HPLC Determination of Sulfadiazine Residues in Coho Salmon (*Oncorhynchus kisutch*) with Confirmation by Liquid Chromatography with Atmospheric Pressure Chemical Ionization Mass Spectrometry

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High-performance liquid chromatography and postcolumn derivatization with fluorescamine were used to determine sulfadiazine (SDZ) residues in muscle and skin from salmon dosed with Tribrissen. Determinations in the fish ranged from 0.2 to 21 600 ng of SDZ/g; in each fish tested, the concentration of SDZ in the skin was at least twice that in the muscle. Liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (LC−APCI/MS) was used to confirm SDZ residues. In-source collision induced dissociation was optimized to produce a mass spectrum containing the protonated molecule and four fragment ions. Sulfadiazine was confirmed in muscle fortified with 10 ng of SDZ/g and in muscle from fish dosed with SDZ by the accurate agreement of ion intensity ratios generated from muscle compared with those generated from SDZ standard injections.

Keywords: *Sulfadiazine; salmon; Oncorhynchus kisutch; liquid chromatography; mass spectrometry*

INTRODUCTION

Sulfadiazine is a drug used in some countries to prevent or treat bacterial diseases in salmon raised in aquaculture (Hormazabal and Rogstad, 1992; Gentleman *et al*., 1993). Because SDZ is not approved for aquaculture use in the United States, SDZ is on the Food and Drug Administration's priority list for fish drugs and chemicals scheduled for methods development; the desired target level for the determination of SDZ in salmon is 1−10 ng of SDZ/g. Gehring *et al*. (1995) developed and validated a high-performance liquid chromatography (HPLC) method with postcolumn fluorescamine derivatization and fluorescence detection to quantitate SDZ in muscle at 1 ng/g in coho salmon and 10 ng/g in Atlantic salmon. For the present study, we applied this method to determine residues in muscle and skin of coho salmon dosed with SDZ.

In addition to a determinative method, a confirmatory method was desired. For regulatory confirmation, mass spectrometry (MS) is the technique of choice. Because of the high sensitivity required and the desire to avoid derivatization reactions, we chose liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (LC−APCI/MS) to confirm SDZ in salmon muscle fortified with 10 ng of SDZ/g and in muscle from salmon dosed with SDZ. We have used LC coupled with APCI/MS detection for the analysis of pesticides in groundwater (Doerge and Bajic, 1992), sulfonamides in milk extracts (Doerge *et al*., 1993), and β-agonist drugs in plasma (Doerge *et al*., 1995). These studies demonstrated the use of a single quadrupole mass spectrometer to produce, by in-source collision induced dissociation (CID), diagnostic fragment ions in a manner analogous to that observed in the collision cell of a triple quadrupole mass spectrometer. In a subsequent study, we demonstrated that a single quadrupole instrument could provide LC/MS data with accuracy and precision consistent with the criteria used by regulatory agencies for confirmatory analyses as previously applied to electron impact (EI) ionization and MS/MS data (Doerge *et al*., 1996). In the present study, we used in-source CID, produced by varying the cone voltage (the potential difference between the sampling cone and the skimmer) to generate diagnostic ions from SDZ as previously described (Doerge *et al*., 1993). The ability to do this for multiple ions reproducibly and with precision with respect to an authentic standard is the basis of the confirmatory method described here.

MATERIALS AND METHODS

Fish Dosing. Fish were dosed at Salt Spring Aquafarms, Salt Spring, British Columbia, under the supervision of the University of British Columbia. Salmon kept in commercial ocean pens were administered Tribrissen, a mixture of 5 parts sulfadiazine and 1 part trimethoprim (Burroughs Wellcome, Kirkland, Canada), via surface-coated 5 mm medicated feed pellets at the rate of 40 mg of Tribrissen/kg of fish daily for 10 days. Tribrissen was administered in two doses of 20 mg/kg in the morning and 20 mg/kg in the late afternoon. Thus, the fish received 33.3 mg/kg of SDZ and 6.7 mg/kg of trimethoprim daily. Five fish were removed on the 10th, 15th, 20th, and 25th days after initiation of treatment. These fish were weighed, gutted, frozen at −20 °C, packed in dry ice, and shipped to the National Center for Toxicological Research where they were stored at −70 °C. Commercially purchased, wild-caught salmon for controls were also stored frozen at −70 °C.

Apparatus. For the determinative procedure, the HPLC system and other apparatus were identical to those described previously by Gehring *et al*. (1995).

