Determination of Multi-Class Antibiotics Residues in Farmed Fish and Shrimp from Sri Lanka by Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS)

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Abstract: Antibiotics have been used to control the aquatic environment in both therapeutic and prophylactic ways. Antibiotics are particularly difficult to extract due to their strong interactions with biological matrices. In this study, UPLC-MS/MS method was developed and validated for quantitative confirmatory analysis of multi-class antibiotics residues in fish and shrimp. Fourteen antibiotics belonging to sulphonamides, β-lactams, quinolones, sulfones and macrolides were determined within one chromatographic run. The samples were suspended in 0.1 M HCl, and the analytes were extracted into ethyl acetate. The extracts were defatted with cyclohexane. The limits of quantification (LOQ) ranged from 0.24 to 1.32 μg kg^{-1} for fish and 0.42–1.62 μg kg^{-1} for shrimp samples. The recoveries ranged from 75 to 105%. The method was applied to the analysis of farmed freshwater Tilapia fish (*Oreochromis niloticus*) and shrimp (*Penaeus monodon*) collected in Sri Lanka. Sulfacetamide (4.31 ± 0.70 μg kg^{-1}) and sulfamethoxypyridazine (0.75 ± 0.15 μg kg^{-1}) were detected in the fish, and sulfapyridine (0.21–0.56 μg kg^{-1}) and sulfadoxine (0.35–1.44 μg kg^{-1}) were detected in the shrimp samples. The concentrations complied with the EU regulation limits for veterinary drug residues in seafood and did not pose a risk in terms of food safety.

Keywords: antibiotics; aquaculture; food safety; LC-MS/MS

Key Contribution: The extraction with ethyl acetate allowed the simultaneous extraction of multi-class antibiotics from fish and shrimp samples and the development and validation of a sensitive multi-residue UPLC-MS/MS analytical method.

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1. Introduction

The development of intensive aquaculture in recent decades has resulted in an increase in the occurrence of infectious diseases and mortality of farmed fish and shrimp [1,2]. This led to the extensive use of antibiotics [3], notably tetracyclines, sulphonamides and quinolones, and, consequently, to their residues in aquaculture products [4]. As a result, different regulators, such as the European Commission [5], Food and Drug Administration (FDA) [6], Environmental Protection Agency (EPA), [7] and government ministries of various countries established the maximum residual limits (MRLs) of antibiotics in food of animal origin. However, such regulations are still absent in most developing countries [8].

Aquaculture accounts for nearly half of the world's food fish production, while in South Asia, its contribution exceeds 70% [9]. The presence of veterinary drug residues in seafood from South Asian Countries inspected by the EU, USA, Canada and Japan from 2000 to 2009 was reported [10]. The presence of some antibiotic residues was detected [11–13]. Chloramphenicol was detected in fish from Bangladesh (5 ng kg^{-1}) and in shrimp from India

(32 ng kg^{-1}) and Indonesia (45 ng kg^{-1}) [14]. Concentrations of 60 ppm of oxytetracycline and 4 ppb of ciprofloxacin were found in imported shrimp samples [15]. The potential presence of antibiotic residues creates a need to monitor the goods on site before they are exported.

In terms of analytical methodology, most of the studies used LC-MS/MS following a dedicated sample preparation to decrease the interferences and minimize the possible matrix effects [16,17]. The need to address the different physicochemical properties of the various antibiotic groups and the complexity of the extraction and cleanup processes make these methods tedious and time-consuming [18–21]. A QuEChERS (quick, easy, cheap, effective, rugged and safe) approach alone or in connection with cleanup by solid phase extraction (SPE) has been the most often proposed [22–24]. The methods developed have typically addressed, at a time, only a few compounds, usually belonging to a single class of antibiotics [25–27]. Consequently, a multiclass antibiotics survey would not only require a large sample amount but would also be time-consuming and costly [28,29]. To achieve a fast and simultaneous extraction of various antibiotics, some authors used EDTA-acetonitrile solution or ammonium acetate buffer to extract antibiotics without a cleanup step [30,31].

