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Multiresidue determination of sulfonamides in edible catfish, shrimp and salmon tissues by high-performance liquid chromatography with postcolumn derivatization and fluorescence detection

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Abstract

A liquid chromatographic (LC) method for determining 14 sulfonamide (SA) (sulfanilamide, sulfadiazine (SDZ), sulfathiazole, sulfapyridine, sulfamerazine (SMR), sulfamethazine (SMZ), sulfamethizole, sulfamethoxypyridazine, sulfachloropyridazine (SCP), sulfamonomethoxine, sulfadoxine, sulfamethoxazole, sulfadimethoxine (SDM), and sulfaquinoxaline (SQX)) residues in edible catfish, shrimp and salmon tissues was developed and validated at 5, 10 or 20 ng g^{-1}. The method was then used to determine residues in tissues of catfish, shrimp and salmon dosed with six selected sulfonamides (sulfadiazine, sulfamerazine, sulfamethazine, sulfachloropyridazine, sulfadimethoxine and sulfaquinoxaline). All assays were within U.S. Food and Drug Administration guidelines for recovery and intra-assay variability. The method was developed to determine possible sulfonamide residues in aquacultured catfish, shrimp and salmon produced for food.
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Keywords: Sulfonamides; Catfish; Shrimp; Salmon; Liquid chromatography

1. Introduction

Sulfonamides (SAs) are relatively inexpensive, widely available antibacterials. Residues of these compounds in animal foods, including those raised in aquaculture, intended for human consumption are of toxicological and regulatory concern: sulfamethazine (SMZ) has been shown to produce thyroid follicular tumors in rodent bioassays [1], sulfonamides can cause allergic reactions in humans [2] and the development of antibiotic resistance is a continuing concern [3]. Fourteen sulfonamides (sulfanilamide, sulfadiazine (SDZ), sulfathiazole, sulfapyridine, sulfamerazine (SMR), sulfamethazine, sulfamethizole, sulfamethoxypyridazine, sulfachloropyridazine (SCP), sulfamonomethoxine, sulfadoxine, sulfamethoxazole, sulfadimethoxine (SDM), and sulfaquinoxaline (SQX)) are on the U.S. Food and Drug Administration's (FDA) priority list of fish drugs and chemicals scheduled for analytical chemical methods development. Priority is based on impact on human food safety and extent of known use in industry. The desired level for determining these compounds is 10 ng g^{-1} in edible tissues (fresh weight) of catfish, shrimp and salmon. Although many methods for determining SAs in various matrices exist, none were found that were suited for the above requirements.

A liquid chromatographic (LC) method [4] to determine the 14 SAs at target levels in salmon had previously been developed in our laboratory, but was found unsuitable for application to catfish or shrimp analysis, presumably due to larger fat content in these species. In the present method, a strong cation exchange (SCX) solid phase extraction (SPE) isolation was developed to replace the second liquid–liquid extraction in the previous method. The present method was then validated at 5, 10 and 20 ng g^{-1} (1/2×, 1× and 2× target level) in catfish and shrimp and also at 10 ng g^{-1} in salmon. Additionally, the method was used to determine six representative SA residues in tissues

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of dosed fish and shrimp; these six, sulfadiazine, sulfamerazine, sulfamethazine, sulfachloropyridazine, sulfadimethoxine and sulfaquinoxaline were selected by the Center for Veterinary Medicine (CVM).

2. Experimental

2.1. Dosing of catfish, shrimp and salmon

Channel catfish were dosed at the Stuttgart National Aquaculture Research Center. Individual fish were orally dosed once with a single SA via capsule inserted into the stomach with a tube and plunger as follows: 4.7 mg SDZ kg^{-1}, 0.4 mg SMR kg^{-1}, 1.5 mg SMZ kg^{-1}, 2.5 mg SCP kg^{-1} or 0.4 mg SDM kg^{-1}. Exposure time was 24 h except for SCP, which was 6 h. Shrimp were dosed at the University of Tucson. Individual tanks of shrimp were given feed medicated with a single SA at each of the following levels: SDZ, 38 and 76 ng g^{-1}; SMR, 78 and 156 ng g^{-1}; SMZ, 210 and 420 ng g^{-1}; SCP, 24 and 48 ng g^{-1}; SDM, 36 and 72 ng g^{-1}; and SQX, 24 and 48 ng g^{-1}. Exposure time was 24 h. Atlantic salmon were dosed at the CVM. Individual fish were orally dosed once with a single SA via capsule as follows: 4.7 mg SDZ kg^{-1}, 0.4 mg SMR kg^{-1}, 1.5 mg SMZ kg^{-1}, 10 mg SCP kg^{-1}, 15 mg SDM kg^{-1} and 10 mg SQX kg^{-1}. Exposure times for SDZ, SMR, SMZ and SDM were 24 h, for SCP and SQX, 12 h. Dosing was estimated based on previous pharmacokinetic studies of SDM [5] and SCP [6] in catfish and uptake and decline of SDM [7] in salmon.

