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High-performance liquid chromatographic analysis of Romet-30 in Chinook salmon (*Oncorhynchus tshawytscha*): wash-out time, tissue distribution in muscle, liver and skin, and metabolism of sulfadimethoxine

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Abstract

A high-performance liquid chromatographic (HPLC) assay was developed for the determination of Romet-30 residue levels in Chinook salmon muscle and liver tissues. The extraction recoveries averaged 66, 78 and 83% for ormetoprim (OMP), sulfadimethoxine (SDM) and N^4-acetyl-SDM (N^4-Ac-SDM), respectively, in muscle tissue; 61 and 72% for SDM and N^4-Ac-SDM, respectively, in liver tissue. The HPLC assay had a lower detection limit of 0.05 μg/g for OMP, SDM and N^4-Ac-SDM in muscle tissue, and a lower detection limit of 0.20 μg/g for SDM and N^4-Ac-SDM in liver tissue. OMP could not be quantified in liver due to the presence of substantial amounts of co-extracted endogenous substances. The HPLC assay was applied to the analysis of Romet-30 residues in Chinook salmon after gastric intubation with Romet-30 at water temperatures between 8.0 and 9.0°C. Different disposition characteristics were found in muscle and liver tissues. The presence of N^4-Ac-SDM in liver tissue was confirmed by MS and MS–MS analyses. An estimation of SDM and N^4-Ac-SDM residue levels in skin tissue was obtained and the disposition of these two compounds was similar to the disposition in muscle tissue.

1. Introduction

Intensive aquaculture and occasional poor fish husbandry practices predispose fish to various infectious diseases due to a variety of stress factors [1]. These adverse conditions enhance the opportunities of introduction, transfer and rapid dissemination of fish infections in the aquaculture industry [2]. Antimicrobial compounds are thus commonly used for the treatment and prevention of disease outbreaks.

Romet-30 (trademark of Hoffmann-La Roche, Missisauga, Ontario, Canada) is a potentiated sulfonamide consisting of a sulfadimethoxime (SDM)–ormetoprim (OMP) (5:1) mixture. It has been approved in the USA and Canada for the control of furunculosis in salmonids and enteric septicemia in channel catfish [3–5]. This antimicrobial is also effective in controlling en-

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teric redmouth disease [6] and vibriosis [7] in salmonids. Romet-30 is administered in medicated feed at a dosage of 50 mg/kg of fish per day for five consecutive days followed by a 42-day withdrawal period required for salmonids [3] and a 3-day withdrawal period required for catfish [4]. The tolerance levels for SDM and OMP in edible fish tissues are 0.1 μg/g [3,4].

Analytical assays are required for monitoring drug residue levels in edible fish tissue, as well as for drug disposition studies. Due to their sensitivity and selectivity, chromatographic methods are most suitable for the analysis of sulfonamide residues in fish tissues [8–17]. More recently, a capillary electrophoresis–mass spectrometric method was reported for analysis of various antimicrobials, including SDM and OMP, in shellfish tissue [18]. Radioisotope-labelled SDM and OMP have also been used to study the disposition of these two drugs in lobster (*Homarus americanus*) [19,20], channel catfish (*Ictalurus punctatus*) [21–23] and rainbow trout (*Salmo gairdneri* and *Oncorhynchus mykiss*) [24–27]. However, the radioisotope assays reported lacked specificity in that the parent drugs were not separated from their metabolites.

The objective of the present study was to develop an HPLC assay for the determination of wash-out time of Romet-30 in Chinook salmon and its tissue distribution in muscle, liver and skin tissues following gastric intubation. N^4-Acetyl-SDM (N^4-Ac-SDM), a metabolite of SDM, was also determined in these three tissues.

2. Experimental

2.1. Materials

Romet-30 was obtained through Syndel Labs. (Vancouver, Canada) (lot No. 4106). OMP and SDM were obtained from Hoffmann–La Roche (Nutley, NJ, USA). Tricaine methanesulfonate (MS-222) was obtained from Syndel Labs. The internal standard, sulfisoxazole, was obtained from Sigma (St. Louis, MO, USA). Tetrabutylammonium hydroxide (TBAH) (40%) and sodium carbonate were obtained from Aldrich (Milwaukee, WI, USA). Phosphoric acid (85%) was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Sodium sulfate anhydrous granular, sodium hydrogencarbonate, disodium hydrogenorthophosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) and acetic anhydride were obtained from BDH (Toronto, Canada). HPLC-grade methanol, acetonitrile and dichloromethane were obtained from BDH. Purified water was produced using a Milli-Q water purification system (Mississauga, Canada).

