Development & Strengthening of Radio-Analytical & Complementary Techniques to Control Residues of Veterinary Drugs & Related Chemicals in Aquaculture Products

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Introduction

- Under an FAO/IAEA Coordinated Research Project (CRP), 11 individual projects have been initiated to develop methods and mechanisms to control residues of pharmaceutically active substances used in aquaculture production. Selected work is presented in this report. In Singapore analytical approaches harnessing the strength of LC-MS/MS and HRMS are under study to support residue monitoring. In China, a project on "*Development of Multi-residue Methods for Veterinary Drugs and Growth Promoters by LC-MS/MS to Strengthen the Analytical Capability in Aquaculture Products in South China*" is ongoing. In Chile, related work ,"*Multi-residue Method for the Detection of Veterinary Drug Residues and Other Organic Contaminants in Aquaculture Products and Feed by LC-MS/MS and Gas Chromatography-MS/MS Techniques*" is also ongoing. Similar work on "*Development and Validation of a Radioimmunoassay Kit for the Screening of Chloramphenicol (CAP) in Fish and Shrimp Tissues and Related Feeds*" is underway in Brazil.
- New methods have been developed in the 4 countries highlighted and await further validation for full application to national residue monitoring programmes.

Methodology

Singapore study:

⇒A hybrid LC-MS system (Exactive, Thermo Scientific) used to develop a screening method for multiple veterinary drugs.

⇒Gradient mobile phase: H_2O, 10 mM ammonium formate, 0.1% formic acid (solvent A) and MeOH, 0.1% formic acid (solvent B) used.

⇒Homogenized/centrifuged samples (5 g) in MeCN analyzed with or without clean-up by dispersive solid phase extraction.

China Study:

⇒Extracts from minced fish sample (2 g) + 2-nitrobenzaldehyde as internal standard (IS), extracted in ethyl acetate; defatted; analyzed by LC–ESI–MS/MS (4500 ABSCIEX); +/- ESI.

⇒Method validated: linearity, recovery, precision, LOD and LOQ

Brazil Study:

⇒Fish muscle (0.8 g) homogenized, hydrolysed, centrifuged & extracted (ethyl acetate);

⇒Cleaned-up (C-18 cartridge) sample in 1 mL PBS-gelatine buffer analysed by RIA using tritium-labeled CAP (~2 µCi), CAP standard (Sigma), and anti-CAP Abs;

⇒Comparison made between six replicates of blank fish samples (tilapia, catfish and shrimp) and five replicates of samples spiked at 0.25 µg kg^{-1}.

Chile Study:

⇒Samples extracted: modified QuEChERS; Agilent Bond Elut Enhanced Matrix Removal-Lipid (EMR-Lipids); MeCN, ammonium acetate;

⇒Gradient mobile phases: solvent A: 0.1% formic acid + 5 mM ammonium formate in H_2O and solvent B: 0.1% formic acid in 50:50 MeOH:MeCN

