Marker Residue Determination of Tritium-Labeled Ivermectin in the Muscle of Aquacultured Largemouth Bass, Hybrid Striped Bass, and Yellow Perch following Oral Treatment

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ABSTRACT: The residue depletion profiles of tritium-labeled ivermectin and its metabolites in the muscle of aquacultured largemouth bass (LMB), hybrid striped bass (HSB), and yellow perch (YP) following oral treatment are reported. Fish were administered ^{3}H-ivermectin at the dose level of 0.1 mg/kg body weight (7−9 μCi) in a gel capsule via stomach tube. At each postdose withdrawal time, six fish of each species were sedated with buffered MS-222 and blood samples taken. Fish were then euthanized, and fillets with adhering skin (scales removed) and bile samples were collected. The muscle fillets were homogenized in dry ice to a fine powder. Aliquots of tissue, plasma, and bile were assayed for total radioactive residue (TRR). The homogenized muscle was extracted in acetonitrile or methanol followed by high-performance liquid chromatographic (HPLC) analysis to determine the presence of parent ivermectin and its potential metabolites. The highest TRR concentrations (ivermectin equivalents) of 53, 45, and 44 ng/g (ppb) were obtained on postdose day 1 for HSB, LMB, and YP, respectively. The TRR depleted most slowly in HSB to 25 ppb at day 91, followed by YP to 19 ppb at day 42 and then by LMB to 22 ppb at day 35. The total residue of ivermectin and its metabolites by HPLC analysis followed the same depletion pattern in the three species. Additionally, the depletion rate of TRR of ^{3}H-ivermectin in the three species followed the pattern bile > plasma > muscle. The results further indicate that one of the polar metabolites of ivermectin could serve as a potential marker residue as an indication of use, rather than the parent ivermectin.

KEYWORDS: ivermectin, metabolism, depletion, finfish, aquaculture, marker residue

INTRODUCTION

Ivermectin, a broad-spectrum antiparasitic drug, is a member of the family of compounds isolated from the fermentation products of the soil microorganism *Streptomyces avermitilis*, known generically as avermectins.[1−3] This group of compounds has both vermicidal and ectoparasiticidal properties. Ivermectin is approved by the U.S. Food and Drug Administration (FDA)[4] for veterinary use in cattle and swine. The approved doses for cattle and swine are 200 and 300 μg/kg body weight (BW), respectively, by subcutaneous injection. The cattle and swine are not to be treated within 35 and 18 days, respectively, of slaughter. The marker residue (MR) for these species is the parent drug ivermectin. The European Community (EC) has set its maximum residue concentration at 15 ng/g (ppb) in liver or fat of these species.[5] In the United States, the ivermectin tolerances set by the FDA[4] for cattle and swine liver are 100 and 20 ppb, respectively, in muscle for cattle the toleneance is 10 ppb and for swine, 20 ppb. However, it is not approved for use in dairy cattle and fish species.

Avermectins possess a 16-member pentacyclic lactone, a spiroketal moiety, and a disaccharide unit. Avermectin B_1, also known as abamectin, is a natural product produced by fermentation and is a mixture of two homologues that possess a double bond at the position 22/23 and differ by a methylene group in the side-chain substituents at position 25.[6] Ivermectin is the 22,23-dihydroderivative of avermectin B_1 and consists of two closely related components, containing no less than 80% of the 22,23-dihydroavermectin B_{1a} (H_2B_{1a}) and no more than 20% of the 22,23-dihydroavermectin B_{1b} (H_2B_{1b}). Ivermectin is a highly effective anthelmintic at extremely low doses toward a variety of endo- and ectoparasites in many host species, including food and companion animals and humans.[7,8] Ivermectin's mode of action has been shown to be due to release and binding of neurotransmitter γ-aminobutyric acid (GABA) in certain nerve synapses.[9,10] However, it has been demonstrated that the main antiparasitic effect is due to a direct action on glutamine-gated chloride channels.[11−13] At therapeutic dose, ivermectin does not affect mammals significantly, because the drug does not readily pass the blood−brain barrier.[1] Palmer et al.[14] noted no increased mortality in fish when using a single oral dose of 0.2 mg/kg BW (recommended mammalian dose) against salmon lice infestations; the dose caused significant reduction in copepods. Increasing the dose to 0.4 mg/kg BW resulted in a higher mortality rate compared with the controls. This is perhaps due to passage of ivermectin across the blood−brain barrier due to its poor development in fish[15] compared to mammals.