Aquaculture of fish and shrimp is an important export industry in Sri Lanka [32]. Despite a long history, information on the use of antibiotics in aquaculture and possible residues is scarce [14,33]. The aim of this study was to provide the first, to the best of our knowledge, data on multi-class antibiotic residues in aquaculture shrimp and fish in Sri Lanka. For this purpose, a rapid, sensitive and selective method for the simultaneous UPLC-MS/MS determination of 14 antibiotic residues from five different classes in farmed shrimp and tilapia samples was developed and validated.

2. Materials and Methodology

Multi-residue antibiotics determination was performed with a QExactive hybrid Quadrupole Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Bermen, Germany) coupled with Ultimate 3000 UPLC (Thermo Fisher). Chromatographic separation was attained on an Accucore C18 reversed-phase column (150 mm, 2.1 mm i.d, 2.6 µm particle size) from Thermo Scientific (Waltham, MA, USA). A 5804R centrifuge (Eppendorf, Hamburg, Germany) was used for the separation of post-leaching supernatants. Ultrapure water (Direct–Q-R 3 UV, France) was used. Q-Exactive LC-MS/MS data were processed with X-Calibri 4.2 software (Thermo Fisher Scientific).

2.1. Reagents

Sulphonamide antibiotics (sulfamethoxypyridazine, sulfapyridine, sulfa- methoxazole, sulfadoxine, sulfamerazine, sulfacetamide, sulfamonomethoxine) stock solutions (500 mg L^{-1}) were prepared from solid standards and dissolved in LC-MS grade methanol (Honeywell, de Winchester, France). β-Lactam antibiotics (penicilloic acid of amoxicillin, penicillin G salt, ampicillin) standard stock solutions (500 mg L^{-1}) were prepared by dissolving the antibiotics powders in water. Quinolones drugs (sparfloxacin, enrofloxacin) standard stock solutions (500 mg L^{-1}) and sulfones drugs (dapsone) stock standard solution (500 mg L^{-1}) were prepared in LC-MS methanol (Honeywell, France). Erythromycin stock standard solution (500 mg L^{-1}) was prepared in HPLC grade ethanol (Honeywell, France) due to the highest solubility. All the antibiotics were purchased from Sigma-Aldrich (Steinheim, Germany) except sparfloxacin and dapsone (LGC, Wesel Germany). Ethyl acetate (Sigma Aldrich, Steinheim, Germany) was used for antibiotics extraction from fish and shrimp flesh. 0.1% formic acid (LC-MS grade, LGC, Wesel, Germany) solutions in acetonitrile (Honeywell, France) and water were used as the mobile phases.

2.2. Fish and Shrimp Samples

Genetically Improved Farmed Tilapia (GIFT Tilapia) Fish (*Oreochromis niloticus*, $n = 6$) and freshwater shrimp samples (*Penaeus monodon*, $n = 4$) were collected from aquaculture farms located in Kalpitiya, Puttalam District, Sri Lanka. The skin of the fish and the shell of the shrimp were removed, and the edible parts were homogenized. All the samples were ground using a domestic blender, freeze-dried, and stored at −20 °C until analysis. Antibiotic-free fish and shrimp samples from a local fish market in Pau, France, were used as blanks.

2.3. Solid-Liquid Extraction of Antibiotics

A 1-g fresh sample (or 0.25 g dry) was accurately weighed in a 15-mL polypropylene centrifuge tube. 0.3 mL of 0.1 M HCl and 3 mL of water were added, and the mixture was vortexed for 1 min. The prepared solution was treated twice with 1 mL of cyclohexane to remove the fat. Antibiotics were extracted with 1 mL of ethyl acetate. After adding ethyl acetate, the mixture was vortexed for 2 min and centrifuged at 5000 rpm for 10 min. The supernatant was collected in an Eppendorf tube. The extraction was repeated twice. The supernatants were combined and evaporated to dryness at 40 °C under a nitrogen stream. The dried extract was reconstituted with 0.1 mL of 50% (v/v) acetonitrile, centrifuged at 13,000 rpm for 10 min, and transferred to an HPLC vial for LC-MS/MS analysis.

