsay@	@nre.vic.gov.au			
B. Method Perfor						
1. a. Limit of Detec	` , `	mg/kg)	0.1mg/kg			
How was LOI	O determined?					
1 1		20) (// // //)	0.1			
b. Limit of Quantification (LOQ) (mg/kg) How was LOQ determined?		JQ) (mg/kg)	0.1mg/kg			
How was LOQ	determined?					
c. Method sensi						
(The smallest di		oncentration				
that can be measured)						
2. JECFA MRL	2. JECFA MRL Muscle (cattle, sheep and pigs): 0.1mg/kg					
Liver (cattle and sheep): 1mg						
		-	0.3mg/kg (pigs): 1mg/kg			
		heep and pigs)				
		: 0.05mg/kg (t				
3. Is analytical data	a corrected for	recovery?	Yes			
4. How is recovery	estimated		External standard			

(e.g. external standard; internal s	standa	d etc.)			
5. Accuracy					
a. Concentration(s) tested	0.1mg/kg				
b. Concentration(s) measured					
c. Recovery (%)	85%				
6. Precision using fortified Control tissue	1				
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 referent Provide accurate measurement of in The laboratory sample. Data of its	of the a	in this regard a	st when other	chemicals or	
a. Drugs of similar structure or of veterinary drugs that may also along with the analyte of interesting the structure or of the structure of	o be u				
b. Contaminants that are likely to in the sample	o be p	esent			

9. Type of Validation studies a. Single laboratory		
b. Multi-laboratory		
c. AOAC or other official p	rocedure	
C. Information relevant to la	boratory implementa	tion
1. Training and experience rec	ommended for analyte	
2. Critical steps in the method		
3. Information on availability of Equipment	of unusual reagents or	
4. Special reagent or sample st	ability concerns	
5. Reagent handling and safety	concerns (if any)	
6. Literature references or other	er 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:		tment of early immature, immature and mature liver
	fluke in cattle, buffal	o, goats and sheep.
4. Analyte(s) measured: (specify if metabolite)	Triclabendazole, Tric	clabendazole sulphone, Triclabendazole sulphoxide
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, o	etc)
7. Summary of principal steps i	
sample preparation:	carbonate, extracting into ethyl acetate.
8. Summary of principal steps in	n Evaporated residue dissolved in acetonitrile and partitioned with
Extraction procedure:	hexane. Hexane discarded and sample made up to volume with 0.02M ammonium acetate
O Summary of principal stone	. 1
9. Summary of principal steps in Analyte clean-up procedure:	
10. Measurement procedure:	
a. Chemical	LC-MS (screen and confirmation)
1. Instrumentation	
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/Immunoa	issay
1. Technique:	N/A
(e.g. Elisa, RIA,	
Immunochromatog, etc)	NY/A
2. Critical reagents:(e.g. antibody specificity and	N/A
Availability)	
3. Special equipment require	d: N/A
c. Microbiological	
1. Technique:	N/A
2. Organism:	
3. Media:	A.
4. Special equipment require	Su:
11. Sample/Analyte Stability	
Warning (if applicable):	
12. Literature References	

		,			
Available:					
13. Contact for Int	farmation.				
a. Name	Dr Robert Syn	mong			
	Australia	HOHS			
b. Country c. Affiliation		Sold			
d. Address	AMDEL Lilyf				
u. Address	30-40 Halloral	n St. Lilyfield 2040			
e. Telephone	(+61) 2 9818	1033			
f. FAX	(+61) 2 9810 8	8771			
g. Email	Robert_Symons@	@amdel.com			
B. Method Perfor	rmance				
1. a. Limit of Dete	ection (LOD) (mg/kg)	0.01mg/kg			
	D determined?				
b. Limit of Qua	antification (LOQ) (mg/kg)	0.05mg/kg			
How was LOQ					
c. Method sens	itivity				
(The smallest d	lifference in concentration				
that can be me	easured)				
	T				
2. JECFA MRL	() 88(1) 88				
	Liver and kidney (cattle): 0.3mg/kg (sheep): 0.1mg/kg				
Fat (cattle and sheep): 0.1mg/kg					
3. Is analytical dat	ta corrected for recovery?	Yes			
4. How is recovery	y estimated	External standard			
(e.g. external st	tandard; internal standard etc	2.)			
5 Acqueox					

