Experience share on veterinary drug multi-residue analysis

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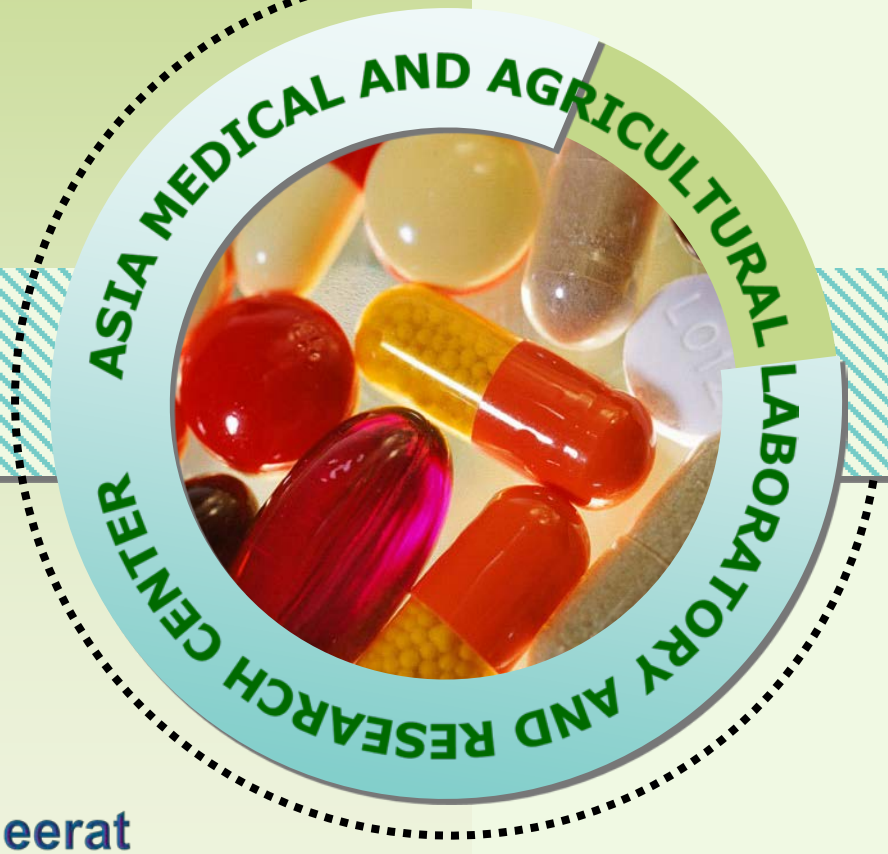


Nittaya Tangpakdeerat
Bangkok, Thailand
E-mail : Nittayatang@gmail.com

AMARC

Outline

1. Introduction
2. Applied methods of analysis
3. Technical Comments

Introduction

PT sample

Sample A

1) 3-amino-2-oxazolidone (AOZ)

2) 5-morpholinomethyl-3-amino-2-oxazolidone (AMOZ)