2.2. HPLC system

The HPLC system consisted of a Beckman Model 100A pump (Fullerton, CA, USA), a Shimadzu SIL-9A auto injector, a Shimadzu SPD-6A UV spectrophotometric detector and a Shimadzu C-R6A Chromatopac data processor (Kyoto, Japan), a Beckman Ultrasphere ion-pair column (5 μm, 250 × 4.6 mm I.D.) (San Ramon, CA, USA), and a guard column with a Brownlee RP-18 cartridge (15 × 3.2 mm I.D.) (Santa Clara, CA, USA). The flow-rate of the mobile phase was 1.0 ml/min. The detection wavelength was at 280 nm. The absorbance range of the detector was at 0.01 AUFS.

2.3. Tandem mass spectrometric (MS–MS) system

The MS–MS experiments were performed on a Sciex API III triple quadrupole mass spectrometer (Thornhill, Canada), equipped with an ionspray interface and an atmospheric-pressure chemical ionization (APCI) source. The samples were delivered by flow injection with 10 μl/min aqueous 50% methanol. A dwell time of 6 ms/u was used for full-scan MS analyses. APCI mass spectra were acquired in the first quadrupole Q1, and selected precursor ions were fragmented in the radio frequency (rf) only quadrupole Q2 at a collision energy of 36 eV. Argon was used as the target gas at a thickness of $5.48 \cdot 10^{14}$ molecules/cm^2. Product ions were examined in the third quadrupole Q3.

2.4. Stock solutions

A mixture of SDM and OMP was prepared by dissolving SDM and OMP in 50 ml of acetoni-

trile to give a concentration of 200 μg/ml for both standards. This stock solution was further diluted to give a series of standard solutions with concentrations of 100, 20 and 2 μg/ml. Sulfisoxazole internal standard solution was prepared in 100 ml of acetonitrile to give a final concentration of approximately 60 μg/ml.

The sodium carbonate–sodium hydrogencarbonate buffer (pH 10) was prepared according to Delory and King [28]. Sodium carbonate stock solution (0.2 *M*) was prepared by dissolving 21.2 g of sodium carbonate in 1000 ml of purified water. Sodium hydrogencarbonate stock solution (0.2 *M*) was prepared by dissolving 16.8 g of sodium hydrogencarbonate in 1000 ml of purified water. The buffer was prepared by mixing 27.5 ml of the above sodium carbonate solution and 22.5 ml of the above sodium hydrogencarbonate solution and further dilution to 200 ml with purified water.

2.5. Synthesis of N^4-Ac-SDM

An excess of 5 ml of acetic anhydride (approximately 53 mmol) was added to 2 g of SDM (approximately 6 mmol) and the mixture was heated on a Thermolyne Dry-bath (Dubuque, IA, USA) at 70°C for 20 min with occasional manual shaking. The excess acetic anhydride was evaporated under nitrogen in a 40°C water bath. The resulting crystals were filtered and washed with water. The product was recrystallized from acetonitrile. Its melting point was determined to be 219–222°C on a capillary melting point apparatus (Arthur H. Thomas Co., Philadelphia, PA, USA), while the Merck Index value is 220–223°C [29]. The purity was determined to be greater than 99.5% by HPLC. A stock solution was then prepared in 25 ml of acetonitrile to give a concentration of 400 μg/ml. This stock solution was further diluted to give concentrations of 200, 100, 20 and 2 μg/ml.

2.6. Extraction procedures

A 5-g sample of muscle or liver tissue, or a 2-g sample of skin tissue, was dissected and placed in a 50-ml centrifuge tube. An aliquot of 200 μl of sulfisoxazole internal standard solution (approximately 12 μg sulfisoxazole) was added along with 300 μl of 0.5 *M* TBAH, 1 ml of pH 10 Na_2CO_3–$NaHCO_3$ buffer and 1 ml of 1 *M* NaOH. After brief manual mixing with a Pasteur pipette, 15 ml of dichloromethane were added and the sample was homogenized at medium speed using a Brinkmann Polytron Model PT 10/35 homogenizer (Rexdale, Canada) for 20–30 s. Granular sodium sulfate anhydrous (2 g) was added to the homogenate and the sample was vortex-mixed for 2 min. The mixture was then centrifuged at 2000 *g* for 15 min. After centrifugation, the top aqueous layer was removed to a test tube, the interfacial solid tissue plug was pushed aside, and the dichloromethane layer was removed. The aqueous layer was returned to the tube containing the tissue plug and an additional 10 ml of dichloromethane were added. The sample was vortex-mixed for 2 min and centrifuged. The dichloromethane layer was removed, combined with the initial dichloromethane extract and evaporated under a nitrogen stream in a 40°C water bath. The residue was reconstituted in 1 ml of HPLC mobile phase. After centrifugation for 5 min at 1450 *g*, the bottom layer was removed and filtered through a 0.45-μm Nylaflo membrane filter (Gelman Sciences, Ann Arbor, MI, USA). An aliquot of 20 μl of the filtrate was injected onto the HPLC system.