2.4. HPLC-MS/MS Analysis

Chromatography was performed using gradient elution. Mobile phases A and B were 0.1% formic acid in water and acetonitrile, respectively. The gradient was: 10% B (0–1 min), linear till 60% B (1–12.5 min); linear till 90% B (12.5–13.5 min), 90% B (13.5–15.5 min), linear till 10% B (15.5–16.5 min), 10% B (16.5–18 min). The injection volume was 10 µL, and the flow rate was 0.4 mL min^{-1}. Data were acquired in positive ionization Parallel Reaction Monitoring (PRM) mode [34]. MS/MS transitions and collision energies are present in Table 1. Standard addition graphs were prepared in duplicate by spiking (1, 5, 10, 50, and 100 µg L^{-1}) the blank samples with antibiotics.

Table 1. MS/MS transitions and collision energies of the compounds.

Compound	Precursor Ion	Product Ions	Collision Energy (v)
Sulfamethoxypyridazine	281.07	156.01	25
Sulfapyridine	250.06	108.04	20
Sulfamethoxazole	254.05	156.01	25
Sulfadoxine	311.08	108.04	25
Sulfamerazine	265.07	92.10	28
Sulfamonomethoxine	281.07	126.07	30
Sulfacetamide	215.05	192.97	45
Enrofloxacin	360.17	316.17	35
Sparfloxacin	393.17	349.18	20
Ampicillin	350.12	106.06	30
Dapsone	249.07	108.04	25
Erythromycin	734.47	158.12	32
Penicilloic acid of amoxicillin	385.13	189.01	30
Penicillin G sodium salt	357.08	160.01	35

2.5. Method Validation

Method validation was performed according to the Eurachem Guideline [35]. The performance characteristics, such as matrix effect, limits of detection (LOD) and quantification (LOQ), linearity, specificity, analytical recovery, repeatability, and reproducibility, were investigated and determined. The matrix effect was estimated by comparing the slope of the external calibration curve [36] with that of the standard addition calibration curve covering the concentrations from 1–100 μg L^{-1} for each antibiotic.

$$\text{Matrix effect (ME)}\ \% = 100 \times \frac{Slope\ of\ the\ matrix\ matched\ curve}{Slope\ of\ the\ external\ standard\ curve}$$

The LODs and LOQs were determined for each antibiotic by analyzing blank samples spiked with a lower concentration (1 μg L^{-1}) of analytes (n = 10). LOD and LOQ values were calculated by using the following equations:

$$\text{LOD} = 3 \times \frac{S}{b} \text{ and LOQ} = 10 \times \frac{S}{b}$$

where "s" is the standard deviation of the concentration measured in the blank and "b" is the mean slope of the standard addition calibration curve.

Linearity was tested for calibration curves (spiked blank samples) by the least-squares linear regression. The specificity of the method was studied using blank samples (n = 21) checked for the presence of interferences. Intraday and interday precision and analytical recovery were established by analyzing blank samples at different concentration levels (n = 7). The analytical recovery was calculated as the ratio between the concentration of the analyte found and the added concentration.

2.6. Statistical Analysis

Statgraphics Centurion XVI v16.1.15 (Manugistics, Rockville, MD, USA) software was used to analyze the data.

3. Results and Discussion

3.1. Optimization of Extraction of Antibiotics

The extraction of antibiotics from fish samples with different solvents such as methanol, water, acetonitrile and EDTA was described [37,38]. Different extraction efficiencies for different classes of antibiotics were reported. Dispersive solid phase extraction (dSPE) cleanup was proposed to reduce the matrix effects but resulted in lower recoveries for some classes of antibiotics, especially for tetracycline compounds [39,40].