3) Sulfamethoxazole

4) Sulfadimidine

5) Sulfaquinoxaline

6) Ciprofloxain

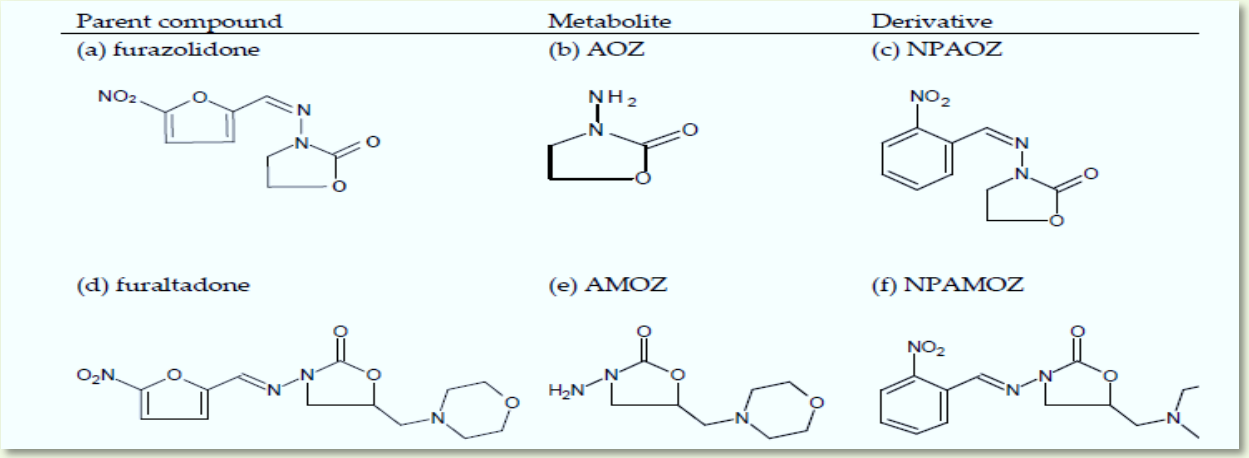
Sample B

Freeze dried chicken contained in sealed foil bag

Introduction

Nitrofurans

Parent drug unstable → **Mild acid in human** → **Metabolites form**



- Broad spectrum synthetic antimicrobial agents
- used to prevent gastrointestinal infections
- mutagenic and carcinogenic

Introduction

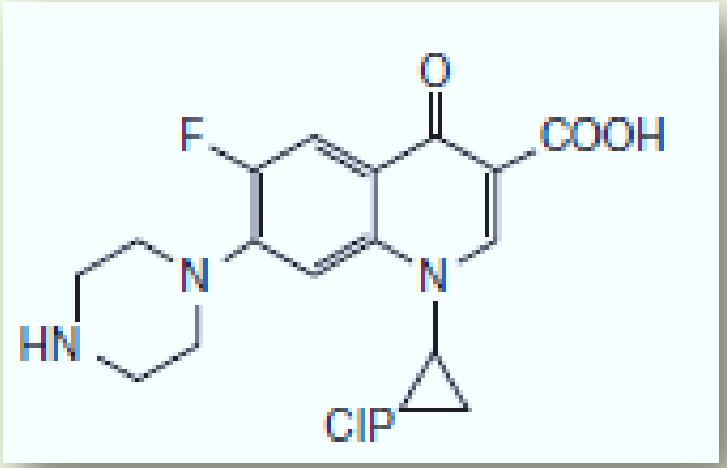
The Minimum Required Perfomance Limits (MRPL)

Compound	(µg/kg)
Nitrofurans Metabolites (SEM, AOZ, AMOZ, AHD)	1

Ref : Commission =Decision 2003/181/EC.

Introduction

Ciprofloxacin is amphoteric compound that is soluble in polar organic solvents, slightly soluble in water and insoluble nonpolar solvents



Nikolaos A. Botsoglou, *Encyclopedia of Chromatography*

Introduction

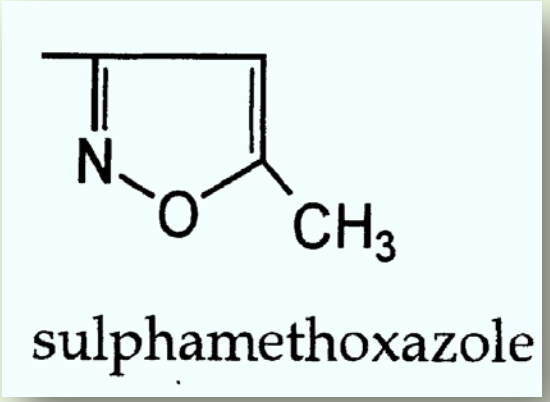
The maximum residue limits (MRL)