2.7. Calibration curves

The calibration curves in muscle tissue were determined in a series of 5-g muscle samples to which were added 200 ml of internal standard solution (60 μg/ml) and appropriate volumes of SDM–OMP and N^4-Ac-SDM standard solutions to give final concentrations of 0.05, 0.10, 0.20, 0.50, 2.00, 5.00 and 10.0 μg/g for each of the standards. The samples were stored at 4°C for 1 h, followed by extraction as described before. The calibration curves were constructed by plotting the peak area ratios of SDM, OMP and N^4-Ac-SDM to the internal standard against the concentrations of SDM, OMP and N^4-Ac-SDM added.

In a similar fashion, the calibration curves of SDM and N^4-Ac-SDM in liver tissue were determined in a range of 0.20 to 25.0 μg/g.

2.8. Assay precision

The intra-assay variability in muscle tissue was determined by the analysis of six muscle samples to which SDM–OMP and N^4-Ac-SDM were added at 0.50 μg/g, and six muscle samples to which SDM–OMP and N^4-Ac-SDM were added at 5.00 μg/g. The analysis of the six muscle samples was completed in one day. The inter-assay variability in muscle tissue was determined at 0.50 and 5.00 μg/g at consecutive daily intervals over a six-day period. Similarly, the intra- and inter-assay variabilities for SDM and N^4-Ac-SDM in liver tissue were determined at 0.50, 5.00 and 15.0 μg/g.

2.9. Extraction recoveries

To six muscle samples were added SDM–OMP and N^4-Ac-SDM to give final concentrations of 0.50 μg/g for each analyte. An additional six muscle samples were prepared at concentrations of 5.00 μg/g for each of SDM–OMP and N^4-Ac-SDM. The samples were extracted as described before, except that the internal standard was added just before the combined dichloromethane extract was evaporated to dryness. Two HPLC mobile phase solutions containing SDM–OMP and N^4-Ac-SDM standards at 0.50 and 5.00 μg/ml along with the internal standard were prepared in parallel, but without undergoing extraction. The recoveries for SDM, OMP and N^4-Ac-SDM were determined by comparison of the peak area ratios of SDM, OMP and N^4-Ac-SDM to the internal standard (sulfisoxazole) from extracted tissue samples with those of SDM, OMP and N^4-Ac-SDM to the internal standard from unextracted standard solutions of identical quantities. In a similar fashion, the recoveries of SDM and N^4-Ac-SDM in liver tissue were determined at 0.50, 5.00 and 20.0 μg/g.

2.10. Confirmation of the presence of N^4-Ac-SDM in liver tissue by MS–MS

A 10-g amount of liver sample from the intubation study was extracted as previously described. After reconstitution, the samples were injected onto the HPLC system. The fraction of the effluent corresponding to the synthetic N^4-Ac-SDM standard was collected. The collected fraction was evaporated to dryness at 37°C in a SpeedVac concentrator (Savant Instruments, Farmingdale, NY, USA). The residues were re-extracted with dichloromethane and evaporated to dryness under a nitrogen stream. The residues were reconstituted with 100 μl of acetonitrile and an aliquot of 1 μl was injected onto the Sciex API III mass system for MS and MS–MS analyses by flow injection.

2.11. Intubation study

Forty-one Chinook salmon were obtained from Salt Spring Aquafarms (Salt Spring Island, Canada). Due to the relatively high mortality rate, probably due to the stress caused by transportation, only 26 fish were available for final analysis. The fish weighed from 615 to 1800 g (1094 ± 57; mean ± S.E.M.). The seawater temperatures ranged from 8.0 to 9.0°C throughout the 20-day study.

The fish were maintained in flowing seawater tanks. After one week of acclimatization, the fish were gastrically intubated with a freshly prepared suspension of Romet-30 in water (25 mg/ml) for 10 days at a dosage of 40 mg/kg per day (standard protocol in British Columbia salmon aquaculture). Before intubation, the fish were lightly anesthesized in a MS-222 bath at a concentration of 43 mg/ml. Sodium hydrogencarbonate (43 mg/ml) was also added to the anesthetic solution in order to buffer the change in pH caused by the addition of MS-222 which otherwise would be irritant to the fish. No obvious regurgitation of the drug was observed after intubation.

Six fish were sampled on each of days 11 (*i.e.*, the first day after the cessation of the intubation) and 14. An additional 7 fish were sampled on

each of days 17 and 20. The fish were sacrificed by a blow to the cranium. Blood, skin, muscle, liver and kidney sampled were collected and kept frozen at −20°C until required for analysis.

2.12. Statistics

All statistical comparisons were performed with analysis of variance (ANOVA) using software NCSS (Kaysville, UT, USA).

