

Overview

- Total Residue Depletion Study in Atlantic salmon
- Tissue Distribution, metabolism, and depletion of radiolabeled SCH 58854
- Emamectin benzoate is formulated in a 0.2% feed mix (SLICE) and administered to fish in treated feed
- The concentrations of [³H]SCH 58854 residues in tissues were highest at early time points (3-72 hr post-final dose) and gradually decreased with time though 90 days post-final-dose
- The highest concentration was found in kidney followed by liver
- Major metabolite was *N*-demethylated compound
- Marker residue of SCH 58854 in edible tissues in Atlantic salmon is free base emamectin B1a (MAB_{1a})
- Determinative Assay of Marker Residue in treated salmon
- The average concentrations of the marker residue in muscle and skin were highest at 72-hr (46 and 59 ppb, respectively) and declined through Day 30 (24 and 36 ppb, respectively)

Introduction

Emamectin benzoate [SCH 58854; 4'-deoxy-4'-(epimethylamino)javermetin B1 benzoate salt] is a semi-synthetic avermectin registered for use to control lepidopterous pests on vegetable crops. It is also highly effective for the treatment and control of sea lice infestations on Atlantic salmon, *Salmo salar*. The structure is shown in Figure 1. The compound, also known as MAB, benzoate, is a mixture of two homologs designated as B1a and B1b. By specification it consists of at least 90% of B1a benzoate and not more than 10% of B1b benzoate.

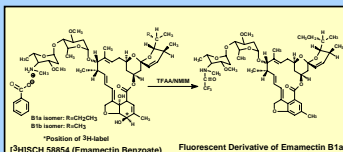


Figure 1. The structure of Emamectin Benzoate and the Fluorescent Derivative of the Marker Residue

Objective

- ❑ To examine the tissue distribution of radioactive residues and the depletion of [³H]SCH 58854 in Atlantic salmon maintained at 5 ± 1 °C following dietary intake of a nominal dose of 50 µg of [³H]SCH 58854/kg/day for 7 consecutive days through 90 days post final dose.
- ❑ To examine the metabolic profiles of [³H]SCH 58854 in Atlantic salmon

Tissue Distribution, Metabolism and Residue Depletion Study in Atlantic Salmon Following Oral Administration of ³H-SCH 58854

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Methods

- Test System: One hundred and twenty-two Atlantic salmon, weighing ~1 kg and maintained at -5 °C
- Dose: ~50 µg/kg weight/day for 7 consecutive days
- Collection: Groups of 10 Fish at 3, 12, 24, and 72 hr and 7, 15, 30, 45, 60, and 90 days post-final dose
- Total Radioactivity Assay of Collected Specimen
- Extraction of Tissue Residues and Metabolite Profiling
- HPLC Column: Primesphere 5 C18, 4.6 x 150 mm, 5 µm
- Mobile Phase: A: 0.1% H₃PO₄ in H₂O; B: CH₃CN (F = 1 mL/min)
- Gradient: 60% A to 70% B in 25 min, to 100% in 1 min (10 min)
- Radioactive Detection: Ramona 5-LS with 600 µL liquid cell using Ready Flow III at 2.5 mL/min
- Radiochromatogram: For muscle and skin tissue residues, fraction were collected and counted by LSC. Reconstructed HPLC chromatograms were prepared using the determined radioactivity in each fraction.
- Residue Depletion Assay Using a Determinative Method for the Marker Residue, MAB_{1a} (Figures 1 and 2)
- SPE Cartridge: Propylsulfonic acid (PRS) Cartridge

Results

- ✓ Maximum mean radioactivity concentrations in edible tissues (muscle and skin) were below 100 ppb at all time points. (Figure 3)
- ✓ The kidney residues were the highest followed by liver residues. (Figure 4)
- ✓ The total residues in kidney, liver, muscle, and skin consisted of SCH 58854 and *N*-demethylated SCH 58854 (L-695,638) as a major metabolite. *N*-formyl SCH 58854 was also detected in minute quantity at early interval. (Figure 5)
- ✓ The average concentrations of the marker residue in the muscle and skin were highest at 72-hr (46 ppb and 59 ppb, respectively) and declined through Day 30 (24 and 36 ppb, respectively). (Figure 6)
- ✓ The overall procedural recovery for the determinative assay was 90.9% ± 2.9 (n = 6) at 40 ppb and 89.2% ± 3.2 (n = 6) at 200 ppb for muscle and 86.5% ± 5.6 (n = 4) at 40 ppb and 82.8% ± 2.9 (n = 4) at 200 ppb for skin.