Compound	in muscle (µg/kg)
Sum of Enrofloxacin and Ciprofloxacin	100

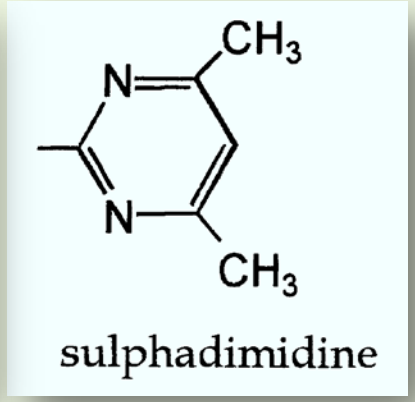
Ref : Commission Regulation (EU) 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, OJ L 15/1, 20.01.2010, p. 29 (ANNEX I).

Introduction

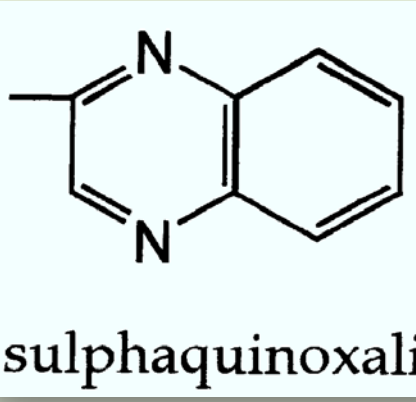
Sulfonamides are one class of antimicrobial agent used widely in the livestock industry to promote growth



sulphamethoxazole



sulphadimidine



sulphaquinoxaline

Journal of Chromatography A, 937 (2001) 49–55

Introduction

The maximum residue limits (MRL)

Compound	in muscle (µg/kg)
Sulfonamides (all substances belonging to the sulfonamide group)	100

Ref : Commission Regulation (EU) 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, OJ L 15/1, 20.01.2010, p. 1–2 (ANNEX I).

Applied methods of analysis

Nitrofurans Metabolite (AOZ, AMOZ)

Freeze dried sample + DI water

↓

Stir for 15 mins

↓ - d4-AOZ, d5-AMOZ

0.2 M HCl 10 mL, 0.1 M 2-NBA 0.1mL

↓

Incubated at 55 ^{0}C, 4 hrs

↓

add 0.3 M $Na_3PO_4.12H_2O$ 2 mL (adjust pH 7±0.5)

↓

Ethyl acetate 5 mL, 2 times

↓

Evaporated to dryness at 40 ^{0}C

↓

Reconstituted solution 0.5 mL

↓

PTFE syringe filter

↓

LC-MS/MS

Ref : In-house method based *on Journal Chromatogr.B* 856 (2007), p.178-189

Applied methods of analysis

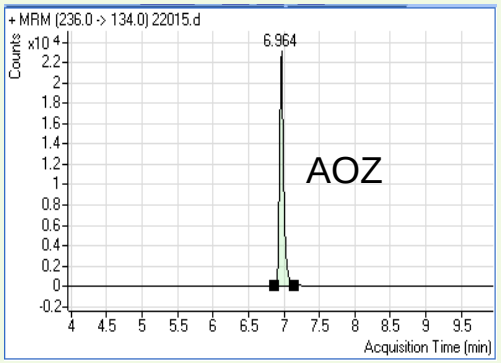
Determination technique

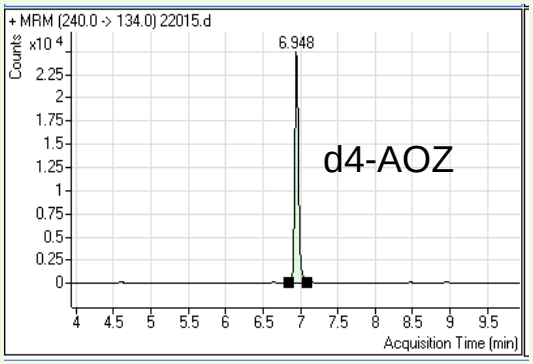
LC-MS/MS	:	Agilent model HP1200, 6410A
Column	:	Symmetry C18, 3.9 mm x 150 mm
Flow rate	:	0.4 mL / min
Run time	:	10 min
Column temperature	:	40 ^{0}C
Injection volume	:	10 μL
Mobile phase A	:	0.1 % Formic acid in DI water
Mobile phase B	:	0.1 % Formic acid in Acetonitrile
Ion source	:	ESI
Ion Polarity	:	Positive
Delta EMV	:	600 V
Gas Temp	:	325 ^{O}C
Gas Flow	:	10 L/min
Nubulizer	:	50 psi
Capillary	:	4500 V