For the confirmatory procedure, the mass spectrometry system consisted of a GP40 gradient pump (Dionex, Sunnyvale, CA), an Inertsil 5 μm ODS-2, 150 × 4.6 mm i.d. analytical column (MetaChem Technologies, Redondo Beach, CA), and a VG Platform single quadrupole mass spectrometer equipped with an APCI interface and an atmospheric pressure ion source (Fisons-VG Organic, Altrincham, UK).

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S0021-8561(96)00187-2 CCC: $12.00

Reagents and Materials. For the determinative procedure, all reagents and materials were the same as those used by Gehring *et al*. (1995).

For the confirmatory procedure, the mobile phase was acetonitrile/2% acetic acid (20:80). Phosphoric acid (0.2 M) was used to elute SDZ from the propylsulfonic acid (PRS) strong cation exchange solid phase extraction (SPE) cartridges (P/N 1210-2039, Varian, Harbor City, CA). Sulfadiazine in the PRS eluates was concentrated using Waters Sep-Pak Vac 6cc, 1.0 g, trifunctional C_{18} (tC_{18}) SPE cartridges (P/N WAT036795, Waters, Milford, MA). The mass spectrometer was calibrated with the following solutions of poly(ethylene glycols) (PEG) PEG 200, 25 μg/mL; PEG 300, 50 μg/mL; PEG 600, 75 μg/mL; and PEG 1000, 250 μg/mL (Sigma Chemical Co., St. Louis, MO) in acetonitrile/5 mM aqueous ammonium acetate (50:50).

Procedure. Semifrozen 2-cm salmon muscle chunks from an entire filet were chopped in a blender and stirred to mix; salmon skin from one filet was minced into small pieces (<5 mm) with scissors and stirred to mix.

All determinative standards and muscle tissue sample extracts were prepared for and determined by HPLC by the method of Gehring *et al*. (1995). Skin tissue samples were prepared by the same method, but with the following exceptions: To conserve samples, triplicate 6-g samples were used, except control and fortified samples, which were quadruplicate 10-g samples. Skin samples were homogenized with the Ultra-Turrax at 24 000 rpm for 30 s.

Analytical confirmatory standards were diluted with methanol/water (50:50). Samples for the confirmatory method were prepared as for the determinative method up to the point of elution from the PRS cartridge. The PRS cartridge was instead eluted with 4.0 mL of 0.2 M phosphoric acid, and the 4.0-mL eluate was discarded. Then the cartridge was eluted with 5.0 mL of 0.2 M phosphoric acid, and the eluate was quantitatively collected in a test tube. This eluate was loaded onto a 6 cm^3/1 g tC_{18} cartridge which had been preconditioned with 6 mL of methanol followed by 6 mL of distilled, deionized (DD) water. The cartridge was washed with 6 mL of DD water and eluted with 2.0 mL of methanol; the 2.0 mL of eluate produced was discarded. The cartridge was then eluted with another 2.0 mL of methanol. The resulting 2.0 mL eluate (the extract from 10 g of muscle) was collected in a 5-mL graduated tube, evaporated under a stream of nitrogen to 0.5 mL, and diluted to exactly 1.0 mL with DD water before 20-μL injections into the LC/MS system.

The total LC column effluent was delivered at 1 mL/min into the atmospheric pressure ion source (120 °C) through the heated nebulizer probe (500 °C). The probe and bath gas was nitrogen. The mass spectrometer was calibrated using the PEG calibration solutions over the range of m/z 85−1200. Positive ions were acquired in full scan (m/z 85−300, 1 s cycle time) or selected ion monitoring (SIM) modes. For SIM, the span was 0.02 Da, dwell time was 0.2 s, and interchannel delay was 0.03 s for a total duty cycle of 1.15 s. The sampling cone voltage was changed to produce varying amounts of in-source CID in concert with acquisition of selected ions. The following were typical conditions for acquisition of SDZ-derived ions: for ions of m/z 251, 158, 156, 108, and 92, cone voltages of 26, 31, 38, 44, and 44 V, respectively, were used. Full scan mass spectra were obtained from flow injection analysis (FIA) at 1.0 mL/min of 100-ng samples of SDZ. Cone voltages were optimized daily over a small range to produce ion intensity ratios as close to 1.0 as possible in order to minimize the effect of random noise on low intensity ion signals. Muscle from commercially purchased salmon served as control tissue; this tissue was fortified with 10.5 ng of SDZ/g. Preparations from these tissues and from muscles of dosed salmon determined to contain approximately 0.2, 9 and 20 ng of SDZ/g were injected into the LC/MS system.