The optimization of the solvent in this work resulted in the choice of ethyl acetate acidified with 0.1 M HCl, which assured the highest recoveries, exceeding 70% for all the compounds. Ethyl acetate is used for extraction because of its medium polarity allowing the simultaneous extraction of both polar and non-polar compounds. Acidification allowed the denaturation of the proteins and cyclohexane-the removal of fat. Consequently, a potential loss of analytes [41] by additional cleanups, such as solid phase extraction (SPE) [19,26] or liquid extraction (LE) [42,43], could be avoided. The obtained chromatograms for the antibiotics standards are shown in Figure 1 (blank sample chromatography is shown in Figure S1).

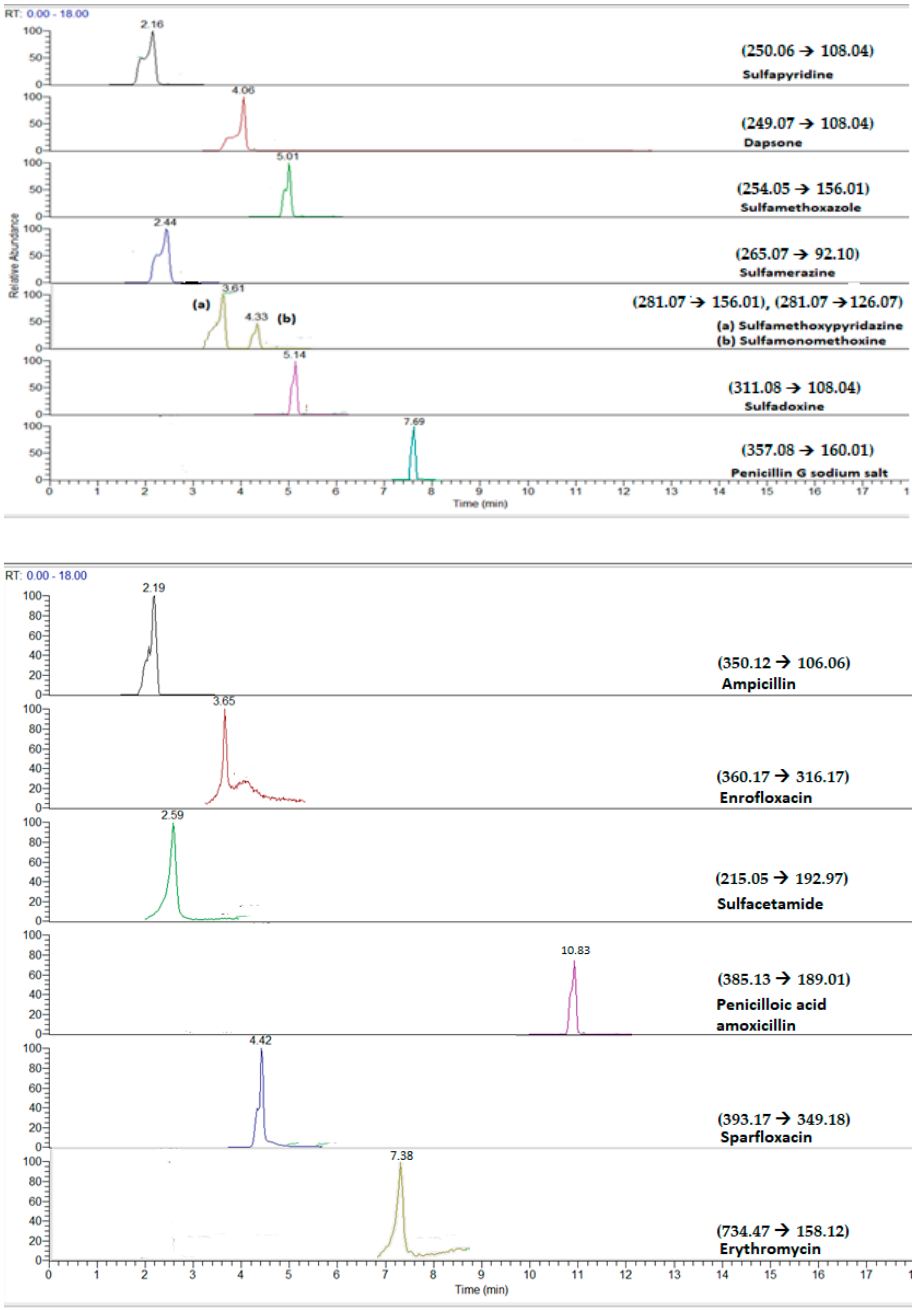

Figure 1. LC-MS/MS chromatograms of the selected antibiotics standards.

3.2. Matrix Effect

The matrix effect, potentially responsible for signal suppression or enhancement, was evaluated by comparing the slopes of the external and standard addition calibration curves covering the concentrations of 1, 5, 10, 50, and 100 µg L^{-1} for each antibiotic. The standard addition calibration curve was prepared by spiking the fish/shrimp samples (preconcentration factor = 50).

The matrix effects are given in Table 2.

Table 2. Matrix effect (ME), limit of detection (LOD), and limit of quantification (LOQ) values.

Antibiotic	Fish			Shrimp		
	ME (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	ME (%)	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
Sulfamethoxypyridazine	45	0.42	1.32	58	0.48	1.62
Sulfapyridine	26	0.31	1.03	33	0.22	0.72
Sulfamethoxazole	21	0.36	1.19	28	0.37	1.26
Sulfadoxine	56	0.10	0.35	74	0.14	0.44
Sulfamerazine	29	0.19	0.63	41	0.25	0.82
Sulfamonomethoxine	73	0.17	0.56	81	0.21	0.68
Sulfacetamide	29	0.30	1.10	39	0.38	1.26
Enrofloxacin	22	0.33	1.11	35	0.36	1.20
Sparfloxacin	37	0.07	0.24	42	0.13	0.42
Ampicillin	80	0.35	1.15	71	0.45	1.50
Dapsone	45	0.26	0.85	53	0.19	0.64
Erythromycin	97	0.25	0.82	82	0.30	1.00
Penicilloic acid of Amoxicillin	35	0.32	1.05	40	0.33	1.10
Penicillin G	18	0.18	0.59	30	0.25	0.80

