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Development and Validation of Multi-Class Multi-Residue Analytical Method for Determination of Veterinary Drugs in Fish by Liquid Chromatography with Tandem Mass Spectrometry (LCMSMS)

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Introduction

Multiresidue analytical methods are preferred in the field of residue analysis to reduce the workload. However, in the drug residue analysis, multiclass applications are very challenging. In this work a multi-residue method for determination of different classes of veterinary drugs; sulfonamides, tetracyclines, macrolides, β- Lactam , qunolines and unauthorized antibiotics such as chloamphenicol in fish was developed.

Experimental

Extraction:

- 2g sample+1 ml of 1 M Sodium citrate at pH 4 + 1 ml of 0.5M Na2EDTA+10ml Acetonitrile- ultraturax for 2 min then shake by hand for 1 min. Extract was centrifuged at 4000 rpm for 10 min
- Sample re-extracted with 10 ml Acetonitrile. Supernatants were combined and evaporated to dryness at 37 ˚C.
- Sample reconstituted in 2 ml methanol/buffer (25/75). The sample was filtered using acrodisc 0.45 µm and 25 µl of the sample was injected into LC-MS/MS system

LC-MS/MS analysis:

Instrument: HPLC: Agilent 1200
MS: API 4000 QTrap with ES(+) and ES(-).
Column: XDB-C18 4.6 x 150 mm, 5 µm particle size.
Mobile phase A: Methanol
Mobile phase B: Methanol/ 10 mM ammonium formate pH 4±0.1 in water (10/90)
Gradient: start at 20% B; 0–3 min from 20 to 90% B; 3–15 min 90% B; 15–15.5 min from 90 to 20% B; 15.5–20 min 20% B at
Flow rate: 0.4 mL/min flow Injection volume: 25 µL
Quantification using matrix-matched standards

Results

Different extraction procedures were tested; extraction using Methanol/ Mcilvaine's Buffer, extraction using Acetonitril / Mcilvaine's Buffer, extraction using Acetonitril /Acetate buffer at pH4 and extraction using Acetonitril/ Acetate buffer at pH4 with 1% EDTA. The mass spectrometric parameters were optimized to give the best sensitivity, two MRM's were chosen for quantification and conformation of most analytes, the selected MRM's were based on the optimized declustring potential and collision energy.The extraction with Acetonitril/ Citrate buffer at pH4 with 0.5M EDTA was selected based on the accepted recovery and CV% results.

Method validation

The method has been validated for twenty veterinary drugs on fish samples at different fortification levels according to the European Commission 657/2002/EC. Fourteen analytes showed accepted performance criteria. Also, the method performance was tested by participating in different proficiency testing rounds (FAPAS, UK); accepted z-scores were obtained.

Table 1 – Summarized recoveries, precisions (CV_R%), Decision limits (CC_α) and detection capabilities (CC_β).

Analyte	Group	25 ug/kg		50 ug/kg		100 ug/kg		150 ug/kg		300 ug/kg		Qtyp	pooled CV%	Cca (ug/kg)	Ccb (ug/kg)
		Mean rec%	CV%	Mean rec%	CV%	Mean rec%	CV%	Mean rec%	CV%	Mean rec%	CV%				
Ceftiofur	B- Lactam	62	11	81	9	72	8	78	7	81	10	75	9	117	132
Chloramphenicol	Antibiotic	89	9	99	7	106	3	94	3	108	3	99	3	0.03	0.07
Chlortetracyclin	Tetracyclines	74	9	72	4	64	11	79	9	96	8	77	8	116	130
Ciprofloxacin	Fluoroquinolones	79	10	80	7	92	14	82	9	86	4	84	9	117	133
Doxycycline	Tetracyclines	109	14	74	6	82	8	77	5	83	4	85	8	116	129
Enrofloxacin	Macrolides	98	9	87	6	79	8	102	7	96	8	92	8	115	128
Erythromycin	Fluoroquinolones	89	10	184	21	75	32	183	18	91	10	124	20	234	267
Flumequine	Fluoroquinolones	81	11	106	4	73	8	112	4	91	3	93	7	214	225
Oxytetracycline	Tetracyclines	91	12	72	9	73	5	71	5	87	3	79	7	115	126
Penicillin V	B- Lactam	75	15	86	8	78	10	79	3	78	4	79	9	67	82
Sulfacetamide	Sulfonamides	87	9	81	7	79	8	78	5	84	7	82	7	114	126
Sulfadiazine	Sulfonamides	84	10	88	7	94	9	121	9	88	8	95	9	116	131
Sulfamerazine	Sulfonamides	76	11	88	6	83	8	84	9	88	3	84	8	115	128
Sulfamethazine	Sulfonamides	80	11	93	7	83	6	83	10	92	2	86	8	115	128
Sulfamethoxazole	Sulfonamides	79	12	84	8	86	7	74	11	84	3	81	9	116	131
Sulfapyridine	Sulfonamides	77	11	91	7	86	9	95	4	85	4	87	8	115	127
Sulfathiazole	Sulfonamides	88	10	86	9	85	12	112	14	93	11	93	11	120	139
Tetracycline	Tetracyclines	86	9	71	8	81	14	81	7	84	10	81	10	118	135
Trimethoprim	Inhibitor	73	6	94	5	79	8	82	3	87	8	83	6	63	73
Tylosine	Macrolides	73	5	74	13	74	10	77	6	78	12	75	10	118	134

Conclusion

Multiresidue multiclass method for determination of twenty veterinary drugs in fish was developed. The described method requires little amount of solvents and sample and could be used in controlling levels of different classes of veterinary drugs in fish samples.