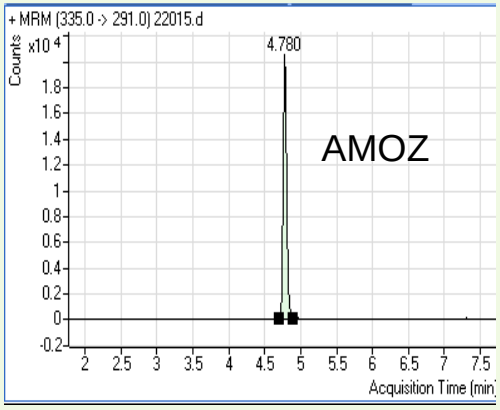
Applied methods of analysis

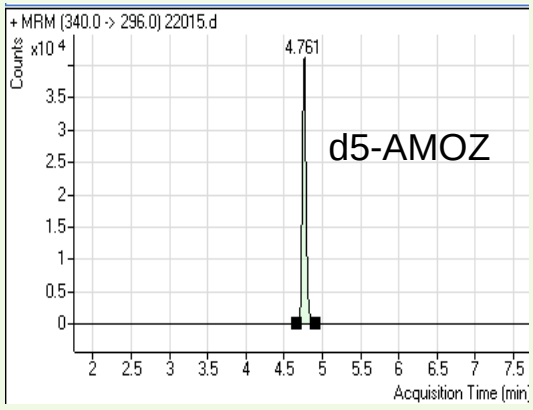
Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Dwell	Fragmentor (V)	Collision Energy (eV)
AOZ	236	134	30	120	6
	236	104	30	120	22
d4-AOZ	240	134	30	110	4
AMOZ	335	291	30	90	3
	335	262	30	90	12
d5-AMOZ	340	296	30	120	7

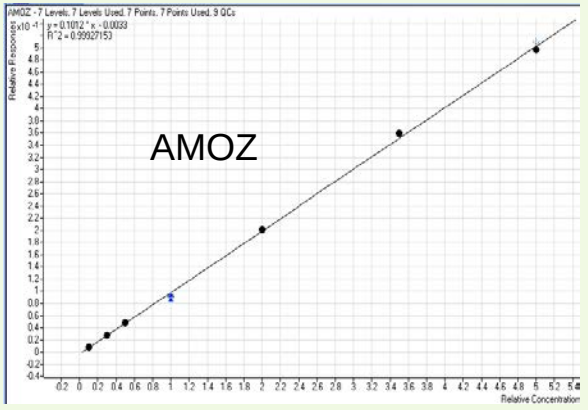
Applied methods of analysis











Applied methods of analysis

- **Fluoroquinolone (Ciprofloxacin)**

Freeze dried sample + DI water
↓
Stir for 15 mins
↓ - d5-NOR
0.2% formic acid in acetonitrile 10 mL, Na_2SO_4 ~1 g
↓
Homogenize 1 min
↓
Centrifuge at 4,000 rcf, 5 mins
↓
Evaporated to dryness at 40 ^{0}C
↓
Reconstituted solution 0.5 mL, hexane 2 mL
↓ mix
Remove hexane, PTFE syringe filter
↓
LC-MS/MS

Ref : In - house method based on
Journal of Food and Drug Analysis
Vol. 18, No.2, 2010, p. 87-97