Most studies reported in the literature on the disposition of ivermectin focus on terrestrial food-producing animals (cattle, sheep, and swine); however, there are a few in finfish species. Hoy et al.[15] studied the disposition of ^{3}H-ivermectin in Atlantic salmon smolts following a single administration via an oral gavage. Ivermectin was slowly absorbed. The highest concentrations of ivermectin equivalent residues were found in lipid-containing organs and reached maximum concentrations at approximately 4 days. Drug elimination was very slow with residue excretion, primarily as parent drug, occurring mainly via the bile. Roth et al.[16] also reported a long withdrawal period in muscle and skin during a depuration study of nonlabeled ivermectin in Atlantic salmon smolts. We reported metabolism and residue depletion of ^{3}H-ivermectin in the muscle of rainbow trout[17] following a single oral dose. The radioactive residue depletion was slow and persisted to 42 days, with primary residue excretion as the 3″-O-demethyl metabolite, which may be a potential marker residue (MR). Recently, we reported total radioactive residue (TRR) depletion of ^{3}H-ivermectin in Atlantic salmon, tilapia, and catfish.[18] The TRR of ^{3}H-ivermectin depleted most quickly from tilapia, a warm-water fish, and most slowly from Atlantic salmon, a cold-water fish. More recently, we also reported metabolism and residue depletion of ^{3}H-ivermectin in the muscle of Atlantic salmon, tilapia, and catfish[19] following a single oral dose. The parent ivermectin was determined to be the MR for these three species.

The sponsor of the compound is required to furnish the FDA with the scientific data necessary for demonstrating that the residues of the compound in the edible products of the treated animals are safe. This usually involves metabolism and depletion studies of a radiolabeled drug in a single species to establish MR. In addition, the sponsor is to provide an analytical method for a nonlabeled drug to monitor its potential unauthorized use and/or for violative residues. In an effort to compare marker residues of ivermectin across a range of aquacultured fish species, the purpose of the current study was to characterize the depletion profile of ^{3}H-ivermectin residues and identify a potential MR in three finfish species: largemouth bass (LMB), hybrid striped bass (HSB), and yellow perch (YP).

■ MATERIALS AND METHODS

Chemicals. Glass-distilled organic solvents (Burdick & Jackson Laboratories, Muskegon, MI, USA) and water from a Milli-Q Plus ultrapure water system (Millipore Corp., Bedford, MA, USA) were used. All chemicals were of high-pressure liquid chromatography (LC) grade, except when noted. Nonlabeled ivermectin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). It had a purity of 94% B_{1a} and was not further purified before use. Tritium-labeled ivermectin B_{1a} (H_2B_{1a}), labeled at the C-22 and C-23 positions, was synthesized by Perkin-Elmer, Life Sciences Products (Boston, MA, USA) with a specific activity of 244.4 mCi/mmol. Ivermectin tritium labeled at the C-22 and 23 positions is stable and appropriate for metabolism studies.[20] Prior to use, its purity was evaluated and repurified to ≥96% by HPLC, using a semipreparative C18 column and acetonitrile as mobile phase.

Apparatus. The HPLC consisted of a series 1100 Agilent Technologies system (Wilmington, DE, USA) fitted with a quaternary pump, an autosampler, a column heater, a solvent degasser, a variable-wavelength UV detector set at 245 nm, and an Easy Chrome data system. An analytical column, a 5 μm Luna C18 (2), 150 × 4.6 mm, and guard cartridge, a 5 μm Luna C18, 4 × 3 mm (Phenomenex, Torrance, CA, USA) were used. Both the analytical and guard columns were in a column heater set at 40 °C. The mobile phase consisted of 85% acetonitrile in water, with a flow rate of 1 mL/min. For purification of ^{3}H-ivermectin, a semipreparative column, a 10 μm Luna C18 (2), 250 × 10 mm, 100 A, was used; the mobile phase consisted of 100% acetonitrile with a flow rate of 2.5 mL/min. For purity determination, a second column was used: a Luna CN, 5 μm, 150 × 3 mm, with a mobile phase of 40:60 ACN/water and flow rate of 1 mL/min; and silica gel Zorbax column, 5 μm, 250 × 4.6 mm, with a mobile phase of 88:12 isooctane/ethanol was also used. The flow rate and column temperature for both columns were 1 mL/min and 40 °C, respectively.