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)	
Veterinary drugs that may also be used along with the analyse of interest	ised
b. Contaminants that are likely to be p in the sample	resent
	Т
9. Type of Validation studies a. Single laboratory	
b. Mullet-laboratory	
c. AOAC or other official procedure	

$C. \ In formation \ relevant \ to \ laboratory \ implementation$

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

Name of drug or chemical: ABAMECTIN
 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:

 $Thawing//Weighing of 20 \ grams \ of \ liver//Homogenization//Weighing \ of 1 \ gram \ of \ homogenized \ liver//Homogenization//Weighing \ of 1 \ gram \ of \ homogenized \ liver//Homogenization//Weighing \ of 1 \ gram \ of \ homogenized \ liver//Homogenization//Weighing \ of 1 \ gram \ of \ homogenized \ liver//Homogenized \ liver//Homogeni$

8. Summary of principal steps in extraction procedure:

 $Extraction\ with\ methanol/acetonitrile//Ultrasonication// \textit{C} entrifugation//Transfer\ the\ supernatant//Evaporation\ under\ nitrogen$

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 20μ l 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; 5μ m) 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

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B. Method performance

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

- 1.b. Limit of quantification (LOQ) (mg/kg): $7.5 \mu q/kq$
- 1.c. Method sensitivity:
- 2. JECFA MRL: $100 \mu g/kg$ (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 μ g/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: $20 \mu g/kg$ (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 Screening method for benzimidazoles in milk by HPLC/UV method:

3. Column/Special equipment:

Inertsil ODS3 desactivated (150x4.6mm; 5μ m) and a guard column Inertsil ODS3

 $(10 \times 3 mm)$

- 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

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http://www.fougeres.afssa.fr/

B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $10 \mu g/kg$
- 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: $100 \mu g/kg$ (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 Screening method for benzimidazoles in milk by HPLC/UV method:

- 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 (125×4mm; 5μ m) + Guard column RP18e (4×4mm)

- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:
- 13. Contact for information:

a. Name: Delepine, Bernard

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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 μ g/kg

- 1.c. Method sensitivity:
- 2. JECFA MRL: 60 μ g/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: $12.5//25//50//100 \mu g/kg$
 - b. Concentration(s) measured:
 - c. Recovery (%):
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $12.5//25//50//100 \mu g/kg$
 - 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

Name of drug or chemical: AZAPERONE
 Drug or chemical class: Butyrophenones
 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 50μ L 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; 5μ m)
- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:
- 13. Contact for information:

a. Name: Roudaut, Brigitte

b. Country: France

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $1 \mu g/kg$
- 1.b. Limit of quantification (LOQ) (mg/kg): $10 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: $100 \mu g/kg$ (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: $10//50//100//200 \mu g/kg$
 - 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: **BENZYLPENI**CILLIN

2. Drug or chemical class: Penicillins3. 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 (125×4mm;5 μ m) with guard column RP18e(4×4mm)

- 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:

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b. Country: France

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WebSite:

B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 3 mg/kg
- 1.b. Limit of quantification (LOQ) (mg/kg): $12.5 \mu q/kq$
- 1.c. Method sensitivity:
- 2. JECFA MRL: 50 μ g/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: $25//50//75//100/\mu_q/k_q$
 - 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: $25//50//75//100 \mu g/kg$ 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: Penicillins3. 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 $65^{\circ}C$ 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;5 μ m) 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 $^{\circ}C$ 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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $3 \mu q/kq$
- 1.b. Limit of quantification (LOQ) (mg/kg): $25 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: 50 μ g/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: $25//50//100//200 \mu g/kg$ (n=4x12)
 - b. Concentration(s) measured: $24.7//49.9//99.1//203.1 \mu g/kg (n=4x12)$