2.2. Chemicals and standards

Acetone, acetonitrile, methanol and methylene chloride, all LC grade, and glacial acetic acid, reagent grade, were from J.T. Baker (Phillipsburg, NJ, USA). Diethylene glycol (DEG), reagent grade, was from Fischer Scientific (Fair Lawn, NJ, USA). Solid ammonium acetate was from Fluka (Switzerland). Fluorescamine was from Pierce (Rockford, IL, USA). Two standards, SDZ and sulfadoxine, were US Pharmacopeial Convention (USPC) reference standards (Rockville, MD, USA). All other SAs were from Sigma Chemical Co. (St. Louis, MO, USA). Single stock SA standards were prepared at 100 μg mL^{-1} in acetonitrile, mixed intermediate standards at 1000 ng mL^{-1} in acetonitrile and analytical standards at 50, 100 and 200 ng mL^{-1} in deionized (DI) water. Stock and intermediate standards were stored in low actinic glassware at 4 °C for 6 months; analytical standards were prepared daily.

2.3. Sample preparation

Catfish and shrimp for controls and for recovery experiments were purchased at local markets; control salmon was provided by the CVM.

2.3.1. Homogenization

Tissues were stored at −80 °C. Tissues were homogenized three times by processing approximately 60 g of solidly frozen 2-cm pieces of catfish muscle, salmon muscle and adhering skin or whole tail meat pieces of shrimp for 30 s in a Robot Coupe RSI 2Y1 scientific batch processor (Jackson, MS, USA).

2.3.2. Extraction

Five replicate 10.0-g samples were weighed into 250-mL Falcon polypropylene tubes from Becton Dickinson (Lincoln Park, NJ, USA). A 10-mL volume of 0.2% acetic acid–methanol–acetonitrile (85:10:5) was added to each sample. These were homogenized for 30 s at 20,000 rpm with a Tekmar (Cincinnati, OH, USA) Ultra-Turrax T25 homogenizer. A 90-mL portion of acetonitrile was added to each, and the samples were shaken at low speed on an Eberbach shaker (Ann Arbor, MI, USA) for 10 min. The samples were centrifuged for 10 min (centrifuge speed not critical), and the supernatants were decanted into 250-mL separatory funnels containing 100 mL DI water and 2 mL DEG. A 30-mL portion of acetonitrile was added to each Falcon tube, and the samples were shaken, centrifuged, and decanted into the separatory funnels as before.

2.3.3. Liquid–liquid partition

A 60-mL portion of methylene chloride was added to the combined supernatants in each separatory funnel. The funnels were shaken by hand for 3 min and left to separate for 15 min. The bottom layer of each was collected in a 500-mL round bottom flask containing several boiling chips. A 40-mL portion of methylene chloride was added to each funnel and the funnels were shaken, left to separate, and collected as before. These samples were then concentrated to 2–3 mL at 65 °C using a Büchi RE 121 rotary evaporator from Brinkman Instruments Inc. (Westbury, NY, USA).

2.3.4. Solid phase extraction

Varian (Harbor City, CA, USA) 3-mL (500 mg) SCX SPE cartridges were conditioned with, in succession, 2.5 mL acetone, 2.5 mL 0.2% acetic acid, 2.5 mL acetone. A 5-mL portion of methylene choride–acetone (60:40) was added to and mixed with the concentrated sample in each 500-mL flask and the sample loaded onto the cartridge. The flasks were then each rinsed with a 5-mL portion of acetone–methylene chloride (60:40) which was washed through the cartridge. Each flask was finally rinsed with 5 mL acetone which was washed through the cartridge. The SAs were then eluted from the cartridges with 5 mL acetone–0.4 M ammonium acetate (50:50). The acetone was removed from the eluates by evaporation under a stream of nitrogen to exactly 2 mL and reserved for LC determination.

2.4. Liquid chromatography

The liquid chromatograph consisted of a Hewlett-Packard (Memphis, TN, USA) Series 1050 quaternary pump and autosampler (50-μL injections). Separation was with a Waters Corp. (Milford, MA, USA) Symmetry C18, 3.5 μm, 150 mm × 4.6 mm I.D. analytical column. The initial mobile phase"A" was aqueous 2% acetic acid–methanol–acetonitrile (85:10:5). The acetonitrile content of the mobile phase was increased linearly to 15% by pumping mobile phase "B" over 25 min and holding for 5 min; mobile phase "B" was aqueous 2%

acetic acid–methanol–acetonitrile (75:10:15). The initial mobile phase was then pumped for 10 min before the next sequence was begun. The mobile phase flow rate was 1 mL min^{-1} at all times. The SAs were derivatized postcolumn with a solution (prepared daily) of 100 mg of fluorescamine dissolved in 200 mL acetonitrile-initial mobile phase (50:50). The derivatization solution was pumped at 0.2 mL min^{-1} by a Pickering Laboratories (Mountain View, CA, USA) PCX 3100 postcolumn reaction module; the reactor was equipped with 0.016 in I.D. × 35 ft PTFE tubing and was heated to 70 °C. Detection was by fluorescence (excitation wavelength, 400 nm; emission wavelength, 495 nm) using a Waters 470 scanning fluorescence detector (gain, ×100). Data were collected by a Hewlett-Packard ChemStation data collection station.