The ANOVA test was applied to compare the slope of the external calibration and standard addition curves at a confidence level of 95%. Significant differences were not found in the sulfamethoxazole, sulfapyridine, sulfamerazine, sulfacetamide, penicillin G and enrofloxacin (value > 0.050 at 95% significance level) in fish samples. However, ANOVA showed that the slope of an external calibration curve and a standard addition calibration curve of the sulfamethoxypyridazine, sulfadoxine, sulfamonomethoxine, sparfloxacine, ampicillin, dapsone, erythromycin, penicilloic acid of amoxicillin, and enrofloxacine were significantly different at a 95% significance level (value < 0.050 at 95% significance level) in fish samples. The observed matrix effect of the shrimp samples varied between 28 and 82%. Significant differences were not found for penicillin G and sulfamethoxazole at a 95% significance level (value > 0.050 at a 95% significance level). Other antibiotics showed a higher matrix effect. Therefore, the understanding of the matrix effect is important when analyzing simultaneously for multi-class antibiotics. Accurate results could only be obtained using the method of the matrix-matched calibration curve. The regression coefficients higher than 0.99 showed good linearity for all antibiotics analyzed.

3.3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ values are given in Table 2. The LOD and LOQ values for fish samples were between 0.07–0.42 μg kg^{-1} and 0.24–1.32 μg kg^{-1} and 0.13–0.48 μg kg^{-1}, and 0.42–1.62 μg kg^{-1} for shrimp samples, respectively. Similar values were reported in a multiclass antibiotics analysis in shrimp and fish samples by Dickson et al. [44]. Pashaei et al. [38] studied the residues of 15 human pharmaceuticals in fish and shrimp samples; the LOD and LOQ values were 0.017–1.371 μg/kg and 0.051–4.113 μg/kg, which is higher than in this study. The LOD and LOQ values are below the EU regulation limits for food of animal origin, which demonstrates the suitability of the developed method for the detection and determination of all the tested antibiotics [5].