- c. Recovery (%): 74 +/- 6 % (n=48)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $25//50 \mu g/kg$ (n=12) b.RepeatabilityWithinlabCV: 7.9 %//5.6 % (n=12)

Name of the Quantitative determination of 8 penicillins in pig muscle by method: 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 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: Penicillins3. 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//G rinding//W eighing 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; 5μ m) 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 $^{\circ}$ 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

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 2.5 μ g/kg

1.b. Limit of quantification (LOQ) (mg/kg): 4.0 μ g/kg

1.c. Method sensitivity:

- 2. JECFA MRL: 4 μ g/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: $4//8//16//32//64 \mu g/kg$ (n=4)
 - b. Concentration(s) measured: $4.16//7.77//15.34//29.69//62.13 \mu g/kg$ (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: $4//16 \mu g/kg$ (n=16) b.RepeatabilityWithinlabCV: 7.9 %//3.5 % (n=16)

Name of the 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 50μ L 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;5 μ m)

- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:
- 13. Contact for information:

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $1 \mu g/kg$

- 1.b. Limit of quantification (LOQ) (mg/kg): 10 μ g/kg
- 1.c. Method sensitivity:
- 2. JECFA MRL: 25 μ g/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: $10//50//100//200 \mu g/kg$
 - 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

Name of drug or chemical: CEFTIOFUR
 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 $50^{\circ}C$ with pH 9 dithioerytritol buffer for converting ceftiofur into

desfuroy|ceftiofur//

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;5 μ m) 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 $+4^{\circ}C$ 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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 70 μ g/kg in muscle and 13 μ g/kg in milk
- 1.b. Limit of quantification (LOQ) (mg/kg): 250 μ g/kg in muscle and 50 μ g/kg in milk
- 1.c. Method sensitivity:
- 2. JECFA MRL: 1000 μ g/kg in muscle and 100 μ g/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: $1000 \mu g/kg (n=8 \text{ musc/e})/100 \mu g/kg (n=8 \text{ milk})$
 - b. Concentration(s) measured: 950 μ g/kg (n=8 in muscle)//98.4 μ g/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: $1000 \mu g/kg (n=8 \text{ in muscle})//100 \mu g/kg (n=8 \text{ in milk})$

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): Chloramphenical

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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 0.16 μ g/kg (CCalpha)
- 1.b. Limit of quantification (LOQ) (mg/kg): 0.25 μq/kq
- 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: $0.25//0.5//1.0//2.0 \mu g/kg$
 - b. Concentration(s) measured:
 - c. Recovery (%):
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $0.25//0.5//1.0//2.0 \mu q/kq$
 - b. Repeatability Within lab CV:

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): Chloramphenical
- 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 (125×4mm; 5μ m) + Guard column RP18e (4×4mm)

- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:

13. Contact for information:

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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): $0.5 \mu g/kg$
- 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 (125×4mm; 5μ m) + Guard column RP18e (4×4mm)

- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:
- 13. Contact for information:

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not valid.

B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): <1 uq/kq
- 1.b. Limit of quantification (LOQ) (mg/kg): $1 \mu g/kg$
- 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: $1.0//2.5//5.0//10.0 \mu g/kg$
 - 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 (125 \times 4mm;5 μ m)

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

Stocked standard solutions in methanol stored 1 month at -20 $^{\circ}C$ 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:

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 15 μ g/kg in muscle//170 μ g/kg in kidney

1.b. Limit of quantification (LOQ) (mg/kg): 50 μ g/kg in muscle//300 μ g/kg in kidney

1.c. Method sensitivity:

2. JECFA MRL: $100 \mu g/kg$ in M//600 $\mu g/kg$ 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: $100 \mu g/kg//600 \mu g/kg$ (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: $100 \mu g/kg$ in M//600 $\mu g/kg$ 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