2.5. Recovery experiments

Control fish and shrimp were analyzed to determine that no SAs were detected. Then, five replicate 10.0-g fish or shrimp control samples were weighed into Falcon polypropylene tubes and fortified at 5, 10 or 20 ng g^{-1} with 50, 100 or 200 μL of mixed intermediate standard. The samples were left in contact with the fortification solution for 30 min before being prepared as above.

3. Results and discussion

Under the conditions described, sulfonamides eluted in the following order with approximate retention times in minutes: sulfanilamide, 4.0; sulfadiazine, 6.0; sulfathiazole, 6.6; sulfapyridine, 7.0; sulfamerazine, 7.5; sulfamethazine, 9.3; sulfamethizole, 10.2; sulfamethoxypyridazine, 10.7; sulfachloropyridazine, 13.6; sulfamonomethoxine, 14.3; sulfadoxine, 14.7; sulfamethoxazole, 15.6; sulfadimethoxine, 23.3; and sulfaquinoxaline, 24.1. Retention times varied with ambient temperature; however, the order of elution did not change in the ambient temperature range (20 ± 3 °C) experienced. Peak heights of external standards were used for quantitation. Final sample extracts were concentrated by a factor of 5; that is, the SA residues extracted from 10 g tissue were in a 2-mL final volume. Based on a signal-to-noise (S/N) ratio of at least

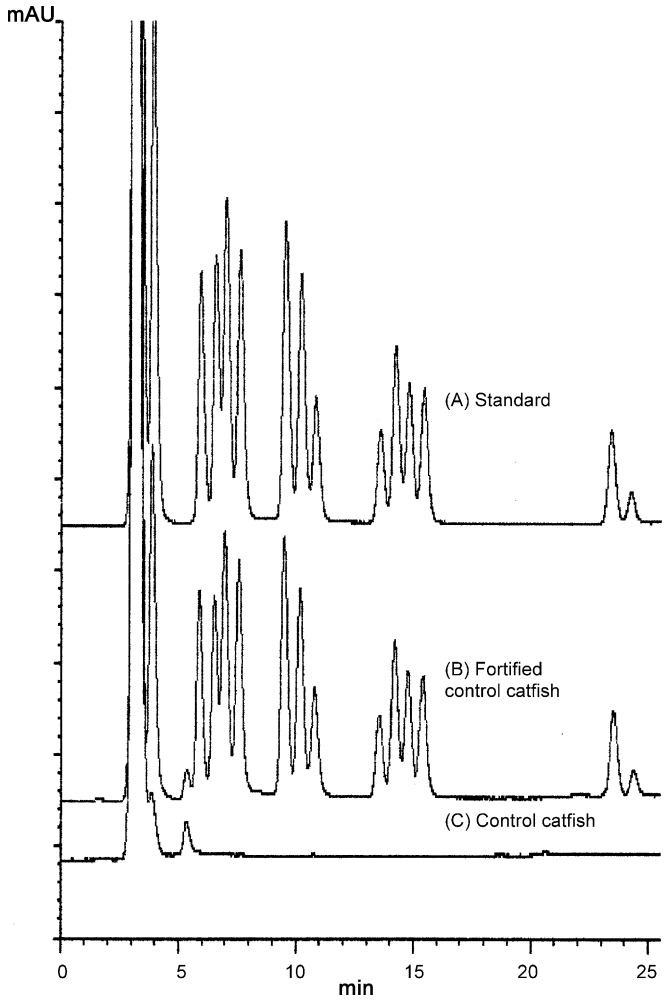

Fig. 1. HPLC chromatograms for 50-μL injections of fluorescamine derivatives of (A) quantitative standard containing 50 ng SAs/mL, extracts of (B) control catfish muscle fortified with 10 ng SAs/g tissue and (C) control catfish muscle.