RESULTS AND DISCUSSION

Sulfadiazine eluted in approximately 6.5 min from the HPLC system; representative HPLC chromatograms are presented in Figure 1 (muscle) and Figure 2 (skin). Sulfadiazine standards ranging in concentration from 1.05 to 1050 ng of SDZ/mL were chromatographed, and

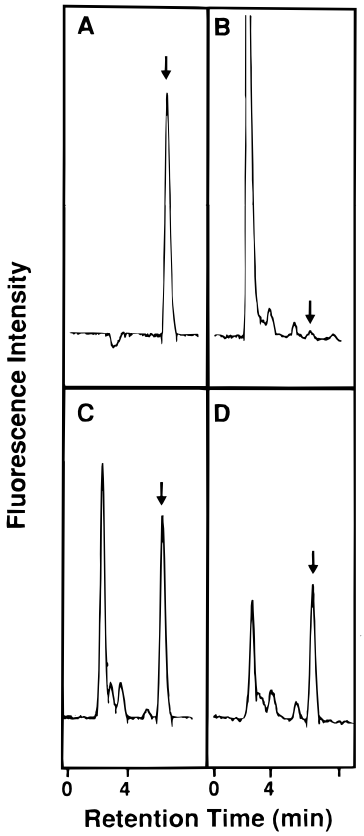

Figure 1. HPLC chromatograms for 10 μL injections of fluorescamine derivatives of (A) quantitative standard containing 8.32 ng of SDZ/mL, (B) fortification control salmon muscle containing 0.2 ng of SDZ/g, (C) salmon muscle (B) fortified with 5.25 ng of SDZ/g, and (D) muscle from dosed salmon containing 3.05 ± 0.59 ng of SDZ/g. Peaks corresponding to SDZ are marked with arrows.

response was linear (r = 0.999 997). Ten replicate injections of a 21.0 ng of SDZ/mL standard demonstrated the reproducibility of the HPLC-postcolumn fluorescamine derivatization (CV = 2%). Interassay variability was measured by determining SDZ residues in a selected muscle sample prepared and determined on three different days spanning a 2-month period (n = 4 for each day). Sulfadiazine in the selected sample averaged 8.32 ± 1.24 ng of SDZ/g (CV = 15%). The limit of quantitation (LOQ) was 1.0 ng of SDZ/g, equivalent to the lowest concentration of standard used. The limit of detection (LOD) was 0.2 ng of SDZ/g, 3 times the noise level of chromatographed extract. Injection volumes of 10 μL were used throughout the determinations; larger injection volumes may be used to further lower detection limits.

Replicate 10-g dosed salmon muscle samples, containing SDZ residues determined to be 0.2 ng of SDZ/g, were fortified with 0.1, 0.5, 1.0, and 2.0 mL of fortification standard (105 ng of SDZ/mL), representing 1.05, 5.25, 10.5, and 21.0 ng of SDZ/g, the target level and twice above (n = 4 for each level). After correcting for the 0.2 ng of SDZ/g present before fortification, recoveries from the fortified muscle samples taken through the determinative procedure averaged 75.4%. Replicate 10-g samples from a composite of skins from coho salmon purchased commercially were fortified with 0.5, 1.0, and 2.0 mL fortification standard representing 5.25, 10.5, and 21.0 ng of SDZ/g (n = 4 for each level). Recoveries from fortified skin averaged 62.4%. Determinations were made using external standards. All recovery data are presented in Table 1.

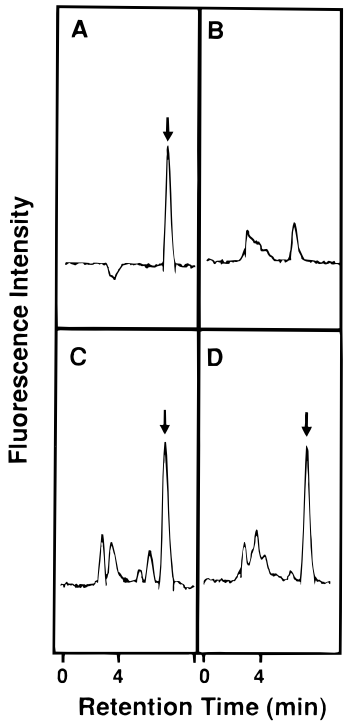

Figure 2. HPLC chromatograms for 10 μL injections of fluorescamine derivatives of (A) quantitative standard containing 4.16 ng of SDZ/mL, (B) fortification control salmon skin, (C) salmon skin (B) fortified with 5.25 ng of SDZ/g, and (D) skin from dosed salmon containing 3.64 ± 0.29 ng of SDZ/g. Peaks corresponding to SDZ are marked with arrows.