A Foxy 200 series fraction collector (Isco, Inc., Lincoln, NE, USA) was used to collect fractions for both analytical and semipreparative columns. LC fractions were mixed with Packard Insta-gel XF, as a liquid scintillation fluid, and counted on a Packard Tri-Carb 3100 Counter (Perkin-Elmer, Life Sciences). A Packard model 301 Sample Oxidizer (Perkin-Elmer, Life Sciences) was used to combust tissue pellets for total residue analysis measurements.

Centrifugations were carried out in a Sorvall RC-5C (rotor HS-4) refrigerated centrifuge (Sorvall Products, Newtown, CT, USA) at 2358 relative centrifugal force (RCF) set at 4 °C for 10 min. A two-speed Waring blender (Waring Commercial, Torrington, CT, USA) with a 40 oz glass jar was used to blend the tissue samples. All liquid transfers were made with Eppendorf digital pipets.

Dose Preparation. The dose was prepared by mixing unlabeled ivermectin with repurified ^{3}H-ivermectin in ethanol to give a specific activity in the range of 155 dpm/ng (61.1 mCi/mmol) to 213 dpm/ng (83.3 mCi/mmol). An appropriate volume of dosing solution, based on fish weight, was transferred to size 4 gel capsules (Torpac Inc., Fairfield, NJ, USA) and ethanol evaporated under the stream of nitrogen. The gel capsules were administered to fish as described below.

Animal Experiments. Largemouth bass (*Micropterus salmoides*), hybrid striped bass (*Morone chrysops* × *saxatillis*), and yellow perch (*Perca flavescens*) were obtained from commercial or private (academic) sources and housed separately in 2000 L recirculating round fiber glass tanks containing fresh water. Water was maintained at a pH of 7.0 ± 0.7 and to a temperature of 20 ± 2 °C for the three fish species. The fish were provided ad libitum access to a commercially available diet and were cultured until they reached mature weight range. Largemouth bass (average = 533 g), hybrid striped bass (average = 1312 g), and yellow perch (average = 275 g) were weighed and then transferred to 80 L flow-through glass tanks, one fish per tank. A maximum of four fish could be treated in a group due to laboratory capacity. The temperature and pH of the fresh water in the experimental tanks was the same as for the holding tanks. The fish were allowed to acclimate for at least 2 days and orally dosed with 0.1 mg/kg BW of tritium-labeled ivermectin in capsules via intragastric feeding tube. Six fish were randomly assigned to predetermined postdose time points: for LMB, 1, 3, 7, 14, 21, 28, and 35 days; for YP 1, 3, 7, 14, 21, 28, and 42 days; and for HSB, 1, 3, 7, 14, 21, 28, 35, and 42 days. Due to high value of TRR at day 42 in HSB, the experiment was continued to include postdose days 56 and 91; however, four fish were used at each of these withdrawal points. On a sampling day, the tank water was drained to about 20 L, and 10−15 g of MS 222, buffered with 2× sodium bicarbonate, was added to the tank water to anesthetize the fish. Each fish was removed from the tank, blood was drawn with a syringe containing anticoagulant (heparin), and the fish were euthanized by decapitation with a sharp knife. The fish was then scaled, and muscle fillets with attached skin (referred to as muscle) were removed and stored at −80 °C. The skin was not removed as muscle with skin attached is considered the edible tissue for laboratory testing of these species in the United States. Samples of scales, bile, liver, and kidney were also collected and stored at −80 °C.

Blending of Muscle Samples. Muscle fillets were semidefrosted and cut into small pieces (∼1 × 1 cm). Half a cup of dry ice pellets was blended to a fine powder in a Warring blender jar. Pieces of tissue were transferred to the jar and homogenized for ∼30 s, producing a dry ice/tissue powder matrix. The dry ice/tissue matrix was transferred to wide-mouth plastic bottles, covered loosely, and stored at −20 °C overnight, allowing the dry ice to sublimate. Sample bottles were then capped tightly and stored at −80 °C.