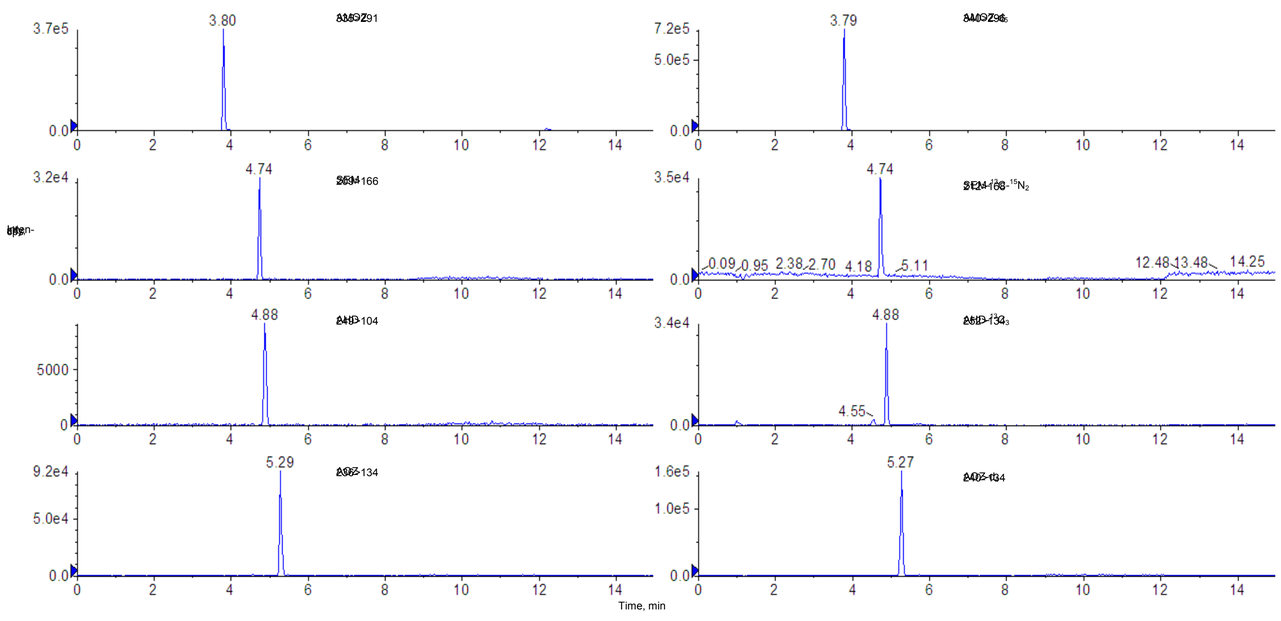
Results

Singapore study:

- Exact masses for 53 drugs including stilbenes, amphenicols, nitroimidazoles, beta-lactams, quinolones, fluoroquinolones, diaminopyridines, lincosamides, macrolides, polypeptides, sulphonamides, anthelmintics and antiseptics obtained.
- Method optimized; LODs: 1 ppm -100 ppm.

China study:

- Method developed for 4 NF metabolites & deuterated analogues as ISs
- Recoveries (0.5, 2.0, 10 µg kg^{-1}) & precision: 87.2% -104 % & 2.7% -10.4 %, respectively.
- LOQs for AMOZ, SEM, AHD &AOZ: 0.25, 0.5, 0.5 and 0.25 µg kg^{-1}, respectively.



TIC of Fish sample spiked with 4 NFMs at 5 µg kg^{-1}

Brazil study:

- RIA detected CAP in tilapia, catfish and shrimp samples; Method optimized (sensitivity and specificity based on CPM); tested on 30 shrimp samples (Rio Grande do Norte, Brazil).
- RIAs: suitable and robust alternative methods for national residue monitoring programmes (Granja, et al, 2008).

Chile study:

- 25 tetracyclines, quinolones, macrolides and amphenicols, among others detected by QTRAP.

Method LOQs and recoveries in fish residue studies

Analyte	LOQ (µg/kg)	Recovery (%)
Basic Green	0.4	115
Basic violet	0.4	117
Malachite green	0.4	115
L. Malachite gr.	0.4	99.9
L. Crystalviolet	0.4	89.9
Ciprofloxacin	20	96.4
Enrofloxacin	20	98.0
Flumequine	20	91.9
Ivermectin	20	72.4
Oxolinicacid	20	95.0
Sarafloxacin	20	75.2

Analyte	LOQ (µg/kg)	Recovery (%)
Doxycycline	40	79.7
Oxytetracycline	40	114
Tetracycline	40	110
Florfenicol	20	78.5
Diflubenzuron	20	96.9
Teflubenzuron	20	99.4
Emamectin. B	2	94.3
Abamectin	20	102
Trimethroprim	20	118
Tylosin	20	94.7
Erythromycin	20	104
CAP	3	103

Conclusions

- **China:** LC-ESI-MS/MS method developed for AMOZ, AOZ, AHD and SEM using multiple deuterated ISs.
- **Singapore:** Multi-residue screening method (HPLC-MS/MS and HRMS) developed; data obtained including retention time, exact masses of precursor and major fragment ions; Generic sample preparation method in place.
- **Brazil:** RIA method developed for analysis of CAP in tilapia, catfish and shrimps (below MRPL); method tested using shrimp samples. Additional/further method development/validation ongoing.
- **Chile:** Multi-residue LC-MS/MS method developed for 25 veterinary drug residues in salmon and preliminary validation done. A modified QuEChERS used.