THE ANALYSES OF MACROLIDES, TETRACYCLINE AND SULFONAMIDE ANTIBIOTICS IN ANIMAL TISSUES USING LC-MS/MS

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INTRODUCTION

Veterinary drugs are used in animal husbandry and aquaculture for therapeutic or disease-preventive reasons and, in some cases, to promote growth of livestock. However, when specified withdrawal periods are not observed, unsafe levels of antibiotic residues may be present in edible products such as milk, eggs, shellfish and meat. Authorities regulate the use of veterinary drugs by setting the maximum residue limits (MRLs) to ensure the safety of the food and facilitate international trade between countries. Residue monitoring plans are used to detect the misuse of authorized veterinary medicines in food producing animals and to investigate the reasons for residue violations. Therefore, it is important to develop simple but accurate methods for the determination of residues of a range of antibiotics in a variety of animal tissues.

Here we describe the results of a successful validation of two separates methods using the ACQUITY UPLC I-Class with FTN Sample Manager coupled to the Xevo TQ-S micro (ES+). The first for the determination of a single class of antibiotics, macrolides, in bovine tissue and a second method for a combination of two classes, tetracyclines and sulfonamides, in shrimp. Validation was performed following the Commission Decision 2002/657/EC guidelines

METHODS

Macrolides in bovine tissue

Weigh 5 g (±0.05 g) sample of homogenized sample into Falcon tube + add internal standards + add 25 mL of 0.1 M Tris buffer

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Vortex mix 1 min + shake 30 min + centrifuge 5 min at 4660 g

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Transfer supernatant into 50 mL tube + add 300 µL Acetic Acid + add 2.5 mL 5% Na_2WO_4

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Leave at 5°C for 1 hour + centrifuge 10 min at 4660 g

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Condition Oasis HLB 6 cc 200 mg with 10 mL MeOH + 10 mL H2O + 5 mL 0.1M Tris buffer

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Load extract + wash with 20 mL 5% MeOH + wash with 5 mL Hexane

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Elute sample with 6 mL of 5% NH_4OH in MeOH + dry + reconstitute in 80/20 H2O/AcN

Column: ACQUITY HSS T3 1.8 µm 2.1 x 100 mm (PN 186003539)
Mobile phases: (A) Water + 0.1% Formic Acid. (B) Acetonitrile + 0.1% Formic Acid

Tetracyclines and sulfonamides in shrimp

Weigh 5 g (±0.05 g) of homogenized sample into Falcon tube + add internal standards + add 25 mL of 25 mM succinate buffer + 25 mg of EDTA sodium salt

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Add 15 mL hexane + ultrasonicate 5 min + shake 30 min + centrifuge 5 min at 4000 RPM

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Discard hexane layer + transfer supernatant into 50 mL tube

↓

Condition Oasis HLB 6 cc 200 mg SPE cartridge (PN WAT106202) with 5 mL MeOH + 5 mL H_2O + 5 mL 25 mM succinate buffer

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Load extract + wash with 10 mL of MeOH/H_2O (5/95 *v/v*)

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Elute sample with 5 mL of MeOH + dry + reconstitute in 1 mL of H_2O/MeOH (70/30 *v/v*)