Table 1. Recovery of Sulfadiazine from Fortified Salmon Tissues

tissue	fortification level (ng/g)	av recovery[a] (%)	CV (%)
muscle	1.05	70.8 ± 7.1	10
	5.25	78.3 ± 4.5	5.8
	10.5	74.7 ± 8.4	11
	21.0	77.8 ± 4.5	5.8
skin	5.25	66.5 ± 7.2	11
	10.5	59.2 ± 1.8	3.0
	21.0	61.4 ± 2.7	4.4

[a] Averages of four replicates.

Determinations of SDZ residues in muscle or skin of dosed salmon ranged from 0.2 to 21 600 ng of SDZ/g. Sample extracts exceeding the established linear range were diluted appropriately prior to chromatography. For muscle, coefficients of variation among replicates (n = 4) averaged 2.3 ± 0.8% for determinations above 100 ng/g and 16.5 ± 8.1% for those below 100 ng/g. For skin, coefficients of variation among replicates (n = 3) averaged 4.7 ± 2.4% for determinations above 100 ng/g and 7.1 ± 6.3% for those below 100 ng/g. Determinations were not corrected for recovery. Data are presented in Table 2.

The concentrations of SDZ in both muscle and skin of dosed salmon varied widely among individual fish on all four test days after dosing was discontinued; similar wide variations have been noted by McErlane (1995). Coefficients of variation among sets of fish for each postdose day ranged from 113 to 222%. All 20 fish tested had higher concentrations of SDZ in the skin than in the muscle. Concentrations in the skin ranged from 2 to 31 times higher than in muscle from the same fish.

High coefficients of variation among replicates were noted for some dosed muscle samples containing SDZ residues ≤3 ng/g. These samples were homogenized by blending and mixing as described under Procedure. We attempted to improve the homogeneity of three muscle samples by more rigorous blending with liquid nitrogen as follows: A 1-gal (approximately 4-L) blender was filled about one-fourth full of liquid nitrogen. The speed of the blender was controlled with a variable rheostat to prevent any liquid nitrogen from splashing out. (**Note**: Liquid nitrogen must be handled with caution to avoid cold injuries. The samples were blended in a fume hood, and protective gear was worn.) The sample was then added to the blender in small amounts (about 10 g at a time). When the entire sample was in the blender, the cover was placed on the blender, and the rheostat was turned high for a total of about 2 min or until the sample was blended to a powdery consistency. When necessary during high-speed blending, the rheostat was turned down, and additional liquid nitrogen was added to maintain the level in the blender cup. To prevent atmospheric moisture condensation on the sample, the blender containing the pulverized sample was placed into a dry atmosphere glovebox until the nitrogen had evaporated (about 15 min). To maintain a dry atmosphere, dry nitrogen was pumped through the vented glove box. Samples were stored in a −70 °C freezer until analyzed. Results of replicate samples (n = 4) homogenized by nitrogen blending are presented in Table 3. Coefficients of variation of SDZ determinations in all three samples were noticeably lower after nitrogen blending. Two skin samples were homogenized similarly by nitrogen blending (Table 2) but required up to 1 h for pulverization, and much sample was lost due to the resulting aerosol. Although nitrogen blending is an effective homogenizing method for both muscle and skin, consideration must be given that it requires added labor, can cause loss of sample, and can be hazardous.

We have observed that the elution profiles of SDZ from different lots of PRS cartridges can vary; therefore, each lot must be evaluated before use. These elution evaluations must be performed with a matrix fortified with SDZ, since the matrix increases the retention time of SDZ on the cartridge. By eluting in 1-mL increments, the analyst can determine the "window" in which SDZ elutes. For the determinative procedure, the eluting solvent was acetonitrile/0.2 M H_3PO_4 (10:90), and the elution window included the 3rd through the 8th mL eluted; thus, the final volume of these samples was 6 mL (extracts from 10 g of muscle and 10 or 6 g of skin in 6 mL of elution solvent).

For LC−APCI/MS confirmation, samples were further concentrated and the H_3PO_4 removed with tC_{18} cartridges. However, to retain SDZ on the tC_{18} cartridge, the solvent for elution from the PRS cartridge was changed to 0.2 M H_3PO_4, and the resulting elution window included the 5th through the 9th mL eluted (extracts from 10 g of muscle in 5 mL of elution solvent). Although our extracts for the determinative and confirmatory procedures were prepared separately, we suggest, to conserve labor, that preparation be done by the method described for the confirmatory procedure only. Sulfadiazine may be determined in as little as 10 μL of the PRS eluate; the remainder of the eluate from positive samples may then be concentrated for MS confirmation.