3. Results and discussion

3.1. Chromatographic conditions

For the analysis of OMP, SDM and N^4-Ac-SDM in muscle tissue, two different mobile phases were necessary to obtain the optimal separation of analytes from the endogenous substances. One mobile phase consisted of acetonitrile–methanol–0.1 *M* phosphate buffer pH 4.0 (12:13:75, v/v/v) and was used for the analysis of OMP. This mobile phase allowed the separation of OMP from the co-extracted endogenous substances eluting early on the chromatograms (Fig. 1). However, N^4-Ac-SDM could not be separated from the late co-eluting endogenous substances with this mobile phase. A second mobile phase consisting of acetonitrile–methanol–0.1 *M* phosphate buffer pH 2.5 (11:23.4:75, v/v/v) was therefore used for the analysis of SDM and N^4-Ac-SDM (Fig. 2).

Sulfisoxazole was chosen as an internal standard because it has a similar structure to SDM and eluted between OMP and SDM with a retention time of approximately 13 min (Figs. 1 and 2). Other sulfonamides were found to elute either too early or too late, or co-elute with endogenous substances.

Preliminary extraction studies with liver samples indicated that substantial amounts of co-extracted endogenous substances were present in the early portion of the chromatograms which coincided with the elution of OMP (Fig. 3). Despite numerous alternations of the extraction protocol and HPLC mobile phases, OMP could not be resolved from co-eluting endogenous substances, thus precluding accurate measurement of OMP in liver tissue. Based on the analytical results in muscle tissue from the intubation study showing that SDM generally exhibited higher residue levels, it was concluded

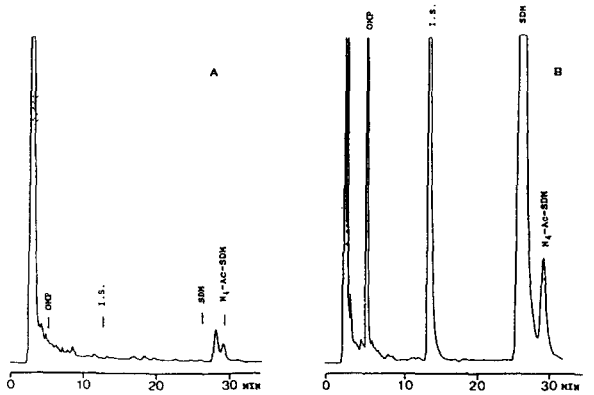

Fig. 1. Chromatograms of (A) a blank Chinook salmon muscle tissue extract and (B) a Chinook salmon muscle tissue extract from the intubation study. Chromatographic conditions: column, Ultrasphere ion pair 5 μm (250 × 4.6 mm I.D.); mobile phase, acetonitrile–methanol–0.1 *M* phosphate buffer pH 4.0 (12:13:75, v/v/v); flow-rate, 1.0 ml/min; ultraviolet detection wavelength, 280 nm; 0.01 AUFS. Peaks: OMP = ormetoprim; I.S. = internal standard; SDM = sulfadimethoxine; N^4-Ac-SDM = N^4-acetylsulfadimethoxine.

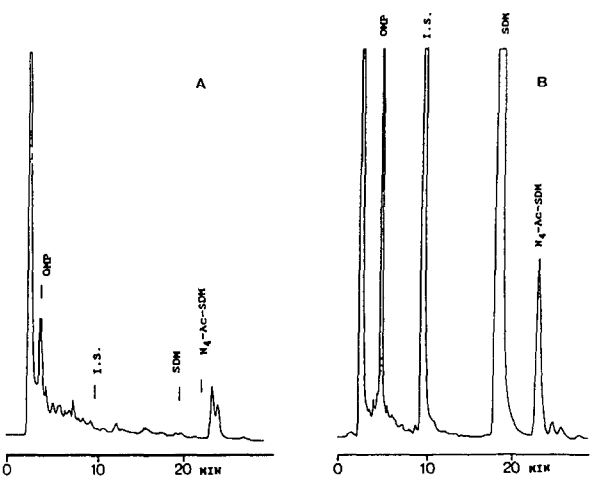

Fig. 2. Chromatograms of (A) a blank Chinook salmon muscle tissue extract and (B) a Chinook salmon muscle tissue extract from the intubation study. HPLC conditions as in Fig. 1, except for the mobile phase: acetonitrile–methanol–0.1 *M* phosphate buffer pH 2.5 (11:23.4:75, v/v/v). Peaks as in Fig. 1.