3.4. Precision and Accuracy

Intraday assays were performed for three different standard additions of each antibiotic, at the low (1 μg L^{-1}), middle (10 μg L^{-1}) and high (100 μg L^{-1}) concentration levels, on three different days (n = 7). Inter-day precision and accuracy were obtained by preparing 5 standard additions on 7 different days (n = 2). Table 3 shows the intraday and inter-day analytical recoveries (AR %) and the relative standard deviation (RSD) for each antibiotic. Results show that RSD was below 20%, which is similar to a previous report [40]. Analytical recoveries of the intraday and inter-day assay were between 75% and 105% for each antibiotic (Table 3). The results compare favorably with many methods reporting analytical recoveries below 60% [26,45,46].

Table 3. Intraday and inter-day analytical recovery (AR %) and intraday and inter-day precision (RSD).

Compound	Tilapia Fish				Shrimp			
	Intra Day		Inter-Day		Intra Day		Inter-Day	
	AR %	RSD	AR %	RSD	AR %	RSD	AR %	RSD
Sulfamethoxypyridazine								
1	102 ± 1	10	105 ± 1	17	101 ± 1	11	96 ± 8	9
5	-[a]	-[a]	102 ± 1	11	-[a]	-[a]	91 ± 7	16
10	89 ± 1	8	82 ± 1	12	88 ± 1	8	81 ± 2	14
50	-[a]	-[a]	96 ± 5	11	-[a]	-[a]	96 ± 6	13
100	87 ± 6	7	83 ± 7	10	84 ± 3	4	80 ± 8	10
Sulfapyridine								
1	94 ± 1	12	93 ± 1	16	87 ± 3	4	93 ± 1	18
5	-[a]	-[a]	92 ± 1	13	-[a]	-[a]	92 ± 7	15
10	89 ± 3	11	79 ± 3	5	87 ± 1	11	79 ± 3	4
50	-[a]	-[a]	93 ± 6	13	-[a]	-[a]	90 ± 5	12
100	88 ± 7	8	84 ± 9	10	86 ± 7	8	83 ± 7	12
Sulfamethoxazole								
1	84 ± 1	13	83 ± 1	14	82 ± 1	13	82 ± 2	13
5	-[a]	-[a]	90 ± 1	18	-[a]	-[a]	91 ± 5	18
10	88 ± 1	11	87 ± 1	14	88 ± 2	13	90 ± 2	14
50	-[a]	-[a]	99 ± 8	17	-[a]	-[a]	76 ± 3	8
100	91 ± 10	12	85 ± 5	5	86 ± 7	8	84 ± 4	5
Sulfadoxine								
1	81 ± 1	10	87 ± 1	9	79 ± 4	12	86 ± 3	9
5	-[a]	-[a]	92 ± 1	17	-[a]	-[a]	86 ± 3	9
10	85 ± 1	7	83 ± 1	7	85 ± 6	8	84 ± 5	7
50	-[a]	-[a]	91 ± 4	9	-[a]	-[a]	92 ± 5	11
100	85 ± 6	7	80 ± 6	8	87 ± 6	6	80 ± 6	9
Sulfamerazine								
1	89 ± 1	7	95 ± 1	10	89 ± 6	7	81 ± 4	6
5	-[a]	-[a]	102 ± 1	6	-[a]	-[a]	92 ± 4	5
10	92 ± 1	4	101 ± 1	7	91 ± 4	5	95 ± 4	4
50	-[a]	-[a]	100 ± 5	10	-[a]	-[a]	80 ± 5	12
100	91 ± 5	5	94 ± 6	6	89 ± 4	6	87 ± 7	8
Sulfamonomethoxine								
1	89 ± 1	1	89 ± 1	1	84 ± 1	2	85 ± 1	2
5	-[a]	-[a]	93 ± 1	2	-[a]	-[a]	94 ± 3	2
10	81 ± 1	8	79 ± 1	2	79 ± 2	7	79 ± 2	5
50	-[a]	-[a]	77 ± 1	4	-[a]	-[a]	78 ± 1	12
100	97 ± 10	11	79 ± 4	3	91 ± 5	13	86 ± 8	9
Sulfacetamide								
1	97 ± 2	10	101 ± 1	7	89 ± 8	8	101 ± 8	7
5	-[a]	-[a]	92 ± 3	7	-[a]	-[a]	92 ± 3	7
10	94 ± 2	15	92 ± 1	12	94 ± 2	15	90 ± 6	11
50	-[a]	-[a]	88 ± 4	8	-[a]	-[a]	84 ± 3	5
100	82 ± 5	6	82 ± 7	10	82 ± 5	7	82 ± 6	11
Enrofloxacin								
1	89 ± 2	19	92 ± 1	14	85 ± 3	19	88 ± 1	12
5	-[a]	-[a]	100 ± 1	12	-[a]	-[a]	94 ± 3	14
10	87 ± 1	16	90 ± 2	18	82 ± 2	13	97 ± 2	13
50	-[a]	-[a]	91 ± 4	9	-[a]	-[a]	87 ± 2	3
100	94 ± 7	10	97 ± 7	7	96 ± 6	6	96 ± 7	8