Applied methods of analysis

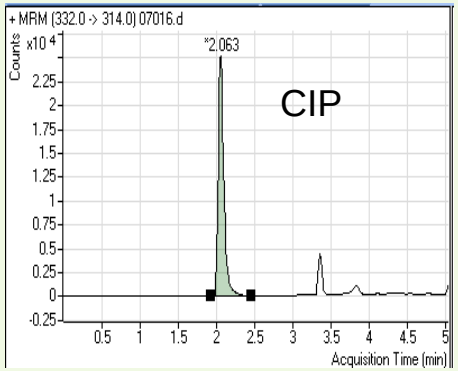
Determination technique

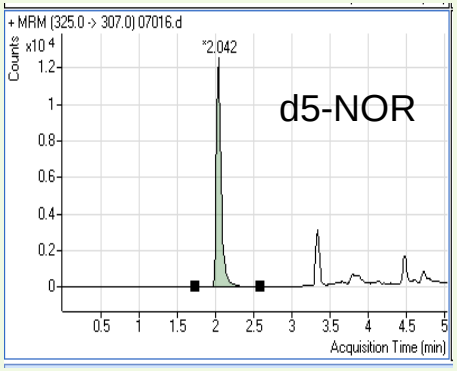
LC-MS/MS	:	Agilent model HP1200, 6410A
Column	:	Symmetry C18, 3.9 mm x 150 mm
Flow rate	:	0.5 mL / min
Run time	:	7 min
Column temperature	:	35 ^{0}C
Injection volume	:	20 μL
Mobile phase A	:	0.1 % Formic acid in DI water
Mobile phase B	:	Acetonitrile
Ion source	:	ESI
Ion Polarity	:	Positive
Delta EMV	:	500 V
Gas Temp	:	325 ^{O}C
Gas Flow	:	10 L/min
Nubulizer	:	45 psi
Capillary	:	3500 V

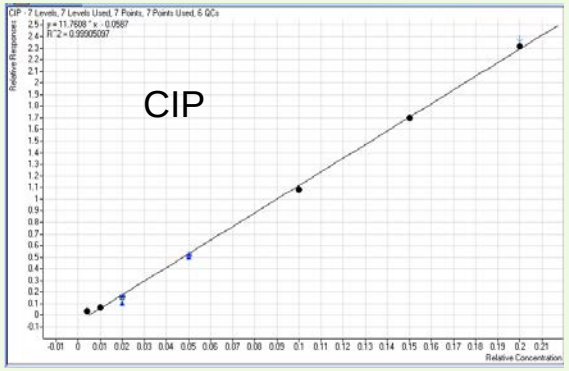
Applied methods of analysis

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Dwell	Fragmentor (V)	Collision Energy (eV)
CIP	332	314	25	100	20
		288	25	100	15
d5-NOR	325	307	25	100	20

(CIP = Ciprofloxacin, d5-NOR = d5-Norfloxacin)







Applied methods of analysis

Sulfonamides (Sulfamethoxazole, Sulfadimidine, Sulfaquinoxaline)

Freeze dried sample + DI water

↓

Stir for 15 mins

↓ - d4-SMR

0.2 M HCl 5 mL, vortex

↓ Incubate at room temp 30 mins

0.3 M citric acid 20 mL

↓ adjust pH ~ 4

Vortex, centrifuge at 4000 rcf, 5 mins

↓

Load SPE cartridge (OASIS HLB 200 mg/6 mL)