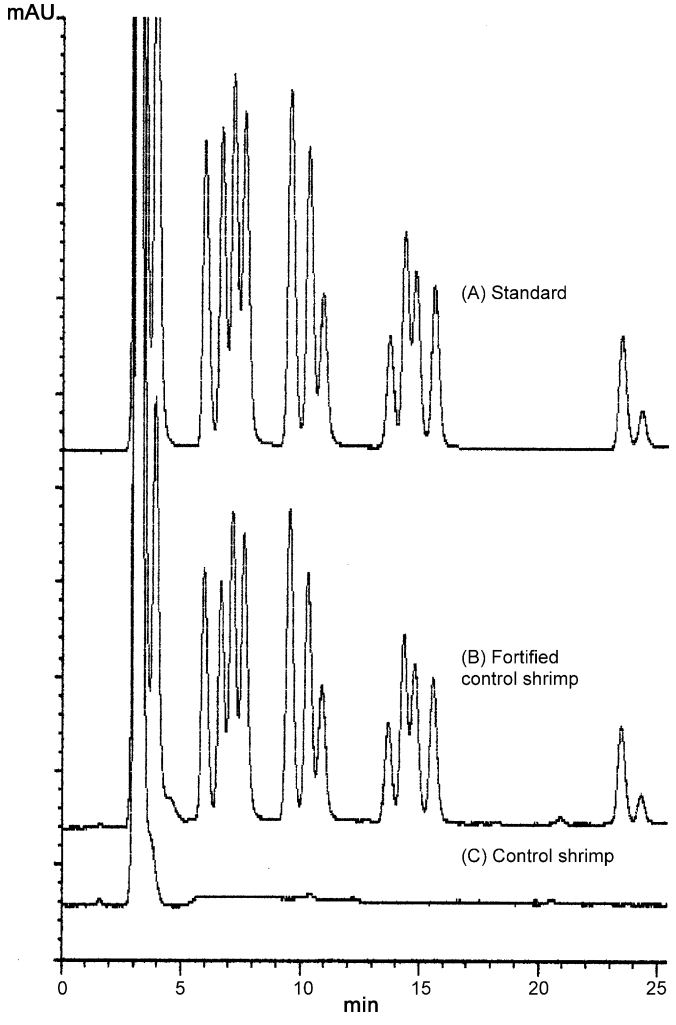

Fig. 2. HPLC chromatograms for 50-μL injections of fluorescamine derivatives of (A) quantitative standard containing 50 ng SAs/mL, extracts of (B) control shrimp edible tissue fortified with 10 ng SAs/g tissue and (C) control shrimp edible tissue.

10:1, limit of quantitation was 1 ng g^{-1}; an exception was SQX with a S/N of 5:1. Three aquaculture drugs, all having primary amine groups, were tested for interference with the SAs by chromatography of standards: Acriflavin produced two peaks with retention times of approximately 9.1 and 9.5 min, both near that of SMZ with a retention time of approximately 9.3 min, benzocaine produced one non-interfering peak approximately 1 min before that of SDM, and tricaine methanesulfonate produced one non-interfering peak at approximately 12 min.

Chromatograms demonstrating recoveries of SAs from control catfish, shrimp and salmon tissue fortified at10 ng g^{-1} are demonstrated in Figs. 1–3, respectively. Corresponding recovery data are presented in Tables 1–3. All recoveries were within FDA guidelines [8] for recovery (60–110% when the marker residue is under 0.1 ppm). Catfish and shrimp were fortified at 5, 10 and 20 ng SAs g^{-1}, while salmon was fortified at the 10 ng g^{-1} level only; recoveries from this single 10 ng g^{-1} fortification averaged 87.6 ± 4.7%, comparable to previous 10 ng g^{-1} average recoveries of 84.6 ± 7.7% found in a previous validation [4]. All assays met FDA guidelines for intra-assay variability (CV ≤ 20% when the marker residue is under 0.1 ppm). To demonstrate inter-assay variability, catfish and shrimp were later fortified a second time with 10 ng SAs g^{-1}. Recoveries averaged 91.6 ± 7.4% from catfish and 85.3 ± 5.8% from shrimp, compared with earlier average 10 ng g^{-1} recoveries of 79.7 ± 6.4% from catfish and 82.9 ± 6.2% from shrimp.

Chromatograms representing determinations of SAs from dosed catfish, shrimp or salmon are presented in Figs. 4–6. Assays consisted of five replicate samples, one control, one fortified control and one reagent blank. All recoveries and CVs met FDA guidelines for intra-assay variability; data are presented in Tables 4–6. Dosed tissues (1 sample each) were initially screened to determine approximate levels of SAs. To demonstrate the efficacy of the method, the CVM's suggested levels were approximately 8 (between 1/2× and 1× target) ng g^{-1} and approximately 16 (between 1× and 2× target) ng g^{-1}. When dosed tissues were not within this desired range, they