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $20 \mu g/kg$

1.b. Limit of quantification (LOQ) (mg/kg): 50 μ q/kq

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: $100 \mu g/kg (n=5)$
 - b. Concentration(s) measured: 91.50 μ g/kg (n=5)
 - c. Recovery (%): 56.1 +/- 6.7 % (n=8)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $50//100//150//200 \mu g/kg$ (n=8) b.RepeatabilityWithinlabCV: 10.8 % for n=5 samples of $100 \mu g/kg$

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

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $2 \mu g/kg$

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: $15 \mu q/kq$ (n=58)

b. Concentration(s) measured:

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

6. Precision using fortified control tissue:

a. Concentration(s) tested: $15 \mu g/kg$ (n=12)

b.RepeatabilityWithinlabCV: 8.4 %

Name of the 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): Danof loxacin
- 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; 5μ m) + 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

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not valid.

B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $5 \mu g/kg$
- 1.b. Limit of quantification (LOQ) (mg/kg): 7.5 μ q/kq
- 1.c. Method sensitivity:
- 2. JECFA MRL: 200 μ g/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: $99.5//201.1//299.3//600.1 \mu g/kg (n=5)$
 - c. Recovery (%):
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $100//200//300//600 \mu g/kg$ 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: Quinolones3. Veterinary use: Antimicrobial

4. Analyte(s) measured (specified if metabolite): Danof loxacin

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;5 μ m;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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $7 \mu_{\rm Q}/k_{\rm Q}$
- 1.b. Limit of quantification (LOQ) (mg/kg): 150 μ g/kg
- 1.c. Method sensitivity:
- 2. JECFA MRL: 200 μ g/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 μ g/kg (n=28)
 - b. Concentration(s) measured:
 - c. Recovery (%): 67 +/- 12 % (n=28)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: 300 μ g/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 (125×4mm; 5μ m) + Guard column RP18e (4×4mm)

- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:
- 13. Contact for information:

a. Name: Delepine, Bernard

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B. Method performance

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

- 1.c. Method sensitivity:
- 2. JECFA MRL: 600 μ g/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: $250//500//750//1000 \mu q/kq$
 - b. Concentration(s) measured:
 - c. Recovery (%): 43.2 +/- 5.3 (n=5)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $250//500//750//1000 \mu_{\rm Q}/k_{\rm Q}$
 - 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 20μ l 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 (125×4mm; 5μ m) with quard column RP18-e (4×4mm)

- 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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 2.5 μ g/kg
- 1.b. Limit of quantification (LOQ) (mg/kg): $7.5 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: $100 \mu g/kg$ (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: $100 \mu g/kg$ (n=6)
 - b. Concentration(s) measured:
 - c. Recovery (%): 77.5 +/- 2.7 % (n=6)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $100 \mu g/kg$ (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 (125 \times 4mm;5 μ m)

- 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:

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 12 μ g/kg in muscle//160 μ g/kg in kidney

1.b. Limit of quantification (LOQ) (mg/kg): 50 μ g/kg in muscle//300 μ g/kg in kidney

1.c. Method sensitivity:

2. JECFA MRL: $100 \mu g/kg$ in M//600 $\mu g/kg$ 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: $100 \mu g/kg//600 \mu g/kg$ (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: $100 \mu g/kg$ in M//600 $\mu g/kg$ 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; 5μ m) + 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

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not valid.

B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $5 \mu g/kg$

1.b. Limit of quantification (LOQ) (mg/kg): $7.5 \mu g/kg$

- 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: $30//50//100//200 \mu q/kq$
 - b. Concentration(s) measured: $29.4//48.7//103.0//198.9 \mu g/kg$
 - c. Recovery (%):
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $30//50//100//200 \mu g/kg$
 - 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: Quinolones3. 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

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not valid.