Sample Preparation for Total Radioactive Residue (TRR) Analysis. The muscle samples were combusted and then counted; however, the plasma and bile samples were directly counted as described below. Triplicate 0.5 g blended samples of muscle were weighed into three combusto-cones and combusted in a continuous flow of oxygen, forming tritiated water using a Packard Sample Oxidizer. Due to high combustion temperatures (3000 °C), this water is in the form of steam, condensed in an air-cooled condenser and collected as tritiated water in the tritium counting vial. Any uncondensed water is collected in the tritium exchange column and flushed down with tritium scintillator (Packard Monophase S) into the same tritium counting vial. Each vial was placed into a Packard Tricarb-3100 counter to measure radioactivity for 10 min. Plasma samples were thawed, and 100 μL was mixed with 10 mL of liquid scintillation fluid (LSF) and the radioactivity measured as above. Similarly, bile samples were thawed and diluted 1:10 with acetonitrile and centrifuged for 5 min. An aliquot of supernatant was counted after mixing with 10 mL of LSF.

Sample Preparation and HPLC Analysis. Muscle (1 g per sample) was assayed in duplicate. Each sample was extracted with 5 mL of acetonitrile or methanol, vortex mixed, and centrifuged. The supernatant was transferred to a 15 mL polypropylene centrifuge tube. The tissue pellet was re-extracted twice more for a total of three extractions. The combined supernatants were evaporated with nitrogen using a Zymark Turbovap LV evaporator set at 50 °C. The extract was concentrated just to dryness, and 1.0 mL of mobile phase added. The sample mixture was sonicated for 5−10 min and vortex mixed. The final extract was centrifuged, and 100 μL of the supernatant was used for HPLC analysis. An HPLC chromatogram of labeled ivermectin standard was obtained to determine the elution position of the ivermectin B_{1a}. Subsequently, HPLC radioactive profiles of the fortified and incurred muscle samples were obtained to characterize ivermectin and its potential metabolites. One milliliter fractions of the column effluent were collected into 7 mL scintillation vials, using the fraction collector. Five milliliters of liquid scintillation cocktail was added to each fraction and vortex mixed. Radioactivity in each fraction was measured for 10 min with the Packard Tricarb-3100 counter. The residual tissue pellets were combusted in the Packard Sample Oxidizer, and the radioactivity was enumerated as above. The extraction solvent was changed from acetonitrile to methanol, because it provided slightly improved ^{3}H-ivermectin recoveries and a less compact pellet, resulting in its easier removal for TRR assay.

■ RESULTS AND DISCUSSION

Total Radioactive Residue Analysis. Recoveries of ^{3}H-ivermectin from fortified control muscle samples were determined throughout the sample analysis. Control muscle was fortified with ^{3}H-ivermectin ranging from 3550 to 3890 dpm (YP), from 2261 to 3756 dpm (LMB), and from 2504 to 3756 dpm (HSB) and was combusted as described above. Background radioactivity from control samples was also determined and subtracted from the fortified sample counts. Average recoveries of 106, 108, and 109% with coefficients of variation (CVs) of 2, 3, and 4%, respectively, were obtained. The mean levels (along with ± SD) of TRR, expressed as nanogram equivalents per gram of muscle of the treated species, LMB, HSB, and YP at various postdose time intervals are plotted in Figure 1. The TRR concentrations peaked on postdose day 1 in all three fish species and were higher in HSB (53 ppb) than in LMB (45 ppb) and YP (44 ppb). The TRR depleted to 22 ppb by day 35 in LMB, to 19 ppb by day 42 in YP, and to 25 ppb by day 91 in HSB. This indicates that the rate of metabolism and depletion of ivermectin in HSB was slower than in the other two species. Due to low concentrations of TRR at days 35 and 42 in LMB and YP, respectively, additional fish were not dosed on subsequent days.
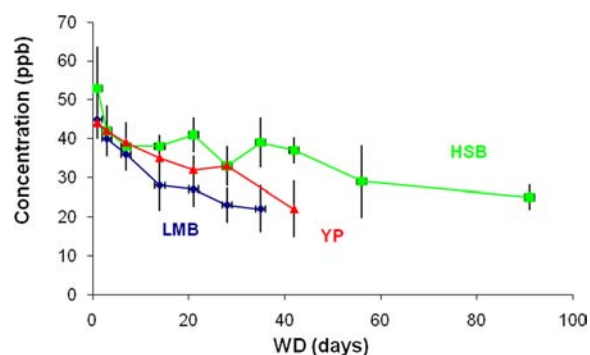
Figure 1. Depletion plot of total radioactive residue (TRR) concentration in parts per billion equivalents of ^{3}H-ivermectin in muscle tissue of HSB, YP, and LMB.