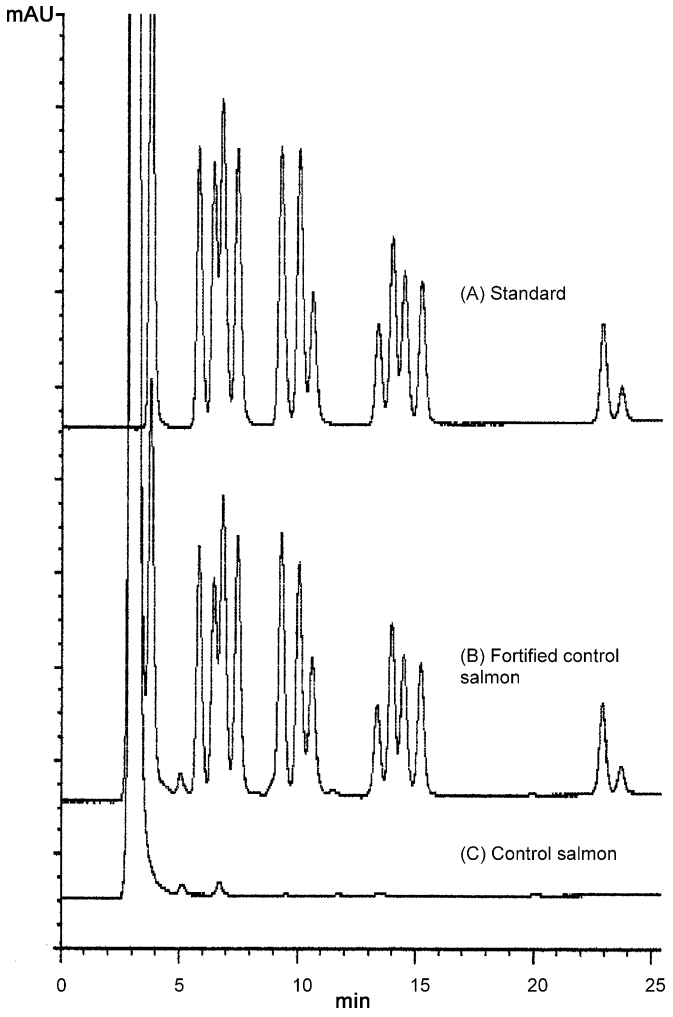

Fig. 3. HPLC chromatograms for 50-μL injections of fluorescamine derivatives of (A) quantitative standard containing 50 ng SAs/mL, extracts of (B) control salmon muscle and skin fortified with 10 ng SAs/g tissue and (C) control salmon muscle and skin.

Table 1
Recoveries of sulfonamides from fortified catfish tissue ($n = 5$)

Sulfonamide	Recovery (%) at indicated fortification								
	5 ng/g			10 ng/g			20 ng/g		
	Mean	S.D.	CV	Mean	S.D.	CV	Mean	S.D.	CV
Sulfanilamide	79.2	4.0	5.1	64.2	1.3	2.1	67.2	1.8	2.7
Sulfadiazine	81.5	1.9	2.4	77.5	2.9	3.7	85.3	3.5	4.1
Sulfathiazole	72.5	2.2	3.1	69.9	2.9	4.1	77.7	3.3	4.2
Sulfapyridine	78.9	2.2	2.8	76.6	2.1	2.8	84.9	3.4	4.0
Sulfamerazine	83.7	2.0	2.4	81.6	2.7	3.3	88.3	3.6	4.0
Sulfamethazine	83.4	2.2	2.7	81.7	2.6	3.1	88.7	3.4	3.8
Sulfamethizole	79.6	2.1	2.6	78.6	2.6	3.3	84.3	3.0	3.5
Sulfamethoxypyridizine	82.3	2.2	2.7	80.6	1.9	2.3	89.0	3.3	3.7
Sulfachloropyridizine	83.9	2.1	2.5	84.6	1.8	2.1	91.2	3.3	3.6
Sulfamonomethoxine	83.8	2.5	3.0	83.8	1.8	2.1	90.2	3.1	3.4
Sulfadoxine	85.4	2.7	3.1	85.3	2.0	2.4	91.9	3.5	3.8
Sulfamethoxazole	89.3	4.5	5.1	87.1	1.5	1.7	91.3	3.3	3.6
Sulfadimethoxine	86.9	2.9	3.3	86.2	2.7	3.1	91.5	3.0	3.3
Sulfaquinoxaline	79.6	2.7	3.4	78.0	2.3	3.0	86.4	3.1	3.6

Table 2
Recoveries of sulfonamides from fortified shrimp tissue ($n = 5$)