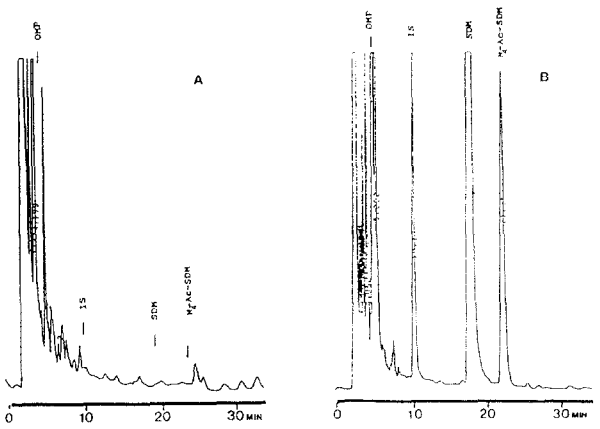

Fig. 3. Chromatograms of (A) a blank Chinook salmon liver tissue extract and (B) a Chinook salmon liver tissue extract from the intubation study. Peaks as in Fig. 1 and HPLC conditions as in Fig. 2.

that SDM represented a more important xenobiotic for the purpose of drug residue detection. For these reasons, only SDM and N^4-Ac-SDM were analyzed in the liver tissue, using the second mobile phase mentioned above.

3.2. Confirmation of N^4-Ac-SDM

During the analysis of liver samples from the intubation study, a significant peak was found to elute after SDM (Fig. 3). Since SDM is extensively metabolized to N^4-Ac-SDM in channel catfish and rainbow trout [22,25], this peak was speculated to be N^4-Ac-SDM. Synthetic N^4-Ac-SDM was found to have the same retention time as the unknown peak. HPLC analysis of an admixture of the synthetic N^4-Ac-SDM and a liver extract from an intubated salmon did not show any evidence of skewing of this peak.

The identity of N^4-Ac-SDM was further confirmed by MS and MS–MS analyses. The dominant protonated molecular ion $[M+H]^+$ (m/z 353) of N^4-Ac-SDM was observed by MS analysis from both the synthetic standard and the collected fraction from the liver samples (Fig. 4). Background ions present in the collected fraction (Fig. 4B) were apparently caused by the co-collected endogenous compounds. Further structural information was acquired by the use of the MS–MS technique. The precursor ion m/z 353 was fragmented in the second quadrupole Q2, and the product ions were analyzed in the third quadrupole Q3. The resulting product ion spectra from both samples showed very similar fragmentation patterns (Fig. 5).

The concentration of N^4-Ac-SDM in muscle tissue was insufficient for detection by MS and MS–MS. However, the chromatographic behavior of the peak in muscle tissue (Fig. 2), and the above described HPLC, MS and MS–MS characteristics obtained in liver tissue confirmed the presence of N^4-Ac-SDM in both liver and muscle tissues.

3.3. Calibration curves, assay precision and recovery studies

The calibration curves for OMP, SDM and N^4-Ac-SDM in muscle tissue were linear ($r^2 > 0.999$) over the concentration range of 0.05 to 10.0 μg/g. Similarly, the calibration curves for SDM and N^4-Ac-SDM in liver tissue were linear ($r^2 > 0.999$) over the concentration range of 0.20 to 25.0 μg/g. The lower detection limits of the assay were determined to be 0.05 μg/g in muscle tissue and 0.20 μg/g in liver tissue at a signal-to-noise ratio of 5. The minimum quantification limits were about 3.5 ng in muscle tissue and 14 ng in liver tissue.

The intra-assay variabilities were less than 5% in muscle tissue for all three compounds, and less than 10% in liver tissue for SDM and N^4-Ac-SDM (Table 1). The inter-assay variabilities were less than 10% in both tissues (Table 2).

The extraction recoveries in muscle and liver tissues ranged from 58 to 84% (Table 3). During extraction, TBAH was added to form a tetrabutylammonium ion-pair with SDM which allowed simultaneous extraction of SDM and OMP into dichloromethane at pH 10 [10]. The addition of granular sodium sulfate anhydrous improved the extraction recoveries.

3.4. Intubation study

The concentration–time curves of OMP, SDM and N^4-Ac-SDM residues in muscle and liver tissues after gastric intubation are presented in