The depletion profiles of TRR of ^{3}H-ivermectin at various postdose intervals in bile, plasma, and tissue for the three species are shown in Figure 2. The bile showed higher
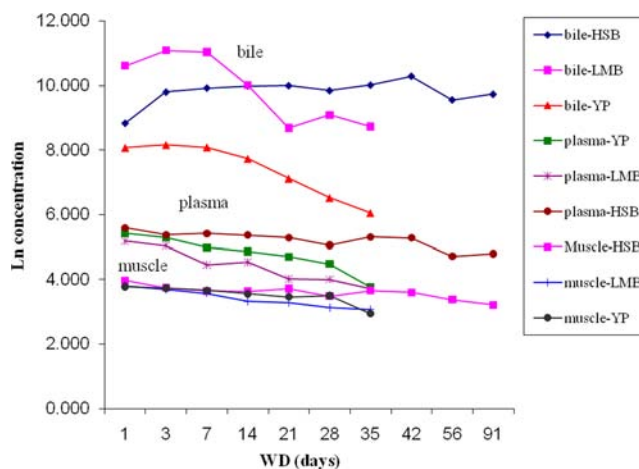
Figure 2. Comparison of the depletion of total radioactive residue (TRR) of ^{3}H-ivermectin in bile, plasma, and muscle of YP, LMB, and HSB: natural log (Ln) of TRR concentration (ppb) versus withdrawal time (WD).

radioactivity levels at all time points followed by plasma and the lowest in muscle; the depletion rate of TRR of ^{3}H-ivermectin in the three species followed the pattern bile > plasma > muscle.

Metabolic Profile of Ivermectin Residues in Muscle. The radioactive residue concentrations determined by HPLC in the three fish species were expressed as the ^{3}H-ivermectin equivalents in ng/g or ng/mL for parent ivermectin and its metabolites. During the analysis of incurred samples of each species, quality control samples comprising a control and a fortified control muscle were assayed. Accordingly, control muscle was fortified with ^{3}H-ivermectin at various concentration levels and carried through extraction and HPLC analysis procedures along with the incurred samples for each species. Mean recoveries of ^{3}H-ivermectin for LMB (565−3756 dpm), HSB (3756 dpm), and YP (3120−3818 dpm) were 87, 85, and 104%, respectively, with the corresponding CVs of 10, 12, and 3%. Tables 1, 2, and 3 indicate concentrations in ng/g (ppb) ivermectin equivalents of labeled ivermectin and its metabolites in HSB, LMB, and YP, respectively, by HPLC analysis. Three metabolites at the retention times (RT) of 3, 6, and 9 min were detected in HSB and LMB, whereas only two at RT of 6 and 9

Table 1. Concentrations of Ivermectin and Metabolites by HPLC Analysis in Muscle of HSB at Various Postdose Times