Figure 3 shows the mass spectrum of SDZ at various cone voltages, and Figure 4 shows the proposed fragmentation reactions. The ions produced under the conditions described above include both SDZ-specific ions (m/z 251, 158, 96) and sulfonamide class-specific ions (m/z 156, 108, 92). These spectra show that it is not possible to obtain a sufficient number of diagnostic

Table 2. Residues of Sulfadiazine in Tissues of Dosed Salmon on Days 0, 5, 10, and 15 after Drug Withdrawal[a]

			sulfadiazine detected (ng/g)			
			muscle		skin	
day	fish no.	weight (kg)	av	CV (%)	av	CV (%)
0	1	2.4	7180 ± 154	2	14700 ± 760	5
	2	2.4	19.9 ± 3.38	17	440 ± 22.7	5
	3	2.5	10200 ± 330	3	21600 ± 462	2
	4	2.7	91.9 ± 4.34	5	749 ± 58	8
	5	3.0	9.07 ± 1.01	11	283 ± 4.2	2
5	1	2.4	8180 ± 224	3	16200 ± 1300	8
	2	2.4	1530 ± 32	2	2920 ± 83	3
	3	2.4	7.36 ± 0.64	9	109 ± 7.2	7
	4[b]	2.5	2.57 ± 0.31	12	45.1 ± 0.9	2
	5[b]	3.2	3.05 ± 0.59	19	60.5 ± 2.1	3
10	1	2.1	8.32 ± 1.24	15	64.8 ± 3.9	6
	2	2.4	20.4 ± 0.58	3	61.5 ± 2.4	4
	3[b]	2.5	1.37 ± 0.33	24	21.4 ± 3.8	18
	4	2.6	0.87 ± 0.22	25	26.7 ± 5.0	19
	5	2.9	190 ± 1.70	1	388 ± 13	3
15	1[c]	1.6	0.2 ± 0.05	25	3.64 ± 0.29	7
	2[c]	1.9	0.2 ± 0.06	29	2.04 ± 0.04	3
	3	2.1	0.91 ± 0.16	18	24.4 ± 0.4	2
	4	2.5	2.24 ± 0.24	11	53.7 ± 3.7	7
	5	2.8	855 ± 21.6	3	2700 ± 58.9	2

[a] Day 0 is the final dosing day. [b] Muscle homogenized by blending with liquid nitrogen. [c] Skin homogenized by blending with liquid nitrogen.

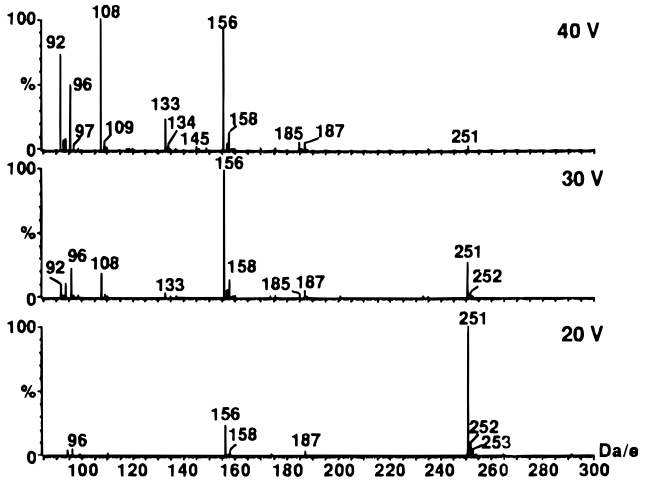

Figure 3. Full scan APCI mass spectrum of SDZ at various cone voltages.

Table 3. Effect of Blending with Liquid Nitrogen on Homogeneity of Sulfadiazine in Salmon Muscle

	sulfadiazine detected (ng/g)			
	before blending with N_2		after blending with N_2	
fish sample	av[a]	CV (%)	av[a]	CV (%)
1	3.20 ± 1.27	40	2.57 ± 0.31	12
2	3.82 ± 1.88	50	3.05 ± 0.59	19
3	2.51 ± 2.23	89	1.37 ± 0.33	24

[a] Averages of four replicates.

ions and the protonated molecule with adequate intensity at any single cone voltage. The selected ion chromatograms generated using this technique showed comparable retention times for SDZ standards and for SDZ in dosed salmon extracts (Figure 5).