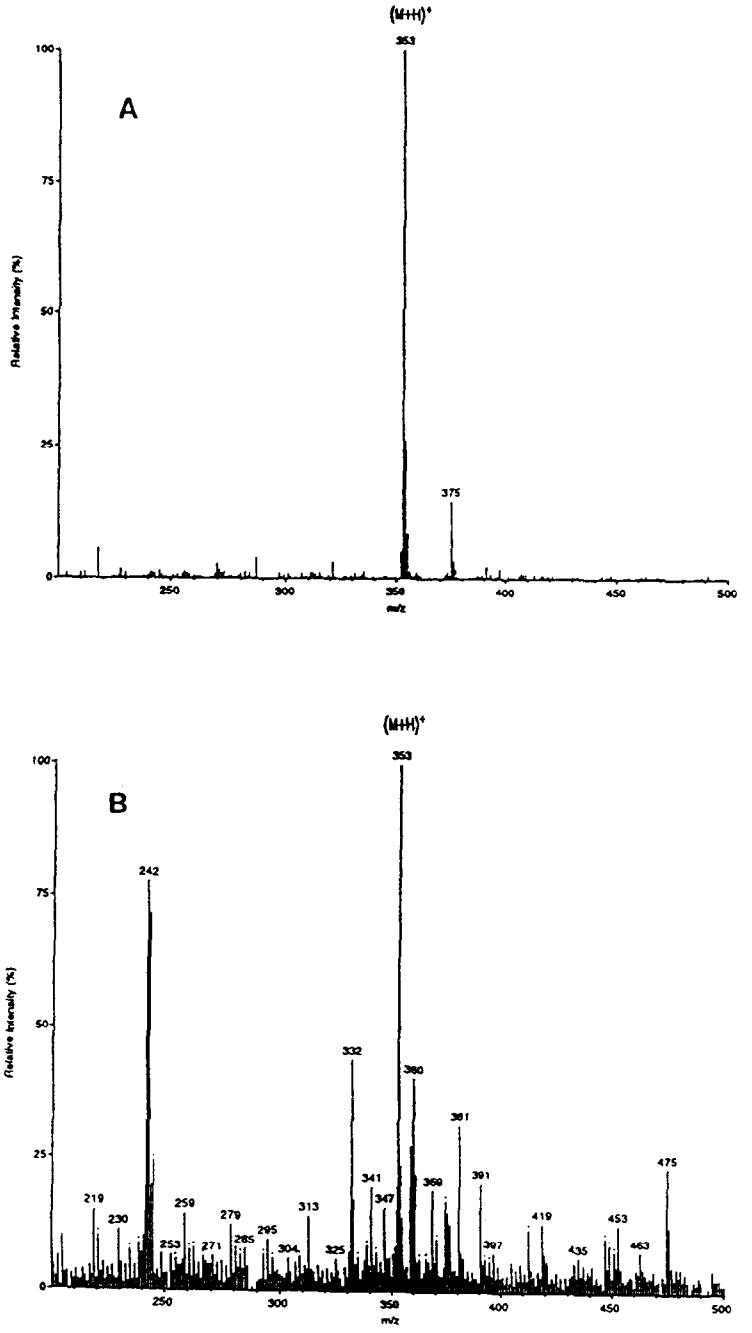

Fig. 4. Flow injection MS analysis of N^4-Ac-SDM: (A) from N^4-Ac-SDM synthetic standard and (B) from HPLC fraction containing N^4-Ac-SDM from liver extract.