Table 3. *Cont.*

Compound	Tilapia Fish				Shrimp			
	Intra Day		Inter-Day		Intra Day		Inter-Day	
	AR %	RSD	AR %	RSD	AR %	RSD	AR %	RSD
Sparfloxacin								
1	87 ± 1	4	96 ± 1	3	90 ± 3	1	86 ± 3	3
5	-[a]	-[a]	87 ± 1	4	-[a]	-[a]	89 ± 2	5
10	86 ± 1	10	84 ± 1	12	85 ± 1	6	86 ± 5	13
50	-[a]	-[a]	87 ± 3	6	-[a]	-[a]	88 ± 2	3
100	91 ± 8	11	88 ± 2	2	91 ± 2	1	88 ± 4	2
Ampicillin								
1	80 ± 1	11	84 ± 1	11	80 ± 2	5	87 ± 6	8
5	-[a]	-[a]	97 ± 3	8	-[a]	-[a]	95 ± 1	5
10	88 ± 1	9	88 ± 1	11	85 ± 3	8	87 ± 4	8
50	-[a]	-[a]	95 ± 3	7	-[a]	-[a]	96 ± 4	8
100	99 ± 9	11	100 ± 9	9	94 ± 8	8	96 ± 6	6
Dapsone								
1	85 ± 1	13	97 ± 1	18	79 ± 1	14	82 ± 1	14
5	-[a]	-[a]	96 ± 2	5	-[a]	-[a]	97 ± 2	4
10	85 ± 1	8	88 ± 2	13	87 ± 7	7	92 ± 3	11
50	-[a]	-[a]	89 ± 2	5	-[a]	-[a]	90 ± 2	5
100	79 ± 5	14	88 ± 8	10	80 ± 3	16	81 ± 7	10
Erythromycin								
1	83 ± 1	3	92 ± 4	5	79 ± 1	14	91 ± 5	8
5	-[a]	-[a]	91 ± 2	6	-[a]	-[a]	91 ± 3	7
10	83 ± 1	5	89 ± 1	14	91 ± 7	10	92 ± 1	13
50	-[a]	-[a]	81 ± 4	9	-[a]	-[a]	83 ± 4	9
100	91 ± 7	19	87 ± 9	11	95 ± 7	18	89 ± 8	10
Penicilloic acid of Amoxicillin								
1	98 ± 2	3	99 ± 4	5	92 ± 5	10	98 ± 5	5
5	-[a]	-[a]	97 ± 1	3	-[a]	-[a]	96 ± 4	9
10	89 ± 1	13	82 ± 3	4	81 ± 4	3	82 ± 1	4
50	-[a]	-[a]	87 ± 3	6	-[a]	-[a]	88 ± 3	6
100	90 ± 5	6	88 ± 5	6	87 ± 4	11	89 ± 5	6
Penicillin G								
1	85 ± 1	17	85 ± 1	17	82 ± 2	15	87 ± 1	18
5	-[a]	-[a]	90 ± 1	4	-[a]	-[a]	87 ± 1	3
10	85 ± 2	5	75 ± 1	7	87 ± 4	4	79 ± 4	6
50	-[a]	-[a]	87 ± 7	15	-[a]	-[a]	84 ± 6	14
100	101 ± 9	18	86 ± 11	13	92 ± 9	10	83 ± 8	10