WD, days	N	^{3}H-IVM, ng/g (±SD)	^{3}H-IVM-3, ng/g (±SD)	^{3}H-IVM-6, ng/g (±SD)	^{3}H-IVM-9, ng/g (±SD)	^{3}H-pellet, ng/g (±SD)	total, ng/g
1	6	29.3 (4.8)	4.8 (2.0)	5.8 (1.4)	4.0 (1.4)	2.3 (1.4)	46.2
3	6	23.3 (2.9)	4.2 (2.0)	4.1 (2.6)	5.6 (0.7)	0.4 (0.1)	40.6[a]
7	6	17.3 (3.0)	4.5 (0.6)	3.2 (0.3)	6.1 (0.9)	0.5 (0.2)	34.1[b]
14	6	12.8 (0.7)	3.0 (0.4)	8.8 (0.9)	8.7 (0.6)	0.8 (0.2)	34.0
21	6	8.3 (1.0)	2.4 (0.2)	8.4 (1.0)	9.7 (1.0)	7.3 (1.4)	36.0
28	6	7.4 (1.1)	2.1 (0.2)	7.3 (0.7)	7.4 (1.0)	7.3 (4.3)	31.5
35	6	7.1 (1.2)	3.1 (0.6)	8.3 (1.2)	10.1 (1.1)	5.4 (2.4)	33.9
42	6	6.3 (1.9)	1.8 (0.1)	8.9 (1.0)	8.7 (1.4)	7.6 (2.2)	33.2
56	4	3.8 (1.2)	ND	9.2 (2.0)	10.2 (3.6)	3.2 (0.9)	26.4
91	4	2.0 (0.3)	0.8 (1.2)	10.6 (0.7)	7.0 (0.9)	1.3 (0.1)	21.8

[a]Includes 2.90 ppb in peak at 8 min. [b]Includes 2.4 ppb at 8 min peak. This peak was not noted at other post-dose times.

Table 2. Concentrations of Ivermectin and Metabolites by HPLC Analysis in Muscle of LMB at Various Postdose Times

WD, days	N	^{3}H-IVM, ng/g (±SD)	^{3}H-IVM-3, ng/g (±SD)	^{3}H-IVM-6, ng/g (±SD)	^{3}H-IVM-9, ng/g (±SD)	^{3}H-pellet, ng/g (±SD)	total, ng/g
1	6	16.1 (2.2)	7.1 (0.8)	7.5 (0.8)	10.9 (1.8)	1.0 (0.4)	42.5
3	6	12.3 (1.5)	6.6 (0.8)	7.5 (0.5)	12.2 (0.8)	0.3 (0.1)	38.8
7	6	8.1 (0.8)	6.2 (1.1)	7 (0.3)	10.9 (1.1)	0.5 (0.2)	32.7
14	6	4.4 (1.7)	6.2 (1.8)	6.6 (1.2)	9.8 (2.4)	0.3 (0.1)	27.4
21	6	3.3 (0.8)	4.8 (0.9)	5.7 (0.6)	8.6 (1.3)	0.4 (0.1)	22.8
28	6	1.8 (1.3)	3.7 (1.2)	5.2 (0.9)	6.9 (1.8)	1.4 (0.8)	19.0
35	6	1.9 (1.4)	3.3 (2.2)	5.1 (1.5)	6.1 (1.4)	1.1 (1.1)	17.5

Table 3. Concentrations of Ivermectin and Metabolites by HPLC Analysis in Muscle of YP at Various Postdose Times

WD, days	N	^{3}H-IVM, ng/g (±SD)	^{3}H-IVM-6, ng/g (±SD)	^{3}H-IVM-9, ng/g (±SD)	^{3}H-pellet, ng/g (±SD)	total, ng/g
1	6	25.1 (1.3)	8.8 (1.2)	4.2 (0.3)	0.8 (0.2)	38.8
3	6	22.3 (2.5)	11.4 (1.6)	5.2 (0.7)	0.8 (0.3)	39.7
7	6	18.8 (3.2)	11.4 (1.9)	5.7 (0.8)	1.3 (0.8)	37.3
14	6	13.6 (2.3)	13.2 (1.3)	7.2 (1.3)	1.1 (0.7)	35.0
21	6	11.1 (1.6)	13.4 (0.8)	6.7 (0.5)	0.9 (0.2)	32.1
28	6	10.1 (2.4)	15.2 (2.0)	7.8 (0.6)	0.7 (0.3)	33.8
42	6	ND	10.9 (5.3)	6.4 (0.9)	0.5 (0.2)	17.7