↓ - pre-condition ACN 5 mL, DI water 5 mL

Load sample

↓ - wash DI water 5 mL

Eluted ACN 5 mL, evaporated to dryness at 40 ^{0}C

↓

Reconstituted solution 1 mL, PTFE syringe filter

↓

LC-MS/MS

Ref : In-house method based on Journal Sci. Food Agric. 89 (2009), p. 63-72

Applied methods of analysis

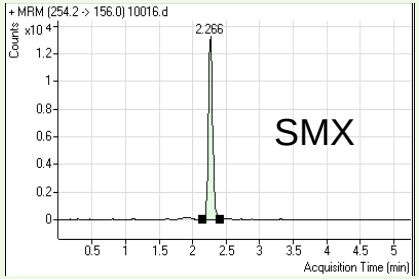
Determination technique

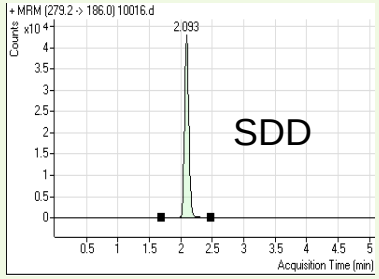
LC-MS/MS	:	Agilent model HP1200, 6410A
Column	:	Symmetry C18, 3.9 mm x 150 mm
Flow rate	:	0.5 mL / min
Run time	:	7 min
Column temperature	:	35 ^{0}C
Injection volume	:	10 μL
Mobile phase A	:	0.1 % Formic acid
Mobile phase B	:	0.1 % Formic acid in acetonitrile
Ion source	:	ESI
Ion Polarity	:	Positive
Delta EMV	:	600 V
Gas Temp	:	325 ^{O}C
Gas Flow	:	10 L/min
Nubulizer	:	35 psi
Capillary	:	5000 V

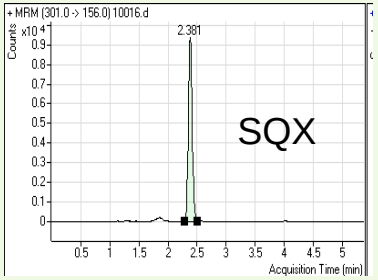
Applied methods of analysis

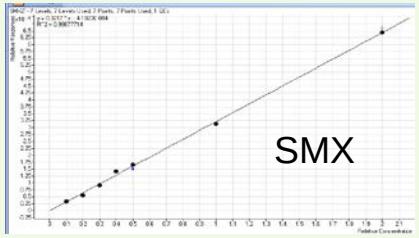
Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Dwell	Fragmentor (V)	Collision Energy (eV)
SMX	254.2	156	15	100	10
	254.2	188	15	100	10
SDD	279.2	186	15	100	20
	279.2	156	15	100	20
SQX	301	156	15	130	10
	301	146	15	130	10
d4-SMR	265.2	109.9	15	100	10

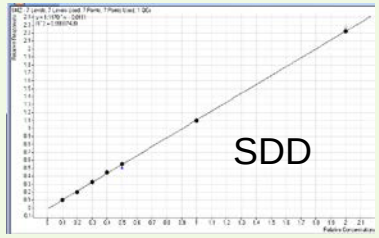
(SMX = Sulfamethoxazole, SDD = Sulfadimidine, SQX = Sulfaquinoxaline, d4-SMR = d4- sulfamerazine)

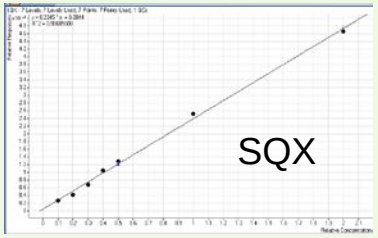








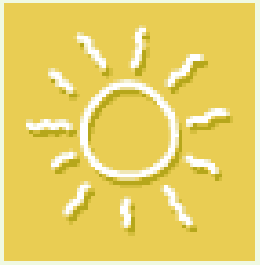




Technical Comments

Factor affecting the test

- Moisture
- pH
- Time
- Temperature



Technical Comments

- Sample handling error
- Inadequate methods
- Equipment failure
- Calculation error
- Reporting error

Technical Comments

- Sample weight
- The preparation of the standard
- Internal standard technique
- Matrix calibration curve
- The mass used in the test

Bangkok, Thailand

ASIA MEDICAL AND AGRICULTURAL LABORATORY AND RESEARCH CENTER

361, 361/1-4 Ladprao Soi
122, Wangthonglang,
Bangkok, Thailand 10310

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AMARC Tel : 66 2 9342381-3

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