B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $1 \mu q/kq$
- 1.b. Limit of quantification (LOQ) (mg/kg): $7.5 \mu g/kg$
- 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: $15 \mu g/kg$ (n=58)
 - b. Concentration(s) measured:
 - c. Recovery (%): 77 +/- 11 % (n=58)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: 15 μ g/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 Screening method for benzimidazoles in milk by HPLC/UV method:

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 method:

3. Column/Special equipment:

Inertsil ODS3 desactivated (150×4.6mm;5µm) and a guard column Inertsil ODS3

 $(10 \times 3 \text{mm})$

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

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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: $100 \mu g/kg$ (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: $100 \mu g/kg$ (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 μ g/kg (n=14)
 - b.RepeatabilityWithinlabCV:

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

- 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; 5μ m) + 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

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not valid.

B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $5 \mu g/kg$
- 1.b. Limit of quantification (LOQ) (mg/kg): 7.5 μ q/kq
- 1.c. Method sensitivity:
- 2. JECFA MRL: 500 μ g/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: $15//30//50//100 \mu g/kg$
 - b. Concentration(s) measured: $15.6//29.7//49.4//100.3 \mu_q/kq$
 - c. Recovery (%):
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $15//30//50//100 \mu q/kq$
 - 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;5 μ m;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

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $7 \mu g/kg$
- 1.b. Limit of quantification (LOQ) (mg/kg): $75 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: 500 μ g/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: $600 \mu g/kg$ (n=12)
 - b. Concentration(s) measured:
 - c. Recovery (%): 66.4 +/- 6.6 % (n=12)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $600 \mu q/kq$ (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 Confirmatory method for 3 aminoglycosides in porcine kidney

by

method: 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 Confirmatory method for 3 aminoglycosides in porcine kidney

by

method: LC/MS

3. Column/Special equipment:

RP18e (125×4mm; 5μ m) + Guard column RP18e (4×4mm)

- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:

13. Contact for information:

a. Name: Delepine, Bernard

b. Country: France

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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 μ g/kg
- 1.c. Method sensitivity:
- 2. JECFA MRL: $5000 \mu g/kg$ (52nd meeting Feb 1999)
- 3. Is analytical data corrected for recovery?
- 4. How is recoverey estimated?
- 5. Accuracy
 - a. Concentration(s) tested: $500//1000//1500//2000 \mu g/kg$
 - b. Concentration(s) measured:
 - c. Recovery (%):
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $500//1000//1500//2000 \mu_q/kq$
 - b.RepeatabilityWithinlabCV:

Name of the Confirmatory method for 3 aminoglycosides in porcine kidney by

method: 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

Name of drug or chemical: IVERMECTIN
 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 20μ l 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 (125×4mm; 5μ m) with guard column RP18-e (4×4mm)

- 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

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 2.5 μ g/kg
- 1.b. Limit of quantification (LOQ) (mg/kg): 7.5 μ g/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
- Accuracy
 - a. Concentration(s) tested: $100 \mu g/kg$ (n=6)
 - b. Concentration(s) measured:
 - c. Recovery (%): 78.8 +/- 3.3 % (n=6)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $100 \mu g/kg (n=6)$
 - 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 Determination of ivermectin residues in milk by HPLC/FLD method:

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

3.	procedure	Ė
3 .	proc	eaure

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 Determination of ivermectin residues in milk by HPLC/FLD method:

3. Column/Special equipment:

Licrospher 100, RP18-e (125x4mm; 5μ m) 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

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $0.5 \mu g/kg$

1.b. Limit of quantification (LOQ) (mg/kg): $1 \mu q/kq$

1.c. Method sensitivity:

2. JECFA MRL: $10 \mu g/kg$ (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: $4 \mu g/kg$ (n=6)
 - b. Concentration(s) measured:
 - c. Recovery (%): 77.7 +/- 6.5 % (n=6)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $4 \mu g/kg$ (n=6)
 - b.RepeatabilityWithinlabCV: 5.7 %

Name of the Determination of ivermectin residues in milk by HPLC/FLD method:

- 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 Determination of avermectin and moxidectin residues in liver by method: HPLC/FLD

A. Descriptive information

Name of drug or chemical: MOXIDECTIN
 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 20μ l 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; 5μ m) 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:

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 0.8 μ g/kg

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

1.c. Method sensitivity:

2. JECFA MRL: $100 \mu g/kg$ (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: $100 \mu q/kq$ (n=6)
 - b. Concentration(s) measured:
 - c. Recovery (%): 79.7 +/- 6.5 % (n=6)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $100 \mu g/kg$ (n=6)
 - b.RepeatabilityWithinlabCV: 4.1 %

Name of the Determination of avermectin and moxidectin residues in liver by method: 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 Confirmatory method for 3 aminoglycosides in porcine kidney

bν

method: 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 Confirmatory method for 3 aminoglycosides in porcine kidney

by

method: LC/MS

3. Column/Special equipment:

RP18e (125×4mm; 5μ m) + Guard column RP18e (4×4mm)

4. Media:

- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:

13. Contact for information:

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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 μ g/kg

1.c. Method sensitivity:

2. JECFA MRL: 20000 μ g/kg (52nd meeting - Feb 1999)

3. Is analytical data corrected for recovery?

4. How is recoverey estimated?

- 5. Accuracy
 - a. Concentration(s) tested: $500//1000//1500//2000 \mu g/kg$
 - b. Concentration(s) measured:
 - c. Recovery (%):
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $500//1000//1500//2000 \mu_{\rm Q}/k_{\rm Q}$
 - b.RepeatabilityWithinlabCV:

Name of the Confirmatory method for 3 aminoglycosides in porcine kidney

by

method: 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 Determination of neomycin in milk by HPLC/FLD method:

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 50 µL 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 Determination of neomycin in milk by HPLC/FLD method:

3. Column/Special equipment:

Licrospher 100, RP18-e (125×4mm;5 μ m) with guard column RP18-e (4×4mm)//post column

reactor

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

Stocked standard solutions in ultra-pure water stored 2 month at $+4^{\circ}C$ and residues of neomycin in milk

stable for 8 months at -20°C

12. Literature references available:

13. Contact for information:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 100 μg/kg
- 1.b. Limit of quantification (LOQ) (mg/kg): 250 μ g/kg
- 1.c. Method sensitivity:
- 2. JECFA MRL: 500 μ g/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 μ g/kg b.RepeatabilityWithinlabCV: 1.4 %

Name of the Determination of neomycin in milk by HPLC/FLD method:

- 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

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

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $25 \mu g/kg$
- 1.b. Limit of quantification (LOQ) (mg/kg): $100 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: 200 μ g/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: $100//200//400//800 \mu_q/kq$ (n=72)
 - b. Concentration(s) measured:
 - c. Recovery (%): 42 +/- 6 % (n=72)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: 200 μ q/kq (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 Screening method for benzimidazoles in milk by HPLC/UV method:

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 Screening method for benzimidazoles in milk by HPLC/UV method:

3. Column/Special equipment:

Inertsil ODS3 desactivated (150×4.6mm;5µm) and a guard column Inertsil ODS3

 $(10 \times 3 mm)$

- 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:

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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: $100 \mu g/kg$ (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: $100 \mu g/kg$ (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 μ g/kg (n=14)
 - b.RepeatabilityWithinlabCV:

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

- 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;5 μ m;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:

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $5 \mu g/kg$

- 1.b. Limit of quantification (LOQ) (mg/kg): $75 \mu g/kg$
- 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 μ g/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%

<u>Name of the</u> Determination of 3 quinolones in fish muscle by HPLC/FLD method:

- 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;5 μ m)

4. Media:

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

Stocked standard solutions in methanol stored 1 month at -20 $^{\circ}C$ 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:

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b. Country: France

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $8 \mu g/kq$ in muscle//80 $\mu g/kq$ in kidney