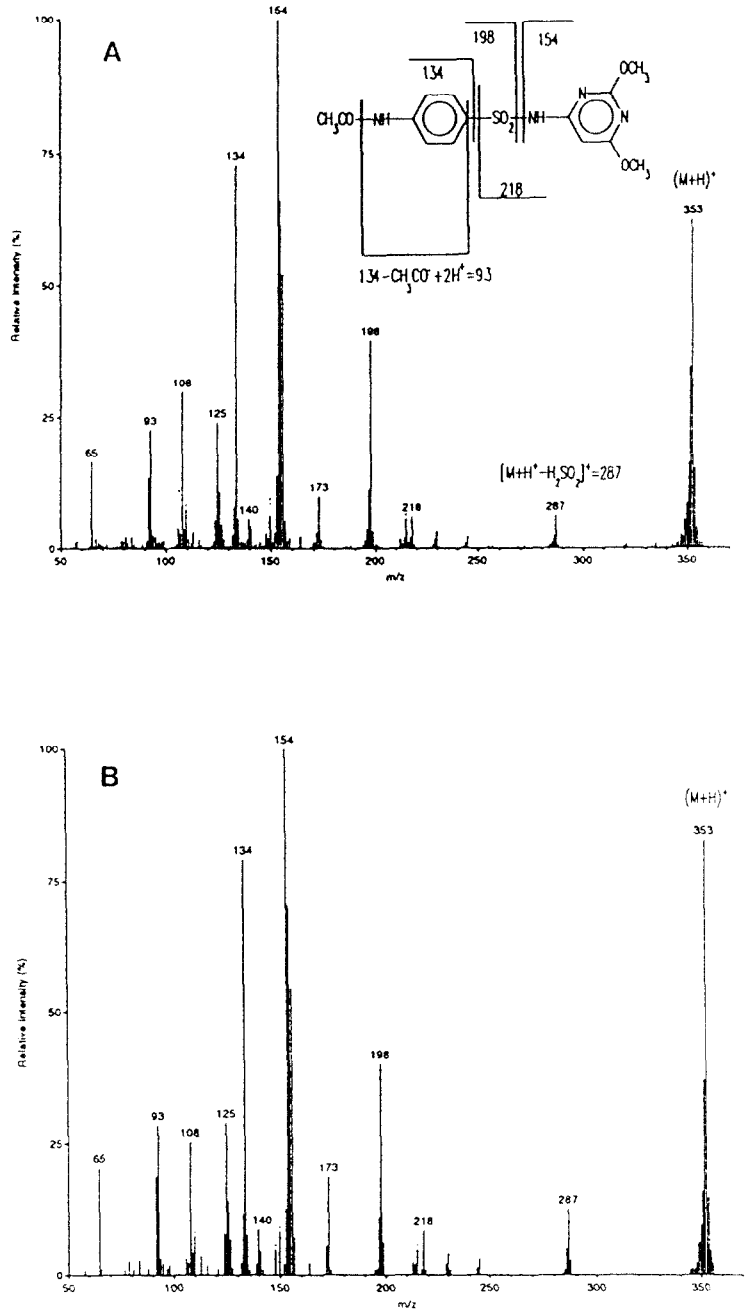

Fig. 5. MS–MS product ion spectra of the protonated molecular ion $[M + H]^+$ of N^4-Ac-SDM obtained by flow injection: (A) from N^4-Ac-SDM synthetic standard and (B) from HPLC fraction containing N^4-Ac-SDM from liver extract. The formation of $[M + H - H_2SO_2]^+$ was confirmed by Pleasance *et al.* [17].

Table 1
Results of intra-assay variability study in muscle and liver tissues

Concentration (μg/g)	Variability of OMP (R.S.D., %)	Variability of SDM (R.S.D., %)	Variability of N^4-Ac-SDM (R.S.D., %)
Muscle tissue[a]			
0.50	3.6	2.8	1.2
5.00	4.8	0.89	0.19
Liver tissue[a]			
0.50	N.D.	4.8	7.2
5.00	N.D.	4.6	3.5
15.0	N.D.	5.1	7.2

[a] Six samples containing OMP, SDM and N^4-Ac-SDM were analyzed at each concentration. N.D. = Not determined.

Figs. 6 and 7. Although OMP and SDM existed in a 1:5 ratio in the drug substances administered, this ratio was not observed from OMP and SDM residue levels in muscle tissue of fish intubated with Romet-30. This observation suggests a difference in the rate of absorption and/or elimination between the two compounds. OMP and SDM were both detectable up to day 20 in muscle tissue. Sulfonamides have been found to be rapidly eliminated initially, but a small persistent residue may remain for a longer period [30]. This finding is also true for the present study where the residue levels of SDM in muscle and liver tissues on days 14, 17 and 20 were not significantly different from one another ($P < 0.05$) (Figs. 6 and 7). SDM was also detectable up to day 20 in liver tissue, however, the overall residue level of SDM in liver tissue was higher than the residue level of SDM in muscle tissue (Figs. 6 and 7).

N^4-Ac-SDM, a metabolite of SDM, was found to have the lowest residue level among the three analytes studied in muscle tissue and was not detected after day 11. We conclude that the distribution of N^4-Ac-SDM into muscle tissue was limited and/or the elimination from muscle tissue was rapid. In contrast, the residue levels of SDM and N^4-Ac-SDM in liver tissue were not

Table 2
Results of inter-assay variability study in muscle and liver tissues

Concentration (μg/g)	Variability of OMP (R.S.D., %)	Variability of SDM (R.S.D., %)	Variability of N^4-Ac-SDM (R.S.D., %)
Muscle tissue[a]			
0.50	7.9	5.4	3.5
5.00	6.7	5.8	3.0
Liver tissue[a]			
0.50	N.D.	9.7	7.8
5.00	N.D.	7.5	5.6
15.0	N.D.	6.7	5.9

[a] Six samples containing OMP, SDM and N^4-Ac-SDM were analyzed at each concentration. N.D. = Not determined.

Table 3
Extraction recoveries from muscle and liver tissues

Concentration of each standard (μg/g)	Recovery of OMP (%)	Recovery of SDM (%)	Recovery of N^4-Ac-SDM (%)
Muscle tissue			
0.50	65 ± 2[a]	76 ± 0.7[a]	82 ± 1[a]
5.00	68 ± 2[a]	79 ± 0.7[a]	84 ± 0.6[a]
Liver tissue			
0.50	N.D.	58[b]	69[b]
5.00	N.D.	64[b]	74[b]
20.0	N.D.	62[b]	74[b]

[a] Presented as mean ± S.E.M. ($n = 6$).
[b] Presented as average values ($n = 2$). N.D. = Not determined.