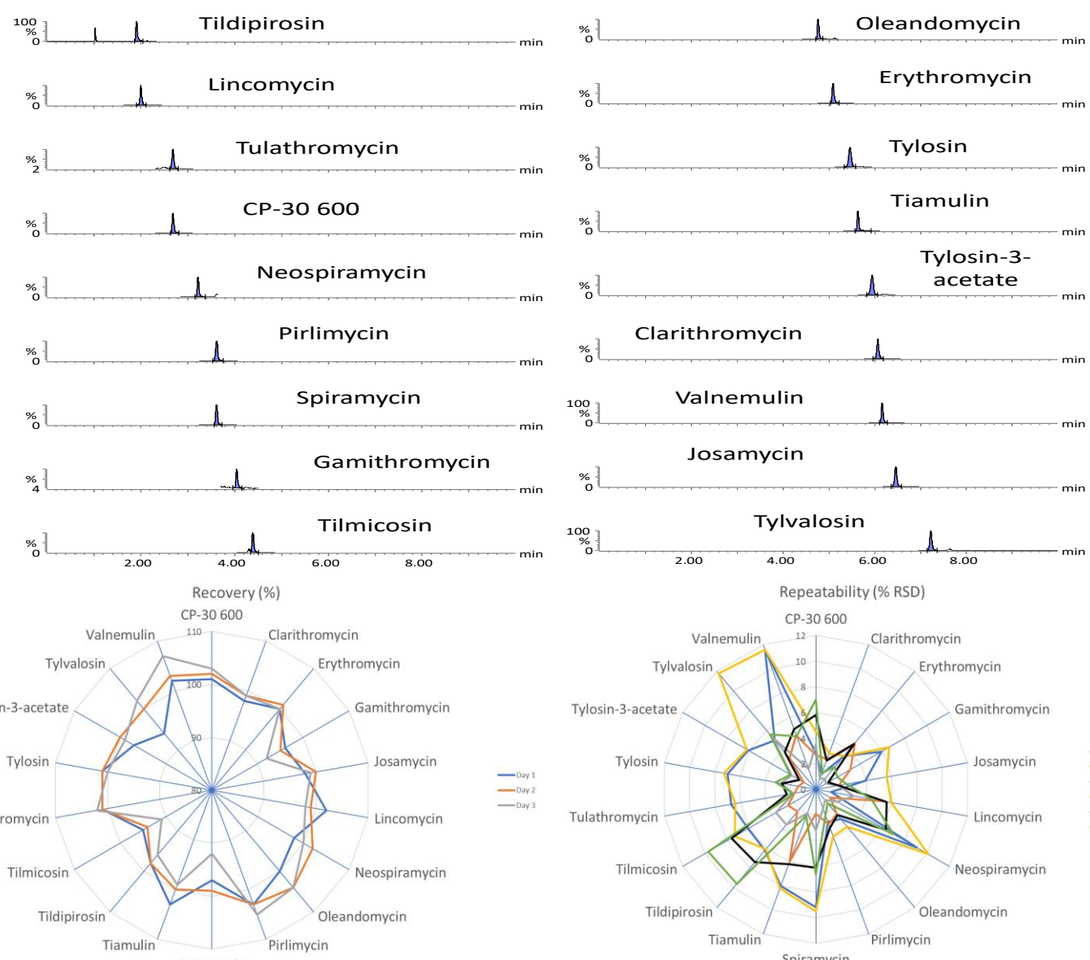
Column: ACQUITY HSS C18 1.8 µm 2.1 x 100 mm (PN 186003533)
Mobile phases: (A) Water + 0.1% Formic Acid. (B) Methanol + 0.1% Formic Acid

Full experimental details are presented in our application notes (see QR codes below)