-[a] = not evaluated.

3.5. Determination of Multi-Class Antibiotics Residues in Farmed Fish and Shrimp

The developed method was applied to the analysis of 6 Tilapia fish samples (*Oreochromis niloticus*) and 4 freshwater shrimp samples (*Penaeus monodon*) collected from Kalpitiya, Sri Lanka. Each sample was analyzed in triplicate. The results are shown in Table 4. Three sulfonamide antibiotics (sulfacetamide, sulfapyridine, and sulfadoxine) were detected in shrimp, while one (sulfamethoxypyridazine) was found in fish.

Table 4. The presence of antibiotics in aquaculture fish and shrimp samples.

Samples	Sulfacetamide (μg kg^{-1})	Sulfapyridine (μg kg^{-1})	Sulfamethoxypyridazine (μg kg^{-1})	Sulfadoxine (μg kg^{-1})
F-1	_	_	0.75 ± 0.15	_
F-2	_	_	_	_
F-3	4.31 ± 0.70	_	_	_
F-4	_	_	_	_
F-5	_	_	_	_
F-6	_	_	_	_
S-1	_	0.35 ± 0.02	_	0.35 ± 0.03
S-2	_	0.21 ± 0.01	_	0.36 ± 0.02
S-3	_	0.56 ± 0.16	_	1.44 ± 0.12
S-4	_	0.26 ± 0.03	_	0.57 ± 0.04

F-Tilapia Fish, S- Shrimp.

A sulfacetamide concentration of 4.31 μg kg^{-1} was found in one fish sample, and sulfamethoxypyridazine was detected at the concentration between LOD and LOQ (0.75 μg kg^{-1}). The other fish samples were free of the targeted antibiotics. In the shrimp samples, sulfapyridine and sulfadoxine were detected in the muscle at the levels of 0.21–0.56 μg kg^{-1} and 0.35–1.44 μg kg^{-1}, respectively. The most common route of administration of antibiotics in aquaculture is by mixing the antibiotics substances with feed samples. Other routes of administration of antibiotics are pond sprinkling and injection [47]. Several studies have revealed that antibiotic accumulation in the aquaculture environment leads to increasing residues in animal tissues, pond water, sediments and aquaculture products [48,49]. Residue antibiotics in aquaculture environments and ecosystems are widely recognized as an emerging threat to both humans and the environment.

4. Conclusions

The extraction with ethyl acetate allowed the simultaneous extraction of multi-class antibiotics from fish and shrimp samples and the development and validation of a sensitive multi-residue UPLC-MS/MS analytical method. The method complies with the analytical requirements in terms of specificity, LOD and LOQ, intraday and interday accuracy and precision. All the detected antibiotic drug residues concentrations found in farmed fish and shrimp from Sri Lanka were below the permitted amount (MRL value) defined by the relevant EU regulation [5] and were lower than the corresponding values reported previously in similar products from South Asian countries [4,50,51]. Further investigation into the possible sources of those antibiotics detected in fish and shrimp muscle samples is needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8030154/s1, Figure S1: Blank sample chromatography.

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