Sulfonamide	Recovery (%) at indicated fortification								
	5 ng/g			10 ng/g			20 ng/g		
	Mean	S.D.	CV	Mean	S.D.	CV	Mean	S.D.	CV
Sulfanilamide	77.3	4.7	6.1	67.3	4.2	6.2	70.0	1.9	2.8
Sulfadiazine	78.3	3.2	4.1	81.2	4.8	5.9	85.5	1.6	1.9
Sulfathiazole	69.8	3.3	4.7	73.4	4.4	6.0	76.4	0.9	1.2
Sulfapyridine	78.6	3.1	3.9	80.3	3.8	4.8	84.1	1.2	1.4
Sulfamerazine	81.4	2.8	3.4	84.8	4.8	5.6	86.9	1.8	2.0
Sulamethazine	80.7	2.5	3.1	85.4	4.9	5.7	86.1	1.7	2.0
Sulfamethizole	74.8	2.3	3.1	81.4	4.5	5.6	83.1	1.8	2.2
Sulfamethoxypyridazine	82.6	3.0	3.7	87.4	4.7	5.4	87.2	1.9	2.2
Sulfachloropyridazine	82.8	2.9	3.5	86.8	4.4	5.1	87.8	2.6	2.9
Sulfamonomethoxine	85.5	3.4	4.0	86.5	4.5	5.2	86.8	2.6	3.0
Sulfadoxine	88.8	4.2	4.8	89.9	4.8	5.3	89.5	2.4	2.7
Sulfamethoxazole	87.9	3.4	3.9	88.7	4.8	5.4	90.5	2.8	3.1
Sulfadimethoxine	86.9	1.8	2.0	86.6	4.8	5.6	87.1	4.1	4.7
Sulfaquinoxaline	84.4	2.2	2.6	81.2	5.5	6.8	82.9	4.8	5.8

Table 3
Recoveries of sulfonamides from salmon tissue fortified at 10 ng/g ($n = 5$)

Sulfonamide	Recovery (%)		
	Mean	S.D.	CV
Sulfanilamide	86.6	14.6	16.9
Sulfadiazine	86.6	4.1	4.7
Sulfathiazole	80.6	3.7	4.6
Sulfapyridine	89.4	3.6	4.0
Sulfamerazine	90.7	4.1	4.6
Sulfamethazine	91.4	4.1	4.4
Sulfamethizole	80.9	4.2	5.2
Sulfamethoxypyridazine	100.6	4.9	4.8
Sulfachloropyridazine	86.7	4.0	4.7
Sulfamonomethoxine	90.2	3.9	4.3
Sulfadoxine	90.0	2.6	2.9
Sulfamethoxazole	89.5	4.1	4.6
Sulfadimethoxine	88.1	3.8	4.4
Sulfaquinoxaline	75.8	3.5	4.6

were diluted by homogenization with an estimated appropriate amount of control tissue and then analyzed. Determination of SQX in catfish was eliminated from the priority listing because none was detected even at the relatively high dose of more than 300 mg kg^{-1}.

During method development and validation, we found some points necessary for success. In an attempt to expedite homogenization, some tissues were processed once only with dry

Table 4
Sulfonamides (ng/g) in edible tissues of dosed catfish ($n = 5$)

Sulfonamide	Low level			High level		
	Mean	S.D.	CV (%)	Mean	S.D.	CV (%)
Sulfadiazine	4.9	0.3	5.2	18.9	1.1	5.9
Sulfamerazine	8.1	0.3	3.8	16.0	1.1	6.7
Sulfamethazine	6.5	0.4	6.8	18.3	1.3	7.3
Sulfachloropyridizine	6.5	0.4	6.3	14.6	1.1	7.6
Sulfadimethoxine	7.5	0.2	2.6	21.0	0.8	3.5