1.b. Limit of quantification (LOQ) (mg/kg): 50 μ g/kg in muscle//300 μ g/kg in kidney

1.c. Method sensitivity:

2. JECFA MRL: $100 \mu g/kg$ in Muscle//600 $\mu g/kg$ 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 μ g/kg in Muscle (n=12)//600 μ g/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: $100 \mu g/kg$ in Muscle//600 $\mu g/kg$ 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

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $35 \mu g/kg$ 1.b. Limit of quantification (LOQ) (mg/kg): $50 \mu g/kg$

- 1.c. Method sensitivity:
- 2. JECFA MRL: $100 \mu g/kg$ (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: $100 \mu g/kg$ (n=5)
 - b. Concentration(s) measured: 99.88 μ q/kq (n=5)
 - c. Recovery (%): 57.1 +/- 6.4 % (n=8)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $50//100//150//200 \mu g/kg$ (n=8) b.RepeatabilityWithinlabCV: 8.9 % for n=5 samples of $100 \mu g/kg$

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; 5μ m) + 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

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not valid.

B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $5 \mu g/kg$
- 1.b. Limit of quantification (LOQ) (mg/kg): $7.5 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: 10 μ g/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. Repeatability Within lab CV:

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;5 μ m;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

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): $2 \mu g/kg$

1.b. Limit of quantification (LOQ) (mg/kg): 15 μ q/kq

- 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 μ g/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: $30 \mu g/kg$ (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: Quinolones3. 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;5 μ m;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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): $1 \mu g/kg$
- 1.b. Limit of quantification (LOQ) (mg/kg): $25 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: 10 μ g/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: $50 \mu g/kg$ (n=58)
 - b. Concentration(s) measured:
 - c. Recovery (%): 71+/- 10 % (n=58)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $50 \mu g/kg$ (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:

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b. Country: France

c. Affiliation: AFSSA - LERMVD, Laboratoire d'études et de recherches sur les

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 30 μ g/kg
- 1.b. Limit of quantification (LOQ) (mg/kg): $100 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: 200 μ g/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: $100//200//400//800 \mu g/kg$ (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	Litaratura	references	or other	ucafu
t).	Lucialuic	references	or omer	useru

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 (125×4mm; 5μ m) + Guard column RP18e (4×4mm)

- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:
- 13. Contact for information:

a. Name: Delepine, Bernard

b. Country: France

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not valid.

B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 117 μ g/kg (n=5)
- 1.b. Limit of quantification (LOQ) (mg/kg): 250 μ g/kg
- 1.c. Method sensitivity:
- 2. JECFA MRL: 600 μ g/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: $250//500//750//1000 \mu g/kg$
 - b. Concentration(s) measured:
 - c. Recovery (%): 45.7 +/- 6.1 % (n=5)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $250//500//750//1000 \mu_q/kq$
 - 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 Determination of 5 sulfonamides in milk by HPLC/FLD method:

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; 5μ m) with guard column RP18-e (4x4mm)

- 4. Media:
- 11. Sample/Analyte stability warning (if applicable):
- 12. Literature references available:

13. Contact for information:

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b. Country: France

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 10 μ g/kg

1.b. Limit of quantification (LOQ) (mg/kg): 50 μ q/kq

- 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: $100 \mu g/kg$ (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 $30^{\circ}C$ //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 50μ L 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 Determination of sulfonamide residues in muscle by HPLC/UV method:

3. Column/Special equipment:

Licrospher 100, RP18-e (125x4mm; 5μ m) with guard column RP18-e (4x4mm)

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

12. Literature references available:

13. Contact for information:

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b. Country: France

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 10 ug/kg

1.b. Limit of quantification (LOQ) (mg/kg): 50 μ g/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: $100//500 \mu q/kq$ (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 (125 \times 4mm;5 μ m)