Results

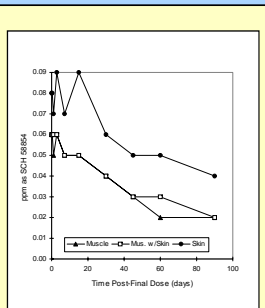


Figure 3. Total ³H Residue Depletion Curves for Muscle, Skin, and Intact Muscle with Skin Tissues

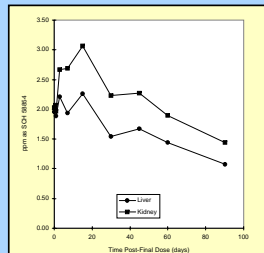


Figure 4. Total ³H Residue Depletion Curves for Liver and Kidney

Figure 2. Flow Diagram of the Determinative Assay for Emamectin benzoate Marker Residue in Fish Tissues

-2.5 g of processed tissue

1. Homogenize with CH₃CN, H₂O, and EtOAc
2. Rinse Probe with EtOAc and combine to the first extract
3. Extract by Shaking and Centrifugation
4. Cleanup of the extract using PRS SPE cartridge
5. Elute with 1% NH₄OAc in CH₃OH
6. Take 1/5 of the methanolic eluate, add H₂O
7. Basify with 2 drops of NH₄OH and extract with EtOAc
8. Evaporate EtOAc to dryness
9. Reconstitute in CH₃CN and sonicate for 10 min in hot water
10. Derivatize with NMIM (N-methylimidazole) and 0.3 mL TFAA(trifluoroacetic anhydride):CH₃CN (1:2) in a hot water bath for 20 min
11. Dilute the mixture to 5 mL with 3 mL CH₃CN and 0.6 mL H₂O
12. Inject 10 µL on HPLC with fluorescence detection
13. Quantitate by comparison with external standards derivatized concurrently

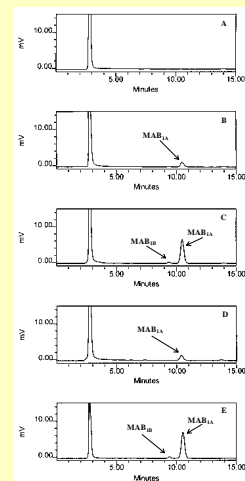


Figure 6. Representative HPLC Chromatograms of the Marker Residue Assay

- (A) Control Muscle
- (B) Fortified Muscle at 40 ppb
- (C) Fortified Muscle at 200 ppb
- (D) Incurred Residue Muscle at 36 ppb (Fish #1, 30-Day)
- (E) Calibration Standard at 20 ng/mL (equiv. 200 ppb)

Acknowledgements

The authors thank Atlantic Fish Health, Inc. for in-life phase of the study and Matthew J. Wisocky and John Ryan for their technical support.

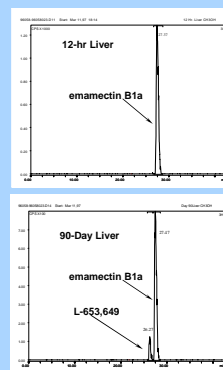


Figure 5. Representative radiochromatogram of 12-hr and Day-90 Liver

Conclusions

- Following a dietary intake of 50 µg of [³H]SCH 58854 in Atlantic salmon for seven consecutive days, the concentrations of the radioactive residues (emamectin B1 benzoate equivalent) in tissues were highest at early time points and gradually decreased with time through 90 days post-final dose: 2-3 ppm in kidney decreasing to 1.4 ppm; ~2 ppm in liver decreasing to 1.1 ppm; 0.069-0.093 ppm in skin decreasing to 0.036 ppm; 0.053-0.065 ppm in muscle decreasing to 0.019 ppm; 0.055-0.064 ppm in muscle with skin decreasing to 0.020 ppm.
- [³H]SCH 58854 (emamectin B1a) represented 98-100% of the radioactive residues at 12-hr post-dose and declined to 83-89% at Day 90.
- Major metabolite was characterized as *N*-demethylated analogue of SCH 58854, which represented <1% of the radioactive residues at 12-hr and increased to 11-17% at Day 90. *N*-formyl analogue of SCH 58854 was also found as a minor metabolite
- The average concentration of MAB_{1a} residues in muscle and skin were highest at 72-hr representing 46 and 59 ppb, respectively. Its levels in the edible tissues declined over time.
- The overall procedural recovery for the determinative assay was 89-91% for muscle and 83-87% for skin.

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