Confirmatory techniques using a single quadrupole mass spectrometer are dependent on chromatographic resolution from potentially coeluting substances that may contain interfering ions and alter the intensity ratio values. The ratio derived from *m/z* 96 differed significantly from that produced by standard SDZ because of a large response at this mass from an unidentified interference present in the reagent blank (not salmon tissue) that eluted just prior to SDZ (retention time 3.86

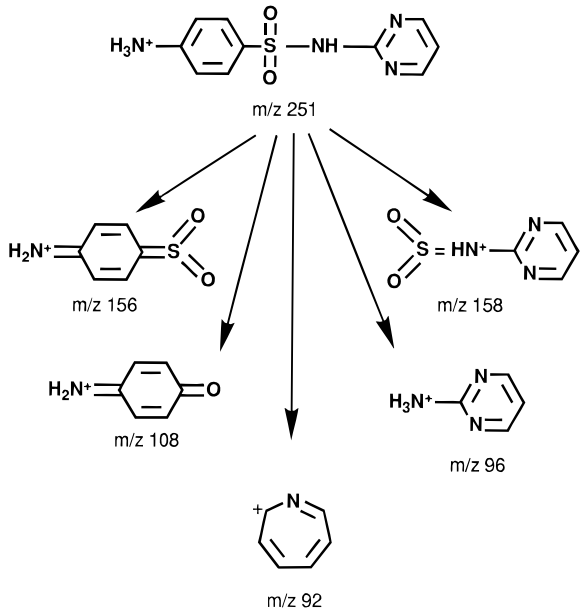

Figure 4. Proposed fragmentation reactions of SDZ.

min, data not shown). The distortion in baseline noise seen in some selected ion chromatograms from the control extract at that time (Figure 5A) is also due to this component; however, as shown in Figure 5, this interference was small with respect to the SDZ signals and had no effect on any other standard/sample ratios at the levels tested (see Tables 4 and 5). However, when incurred salmon samples containing 0.2 ng of SDZ/g were analyzed, the baseline distortions due to the interference were of sufficient magnitude to vitiate all ratio determinations. The baseline dips probably represent the suppression of chemical noise from the mobile phase when significant amounts of other ionizable compounds elute from the column. In a similar manner, it is possible for such suppression by a coeluting substance to interfere with signals from the analyte of interest, even though there are no common ions in the mass spectra.

The limit of confirmation was estimated by extrapolation of the response for the ion with the lowest signal to noise (S/N) ratio of the five ions monitored, *m/z* 158.