significantly different from each other ($P < 0.05$) on the same sampling day. This observation suggests that the liver is the major site for the metabolism of SDM, which confirms previous conclusions made in both rainbow trout and channel catfish [22,24,25]. It has also been reported that N^4-Ac-SDM predominated in bile of rainbow trout and channel catfish [22,24,25], thereby further indicating that hepatic metabolism and biliary excretion are important elimination pathways for SDM in fish. The extensive enterohepatic recirculation of SDM, and particularly N^4-Ac-SDM, reported by Kleinow *et al.* [25] further explains the longer duration of N^4-Ac-SDM residue detected in liver tissue up to day 20, as compared with N^4-Ac-SDM residue in muscle tissue which was not detectable after day 11. N^4-Ac-SDM was also found to be eliminated rapidly at the initial stage but persisted in liver tissue at lower concentrations during the latter sampling periods.

Although gastric intubation was employed to minimize the variations in drug residue levels between individual fish as noted in a previous feeding study [12], significant variations were still

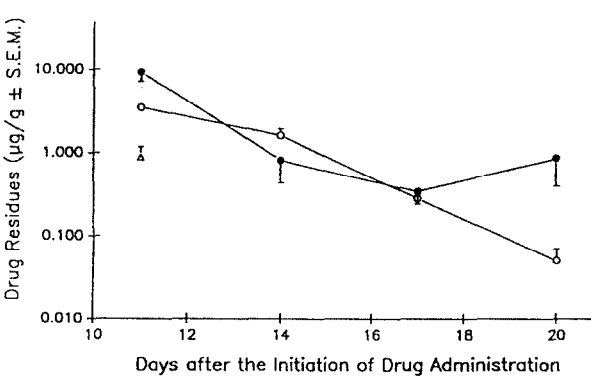

Fig. 6. OMP (○), SDM (●) and N^4-Ac-SDM (△) residue profiles in Chinook salmon muscle tissue after intubation with Romet-30. Data points are presented as mean ± standard error of the mean (S.E.M.).

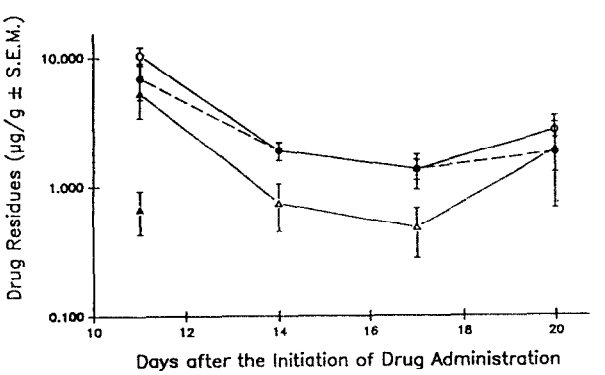

Fig. 7. SDM and N^4-Ac-SDM residue profiles in Chinook salmon liver and skin tissues after intubation with Romet-30. Data points are presented as mean ± standard error of the mean (S.E.M.). ○ = SDM in liver tissue; ● = N^4-Ac-SDM in liver tissue; △ = SDM in skin tissue; ▲ = N^4-Ac-SDM in skin tissue.

observed. These variations in drug residue levels were most likely attributed to biological variations, such as physical and health conditions of the fish, and to differences associated with absorption, distribution and/or elimination of the drug. Due to considerable inter-fish variations in SDM residue level, the wash-out time for Romet-30 could not be reliably determined from the present study. However, the data shows that the absorption and the elimination of sulfonamide antimicrobials in salmon are highly variable processes and depend on a number of factors beyond the control of the producer.

Although salmon skin tissue was more difficult to analyze because of the presence of high levels of endogenous interfering substances in the tissue extract, an estimation of the residue levels of SDM and N^4-Ac-SDM was made. The data (Fig. 7) show that the residue profiles of SDM and N^4-Ac-SDM in skin tissue are very similar to those found in muscle tissue. The overall residue level of SDM was also lower than the residue level of SDM in liver tissue.

The current HPLC assay could not be applied for the analysis in kidney tissue due to the presence of high amounts of endogenous substances, a phenomenon which was also reported by Reimer and Suarez [14].

In conclusion, a sensitive and selective HPLC assay has been developed for the analysis of OMP, SDM and N^4-Ac-SDM in Chinook salmon muscle tissue and for the analysis of SDM and N^4-Ac-SDM in Chinook salmon liver tissue. An *in vivo* intubation study revealed considerable variations in Romet-30 residue levels between individual fish. Since the developed HPLC assay in muscle tissue had a higher sensitivity (0.05 μg/g) than the tolerance level for Romet-30 (0.1 μg/g) [3,4], the muscle tissue appears to be a good target tissue for SDM and OMP residue analyses. The developed HPLC assay can also be used for disposition study of N^4-Ac-SDM.

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5. References

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