min were detected in YP. Figure 3 illustrates a typical HPLC radiogram of control and incurred muscle of postdose day 28 HSB. No interfering peaks are noted at the elution position of ivermectin and its metabolites in the control muscle. The concentration of parent ivermectin peaked by postdose day 1 in all three fish species, HSB (29.3 ng/g), LMB (16.1 ng/g), and
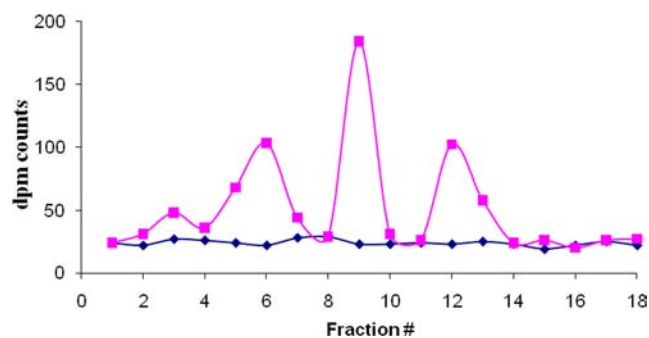
Figure 3. Typical HPLC radiogram of control and incurred HSB muscle of postdose day 28 (counts in disintegrations per minute (dpm) vs fraction numbers): metabolite 1 (RT 3 min); metabolite 2 (RT 6 min); metabolite 3 (RT 9 min); ivermectin (RT 12 min).

YP (25.1 ng/g). It declined to 2 ppb by days 28 and 91 in HSB and LMB; however, it was not detectable in YP at day 42.

The metabolite at RT of 9 min was previously reported to be a major metabolite present in rainbow trout and tentatively characterized as the 3″-O-dimethyl ivermectin B$_{1a}$ metabolite.[17] However, further work, for example, mass spectral characterization, is needed to positively confirm that the metabolite detected at RT 9 min in this study is the same as reported previously. Table 4 shows the percent distribution of ^{3}H radioactivity in ivermectin and three of its metabolites by HPLC (extractable portion only). The percent radioactivity in parent ivermectin was higher than in metabolites until day 14 in both HSB (38%) and YP (40%), whereas in LMB, it was higher only until day 3 (32%). In LMB the radioactivity of the metabolite at RT 9 min increased to 34% by day 7 and was 39% at day 28 and 37% at day 35; in HSB, the radioactivity of the metabolite at RT 9 min increased to 34% by day 21 and continued to increase to 44% by day 56 and was still at 34% by day 91. However, in the case of YP, the radioactivity increased to 43% by day 21 in metabolite at RT 6 min. These results suggest that potential MR for LMB is the metabolite at RT 9 min, whereas for YP it is at RT 6 min. Because there is not a significant difference in percent radioactivity in metabolites at RT 6 and 9 min for HSB, either may have potential for use as MR. However, further experiments using unlabeled ivermectin to treat fish may be needed to isolate, characterize, and confirm the identity of MR in the three fish species.

The residual tissue pellets of extracted samples at all postdose time points of the three species were oxidized on a Packard Sample Oxidizer to determine concentrations of unextracted radioactivity (UExt). The concentrations of pellets in ng/g of ivermectin equivalent radioactivity are also given in Tables 1−3 for the three species. The radioactivity in the unextracted portion (pellets) was relatively low, with most in the extracted fraction. The increase in value for the ^{3}H-pellet in Table 2 for 28 and 35 days could be due to insufficient extraction of the analytes from the tissue samples of these time

Table 4. Distribution of Percent Radioactivity (Extractable Portion) in Parent Ivermectin and Metabolites in HSB, LMB, and YP Muscle[a]

WD, days	% ³H-IVM (RT 12 min)			% ³H-metabolite 1 (RT 3 min)			% ³H-metabolite 2 (RT 6 min)			% ³H-metabolite 3 (RT 9 min)		
	LMB	YP	HSB	LMB	YP	HSB	LMB	YP	HSB	LMB	YP	HSB
1	39	66	67	17	nd	11	18	23	13	26	11	9
3	32	57	62	17	nd	11	19	29	11	32	13	15
7	25	52	56	19	nd	14	22	32	10	34	16	20
14	16	40	38	23	nd	9	25	39	26	36	21	26
21	15	36	29	21	nd	8	26	43	29	39	22	34
28	10	30	30	21	nd	9	30	46	30	39	24	30
35	11	*	25	20	*	11	31	*	29	37	*	35
42	*	nd	24	*	nd	7	*	63	35	*	37	34
56	*	*	16	*	*	nd	*	*	40	*	*	44
91	*	*	10	*	*	4	*	*	52	*	*	34

[a] nd, not detected; *, data not collected.