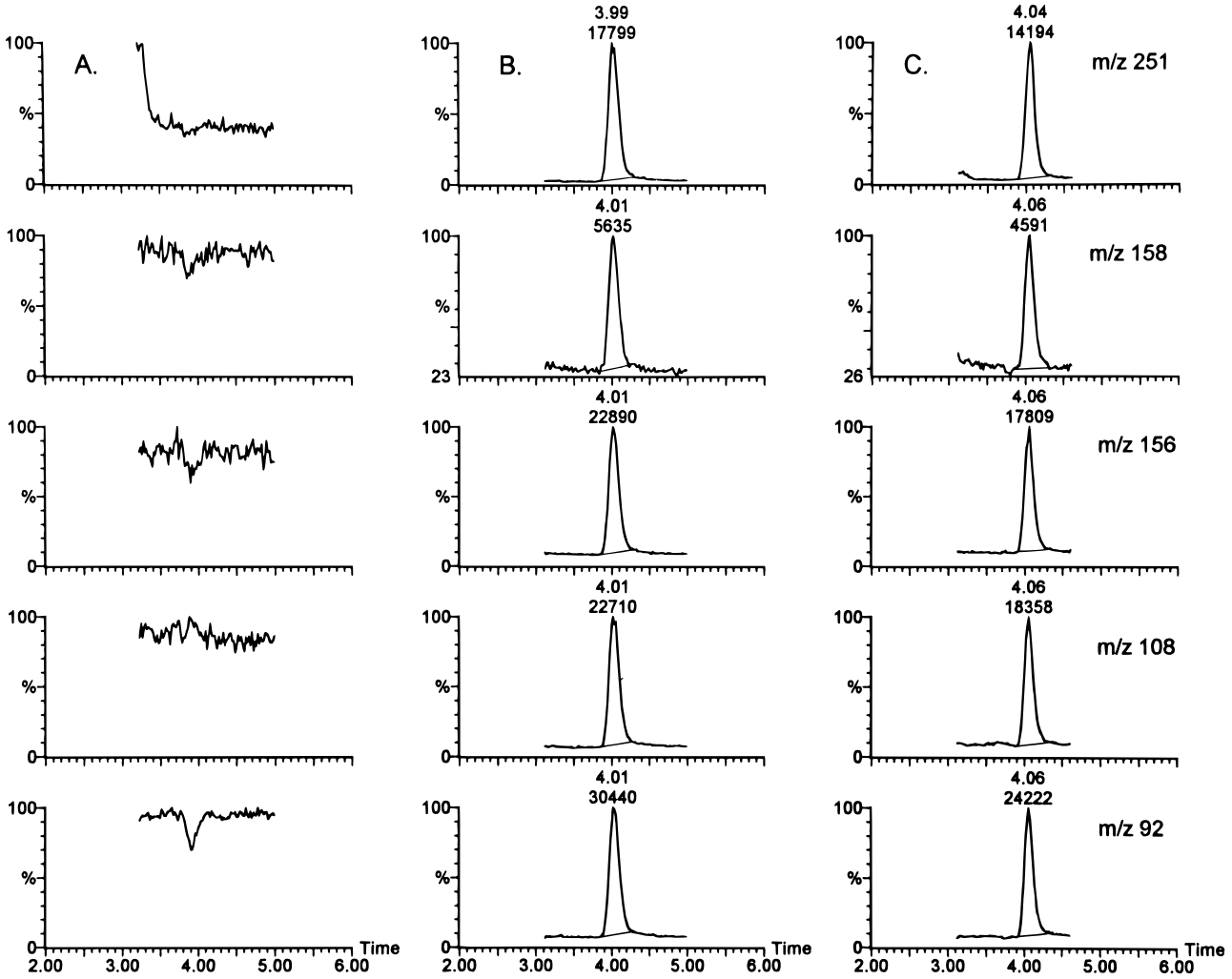

Figure 5. Selected ion chromatograms for SDZ-derived ions in (A) muscle from a representative control salmon purchased commercially, (B) SDZ standard (2.1 ng on-column), and (C) muscle from dosed salmon containing 20 ng of SDZ/g. The integrated areas are listed below the retention time, in minutes, above the respective peak.

Table 4. Ion Intensity Ratios for Sulfadiazine Standards Compared with Those for Sulfadiazine in Salmon Muscle Extracts[a]

	ion intensity ratio			
sample	*m/z* 158/251	*m/z* 156/251	*m/z* 108/251	*m/z* 92/251
SDZ, 2.1 ng[b]	0.21 ± 0.01	1.09 ± 0.01	0.97 ± 0.01	1.10 ± 0.05
fortified salmon, 10.5 ng of SDZ/g[c]	0.22 ± 0.01	1.07 ± 0.05	1.00 ± 0.03	1.16 ± 0.03
percent difference	−4.8	1.8	−3.1	−5.5
SDZ, 2.1 ng[b]	0.29 ± 0.01	1.21 ± 0.02	1.20 ± 0.03	1.55 ± 0.05
dosed salmon, 9ng of SDZ/g[d]	0.30 ± 0.01	1.19 ± 0.03	1.16 ± 0.02	1.50 ± 0.04
percent difference	−3.5	1.7	3.3	3.2
SDZ, 2.1 ng[b]	0.31 ± 0.01	1.26 ± 0.04	1.26 ± 0.03	1.67 ± 0.07
dosed salmon, 20 ng of SDZ/g[b]	0.32 ± 0.01	1.24 ± 0.01	1.26 ± 0.01	1.68 ± 0.02
percent difference	−3.2	1.6	0	−0.6

[a] Ion intensities were determined from the peak areas under the respective selected ion chromatograms using the cone voltages shown in Materials and Methods. Ratios were computed from the areas under the respective chromatograms relative to that for *m/z* 251. Average intensity ratio values, determined from SDZ standards and SDZ in salmon extracts, are expressed as the relative differences between salmon extract- and standard-derived ratios: percent difference = [(standard average − salmon average)/standard average] × 100. [b] Four replicate determinations. [c] Six replicate determinations. [d] Three replicate determinations.

Incurred salmon samples containing 0.2 (S/N < 3) and 9 ng of SDZ/g (S/N > 3) were available and were used to bracket the value. It is estimated that SDZ present in incurred salmon tissues at 2 ng/g would yield a S/N ratio of 3 for *m/z* 158, and this value is taken as the limit of confirmation using the five ion procedure described.