- 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

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B. Method performance

1.a. Limit of detection (LOD) (mg/kg): 9 μ g/kg in muscle//60 μ g/kg in kidney

- 1.b. Limit of quantification (LOQ) (mg/kg): 50 μ g/kg in muscle//300 μ g/kg in kidney
- 1.c. Method sensitivity:
- 2. JECFA MRL: $100 \mu g/kg$ in M//600 $\mu g/kg$ 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: $100 \mu g/kg//600 \mu g/kg$ (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: $100 \mu g/kg$ in M//600 $\mu g/kg$ 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: **TETR***ACYCLINE*

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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 9 μ g/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: $100 \mu g/kg (n=5)$
 - b. Concentration(s) measured: 93.47 μ g/kg (n=5)
 - c. Recovery (%): 66.8 +/- 7.4 % (n=8)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $50//100//150//200 \mu g/kg$ (n=8) b.RepeatabilityWithinlabCV: 8.5 % for n=5 samples of 100 $\mu g/kg$

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 Screening method for benzimidazoles in milk by HPLC/UV method:

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 Screening method for benzimidazoles in milk by HPLC/UV method:

3. Column/Special equipment:

Inertsil ODS3 desactivated (150x4.6mm; 5μ m) and a guard column Inertsil ODS3

 $(10 \times 3 mm)$

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:

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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: $100 \mu g/kg$ (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: $100 \mu g/kg$ (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: $100 \mu g/kg (n=14)$
 - b.RepeatabilityWithinlabCV:

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

- 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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 15 μ g/kg
- 1.b. Limit of quantification (LOQ) (mg/kg): $25 \mu g/kg$
- 1.c. Method sensitivity:
- 2. JECFA MRL: $100 \mu \text{g/kg}$ (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: $25//50//100//200 \mu_{\rm g}/k_{\rm g}$ (n=72)
 - b. Concentration(s) measured:
 - c. Recovery (%): 59.2 +/- 7.6 % (n=72)
- 6. Precision using fortified control tissue:
 - a. Concentration(s) tested: $50 \mu g/kg$ (n=18)
 - b.RepeatabilityWithinlabCV: 12.1 %

Name of the Determination of 4 macrolide residues (spiramycin, method: 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

Name of drug or chemical: TYLOSIN
 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:

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B. Method performance

- 1.a. Limit of detection (LOD) (mg/kg): 15 μ g/kg
- 1.b. Limit of quantification (LOQ) (mg/kg): $25 \mu g/kg$
- 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: $25//50//100//200 \mu g/kg$ (n=72)
 - b. Concentration(s) measured:
 - c. Recovery (%): 63.2 +/- 5.9 % (n=72)
- 6. Precision using fortified control tissue:

a. Concentration(s) tested: $50 \mu g/kg$ (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

Instrumentation
 Detector system
 HPLC
 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)

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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 $\sqrt{}$

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

	Oxytetracycli	Oxytetracycli	Oxytetracycli
	ne (mg/kg)	ne(mg/kg)	ne(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

$\frac{\text{OUTLINE OF SCIENTIFIC ISSUES COMMONLY CONSIDERED IN THE DEVELOPMENT AND }}{\text{VALIDATION OF ANALYTICAL METHODS}}$

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 ± 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 $\,\mu l$ of std. 0.25 $\mu g/ml$,TC chromatographic standard solution produces 3 distinct peaks

and resolution between oxytetracycline and tetracycline or chlortetracycline peaks should ≥ 1.5 .

* Readiness to perform assessment

Test accracy and precission by test linearity of calibration curve in the range of 0.05 - 1.00 $\mu g/ml$ for

oxytetracycline and tetracycline, 0.10 - $2.00~\mu g/ml$ for chlortetracycline every month; gave correlation

coefficient \geq 0.995, and make standard control chart from std. Tetracyclines 0.25 μ g/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 $0.25\,\mu g/ml \geq 3$ 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 $0.10\,\text{mg/kg}$ CV = 7.38%, $0.20\,\text{mg/kg}$ CV = 5.12%, $0.40\,\text{mg/kg}$ 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

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