Table 5. Comparison of Total Radioactive Residues (TRR) in Ivermectin Equivalents Determined by the Sample Oxidizer and by HPLC Analysis at Various Withdrawal (WD) Periods in the Three Species[a]

WD, days	HSB			YP			LMB		
	TRR, ng/g	Ext+Uext, ng/g	% of TRR	TRR, ng/g	Ext+Uext, ng/g	% of TRR	TRR, ng/g	Ext+Uext, ng/g	% of TRR
1	52.7	46.2	87.7	43.8	38.8	88.7	44.7	42.5	95.1
3	42.3	40.6	96.0	42.2	39.7	93.9	40.1	38.8	96.8
7	38.2	34.1	89.3	39.3	37.3	94.9	35.7	32.7	91.6
14	38.0	34.0	89.5	35.0	35.0	100.0	27.9	27.4	98.7
21	41.0	36.0	87.8	32.3	32.1	99.3	26.7	23.2	86.9
28	32.6	31.5	96.6	33.0	33.8	102.5	22.9	19.0	83.0
35	38.9	33.9	87.1				21.7	17.5	80.6
42	36.7	33.2	90.5	19.3	17.7	92.0			
56	29.3	26.4	90.1						
91	24.9	21.8	87.6						
av			90.2			95.9			90.4
SD			3.4			4.9			7.1

[a] Extractable (Ext) samples were processed by HPLC, and unextractable (UExt) samples were processed by the Oxidizer.

periods. Table 5 shows the comparison of TRR determined by the Sample Oxidizer and that recovered from both extractable (HPLC) and unextractable (Oxidizer) samples. The average radioactive residue levels obtained from extractable and unextractable samples over their TRR by the Packard Oxidizer at various postdose time points were 90, 96, and 90% for HSB, YP and LMB, respectively, with CVs of <10%. These results indicate a good extraction efficiency and recovery of radioactivity from the muscle of the three species. The difference in full recovery as compared to TRR could be attributed to extraction inefficiency as well as to the potential presence of additional undetectable minor metabolites in some muscle samples.

As per the Code of Federal Regulations, the MR for ivermectin in cattle and swine is the parent drug, and the FDA has set its tolerance to be 10 and 20 ppb in muscle, respectively, with corresponding withdrawal times of 35 and 18 days.[4] Our previous studies[19] suggested the parent drug to be a potential MR in muscle for Atlantic salmon, tilapia, and catfish, and by postdose day 21, ivermectin was depleted to <10 ppb in all three species. Therefore, it is reasonable to evaluate and consider ivermectin to be the MR in the fish species reported here and in rainbow trout reported previously.[17] In the case of HSB (Table 1) and LMB (Table 2), ivermectin and the three metabolites are depleted to about 10 ppb by postdose day 21. In yellow perch (Table 3), ivermectin and the two metabolites

are depleted to about 15 ppb by day 28. Similarly, in rainbow trout, both ivermectin and its metabolite are depleted by postdose day 28 to <15 ppb and by day 35 to <10 ppb. On the basis of these residue depletion studies, regulatory agencies could consider parent ivermectin as a MR for the seven fish species and potentially assign tolerance levels and withdrawal times comparable to those approved for cattle and/or swine.

In conclusion, the metabolic disposition of ³H-ivermectin in LMB, HSB, and YP has been studied. The results suggest that one of the polar metabolites, consisting of radioactive peaks eluting before the parent drug ivermectin, could serve as a potential marker residue in the muscle of the three fish species: for LMB and YP, a metabolite at RT 9 and 6 min, respectively; for HSB, it could be either one. However, additional experiments with unlabeled ivermectin will be needed to isolate, characterize, and confirm the identity of these metabolites. Previously for rainbow trout, a metabolite at RT 9 min was tentatively characterized as potential MR.[17] However, in the case of Atlantic salmon, tilapia, and channel catfish, the parent ivermectin was suggested to be the potential MR.[19] This work represents our efforts to investigate a variety of finfish species to identify a marker residue (or residues) for monitoring the unauthorized use of aquaculture drugs. In view of the residue depletion data generated in this study and reported previously,[17,19] it is possible to consider the parent

ivermectin to be a MR for all seven fish species, similar to cattle and swine.

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Notes

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