RESULTS AND DISCUSSION

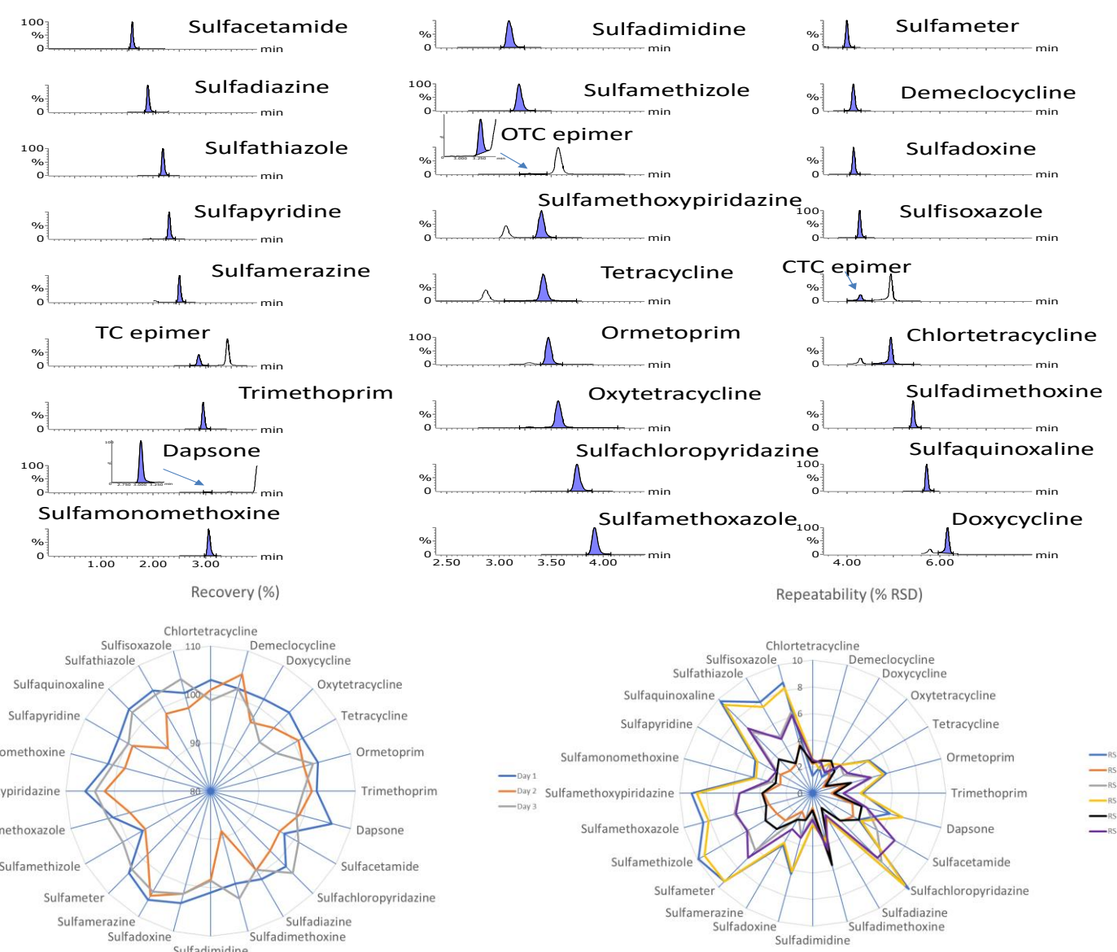
Macrolides in bovine tissue

Chromatograms of a selection of analytes from the analysis of the matrix-matched standard at the lowest calibrated concentration



Compound	MRL/TL (µg/kg)	CCα (µg/kg)	Compound	MRL/TL (µg/kg)	CCα (µg/kg)
CP-30 600	300	329	Spiramycin	200	239
Clarithromycin	5.0	5.2	Tiamulin	100	120
Erythromycin	200	215	Tildipirosin	400	495
Gamithromycin	5.0	5.7	Tilmicosin	50	62
Josamycin	5.0	5.1	Tulathromycin	300	323
Lincomycin	100	109	Tylosin	100	110
Neospiramycin	200	221	Tylosin-3-acetate	50	52
Oleandomycin	5.0	5.2	Tylvalosin	50	56
Pirlimycin	100	106	Valnemulin	50	59

Tetracyclines and sulfonamides in shrimp



Compound	MRL/TL (µg/kg)	CCα (µg/kg)	Compound	MRL/TL (µg/kg)	CCα (µg/kg)
Chlortetracycline	100	104	Sulfadimidine	100	102
Demeclocycline	100	104	Sulfadoxine	100	104
Doxycycline	100	104	Sulfamerazine	100	104
Oxytetracycline	100	104	Sulfameter	100	106
Tetracycline	100	102	Sulfamethizole	100	106
Ormetoprim	5.0	5.2	Sulfamethoxazole	100	106
Trimethoprim	50	51	Sulfamethoxypiridazine	100	106
Dapsone	1.3	1.3	Sulfamonomethoxine	100	105
Sulfacetamide	100	106	Sulfapyridine	100	105
Sulfachloropyridazine	100	105	Sulfaquinoxaline	100	105
Sulfadiazine	100	102	Sulfathiazole	100	104
Sulfadimethoxine	100	108	Sulfisoxazole	100	106

CONCLUSIONS

- The methods described here proved to be fast, sensitive and reliable for the determination of antibiotic residues in two types of animal tissue
- The procedures can also be applied to other animal and fish tissues after suitable validation
- These cost-effective methods can be easily implemented in routine testing laboratories, has been demonstrated as suitable for checking compliance with MRLs and has the potential for screening at much lower concentrations, for example for food business operators' due diligence testing

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