As previously applied to EI and MS/MS data, confirmation of analyte structure by selected ion acquisition requires the presence of at least three ions with intensity ratios generated from an authentic standard agreeing to within ±10% of the absolute value for the ratios produced by the analyte present in the sample extract (Sphon, 1978; Kienhuis, 1993). While the use of selected ion monitoring does effectively ignore portions of the mass spectrum that are available through the use of full scan acquisitions, the requirement for high-sensitivity detection makes it unavoidable. To accommodate the need for multiple ions including the protonated molecule in this SDZ confirmatory method, the mass spectrometer software was used to perform rapid switching of the cone voltage as a step function in concert with acquisition of the respective selected ion. The protonated molecule was acquired at an optimal low cone

Table 5. Variation of Ion Intensity Ratios Obtained on Different Days

	ion intensity ratio[b]			
sample[a]	*m/z* 158/251	*m/z* 156/251	*m/z* 108/251	*m/z* 92/251
SDZ, 2.1 ng	0.27 ± 0.01	0.93 ± 0.02	1.11 ± 0.01	1.19 ± 0.03
dosed salmon	0.27 ± 0.01	0.91 ± 0.01	1.10 ± 0.02	1.18 ± 0.01
percent difference	0	2.2	0.9	0.8
SDZ, 1.5 g	0.28 ± 0.03	0.98 ± 0.01	0.98 ± 0.04	1.01 ± 0.05
fortified salmon	0.30 ± 0.01	1.01 ± 0.02	1.00 ± 0.02	1.02 ± 0.05
percent difference	−7.1	−3.1	−2.0	−1.0

[a] Four replicate determinations for each. [b] Ion intensity ratios were determined from injections of SDZ standards, from SDZ residues in muscle from dosed salmon (analyzed 8 weeks later than in Table 4), and from fortified salmon muscle (analyzed 2 weeks earlier than in Table 4). The cone voltages used varied slightly between experiments.

voltage (24−26 V), and fragment ions were acquired at higher voltages to increase the respective signal. However, it was not possible to increase the intensity for *m/z* 158 relative to the other ions at any voltage. This procedure produced ions with similar absolute intensities (see Figure 5B,C) and served to minimize the variability in the computed ion intensity ratios determined from the peak areas under the respective selected ion chromatograms (Tables 4 and 5). Although small changes in cone voltage were made for daily optimization, conditions were identical for standard and sample ratio determinations on a given day.

A slow, progressive change in the intensity ratios for SDZ in both standards and salmon extracts occurred throughout the day as more extracts were injected. The intensity of the protonated molecule decreased slightly, resulting in a small but significant increase in the ratios for all fragment ions. This occurrence is consistent with an increased amount of fragmentation of protonated SDZ as source conditions change (e.g., deposition of contaminants). The effect of this variation was overcome by interspersing standard and sample injections.

By APCI/MS, highly reproducible ion intensity ratios were obtained from either multiple injections of SDZ standards or muscle extracts from SDZ-dosed salmon (relative standard deviation ≤5%, Tables 4 and 5). The values of the ion intensity ratios determined for SDZ in standards and in salmon extracts containing 9−20 ng of SDZ/g agreed to within 3% of the absolute value and 7.1% relative difference (see Tables 4 and 5). The percent difference between ion intensity ratios for SDZ standards and SDZ in fortified or dosed salmon extracts was calculated using the following procedure: Three to six replicate injections of sample extracts were bracketed by two to three replicate injections of standard. The average ratios for SDZ in the samples were compared with the composite average for those in the standards bracketing the samples. The degree of concordance between sample and standard ion intensity ratios for the five ions monitored by LC−APCI/MS meets the criteria for regulatory confirmation as they have been applied to other types of MS data (Sphon, 1978). Similar analyses were performed on two other days to determine the effect of interassay variability on matching salmon-derived ratios with those generated by authentic SDZ standards. While the determined values for the ratios and the cone voltages used to produce the ratios changed slightly between analyses (presumably due to changes in source contamination or other undefined factors), a similar degree of match between SDZ ratios from standard and salmon extracts was observed on both other occasions (see Tables 4 and 5).

CONCLUSION

While showing good agreement of SDZ concentrations among replicate samples from the same fish, these results show the considerable variability in SDZ concentration among different dosed fish for the same drug-withdrawal day. They show that 100% of the fish tested had higher concentrations of SDZ in the skin than in the muscle, indicating that skin may serve as the edible tissue target organ.

These results show that the LC−APCI/MS method described for confirmation of SDZ residues in salmon muscle meets the generally accepted criteria for a regulatory method. It will be important to determine the reproducibility of these procedures through interlaboratory comparisons using instruments from different sources.

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Received for review March 27, 1996. Revised manuscript received June 21, 1996. Accepted June 26, 1996.[⊗]

JF9601877

[⊗] Abstract published in *Advance ACS Abstracts*, August 15, 1996.