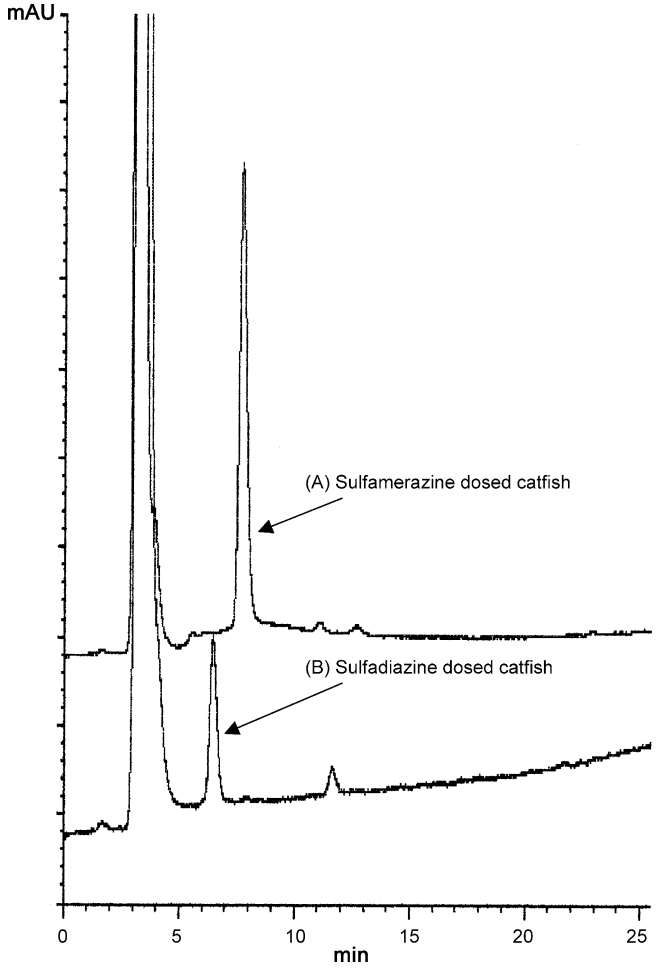

Fig. 4. HPLC chromatograms for 50-μL injections of fluorescamine derivatives of extracts of dosed catfish containing (A) 8.49 ng sulfamerazine/g muscle and (B) 4.81 ng sulfadiazine/g muscle.

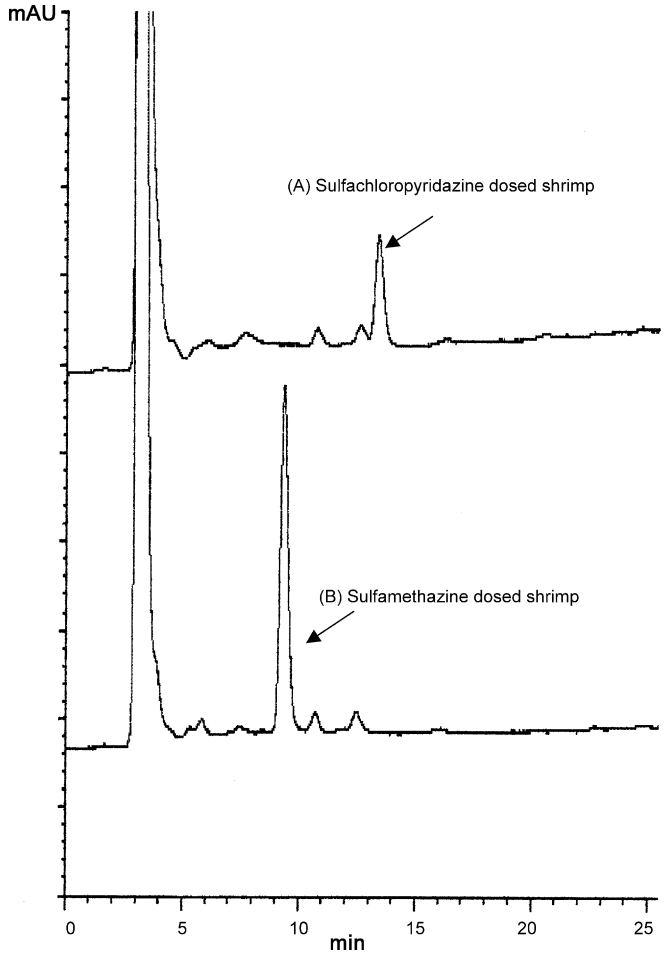

Fig. 5. HPLC chromatograms for 50-μL injections of fluorescamine derivatives of extracts of dosed shrimp containing (A) 8.80 ng sulfachloropyridazine/g edible tissue and (B) 8.34 ng sulfamethazine/g edible tissue.

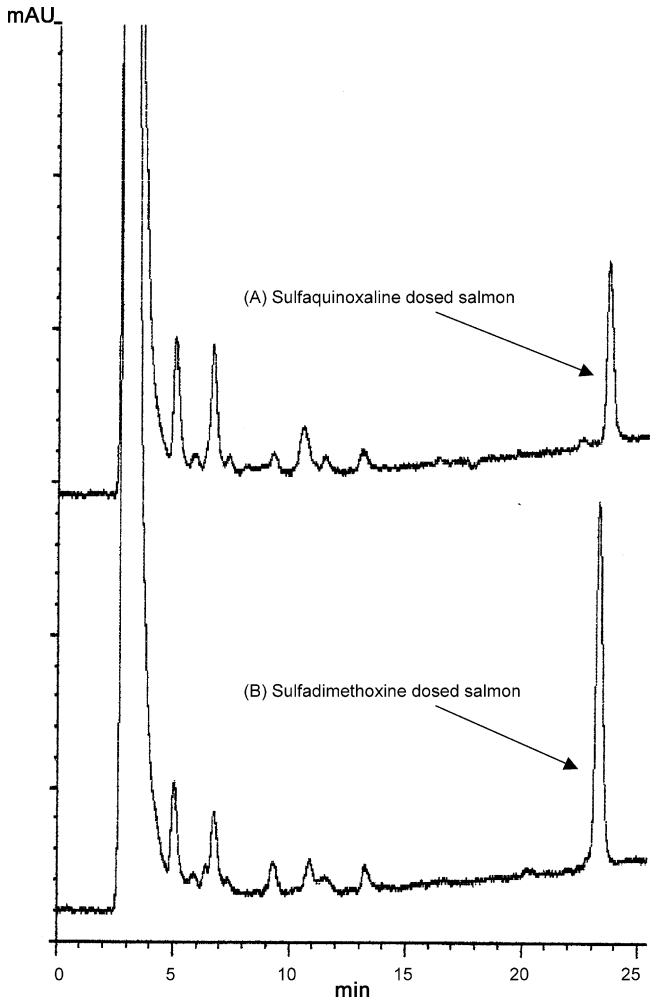

Fig. 6. HPLC chromatograms for 50-μL injections of fluorescamine derivatives of extracts of dosed salmon containing (A) 7.73 ng sulfaquinoxaline/g muscle and skin and (B) 4.98 ng sulfadimethoxine/g muscle and skin.

Table 5
Sulfonamides (ng/g) in edible tissues of dosed shrimp ($n = 5$)

Sulfonamide	Low level			High level		
	Mean	S.D.	CV (%)	Mean	S.D.	CV (%)
Sulfadiazine	8.6	0.5	6.0	17.4	0.7	4.1
Sulfamerazine	6.8	0.1	1.8	19.1	0.4	2.1
Sulfamethazine	8.1	0.3	3.3	19.7	0.4	2.2
Sulfachloropyridizine	8.7	0.2	1.9	15.4	0.5	3.2
Sulfadimethoxine	9.9	0.3	2.9	17.5	0.4	2.5
Sulfaquinoxaline	4.9	0.2	4.0	13.5	0.5	3.6

Table 6
Sulfonamides (ng/g) in edible tissues of dosed salmon ($n = 5$)

Sulfonamide	Low level			High level		
	Mean	S.D.	CV (%)	Mean	S.D.	CV (%)
Sulfadiazine	8.5	1.4	16.0	22.4	1.6	7.1
Sulfamerazine	8.0	0.4	4.9	18.7	2.3	12.2
Sulfamethazine	8.7	0.6	6.4	21.5	0.6	2.6
Sulfachloropyridazine	8.0	0.6	7.8	22.2	0.7	3.0
Sulfadimethoxine	8.5	0.6	6.5	14.4	1.6	10.9
Sulfaquinoxaline	7.6	0.5	6.6	15.7	1.7	11.0

ice. However, since the SAs declined under these conditions, this technique was abandoned. It was important to homogenize three times as described above to homogenize sufficiently. Proper homogenization was particularly important in processing salmon muscle with skin, since SDZ has been shown to be up to 30 times higher in salmon skin than in muscle [9]. During the first liquid–liquid extraction, it was important to extract with at least 60 mL methylene chloride; extracting with less than 60 mL sometimes resulted in the formation of three layers, or the layers appeared inverted. To prevent recovery loss, it was important not to let the extracts go to dryness during rotary evaporation. While eight samples could easily be prepared during an 8 h workday, it was important that the samples were at least completed to the point that they were eluted off the SPE cartridge. They could then be stored frozen overnight and concentrated by evaporation under nitrogen the next day for LC analysis. Ending sample preparation at any earlier step resulted in recovery loss.

In conclusion, the LC method presented enables quantitative determination of 14 sulfonamide residues at levels of 5, 10 and 20 ng g^{-1} in edible tissues of catfish, shrimp and salmon. These residues may be present in aquacultured species produced

for food. In addition, the mobile phases and completed samples are suitable for either screening or confirmation by liquid chromatography/mass spectrometry (LC/MS).

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References

[1] N.A. Littlefield, W.G. Sheldon, R. Allen, D.W. Gaylor, Food Chem. Toxicol. 28 (1990) 157.
[2] A.E. Cribb, M. Miller, A. Tespro, S.P. Spielberg, Mol. Pharmacol. 38 (1990) 744.
[3] H.C. Neu, Science 257 (1992) 1064.
[4] T.A. Gehring, L.G. Rushing, H.C. Thompson Jr., J. AOAC Int. 80 (1997) 751.
[5] C.M.F. Michel, K.S. Squibb, J.M. O'Connor, Xenobiotica 20 (1990) 1299.
[6] F.K. Alavi, L.L. Rolf, C.R. Clarke, J. Vet. Pharmacol. Ther. 16 (1993) 232.
[7] J.A. Walisser, H.M. Burt, T.E. Valg, D.D. Kitts, K.M. McErlane, J. Chromatogr. 518 (1990) 179.
[8] General Principles for Evaluating the Safety of Compounds used in Food-producing Animals, U.S. Food and Drug Administration Memorandum, 1984.
[9] T.A. Gehring, L.G. Rushing, M.I. Churchwell, D.R. Doerge, K.M. McErlane, H.C. Thompson Jr., J. Agric. Food Chem. 44 (1996) 3164.