

Degradation rate of praziquantel and fenbendazole in rainbow trout following oral administration

Zdenka SOUKUPOVA-MARKOVA¹, Veronika DOUBKOVA²,
Petr MARSALEK², Zdenka SVOBODOVA², Ivana PAPEZIKOVA¹,
Stepan LANG³, Stanislav NAVRATIL¹, Miroslava PALIKOVA¹

- 1 Department of Ecology and Diseases of Game, Fish and Bees, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
- 2 Department of Veterinary Public Health and Animal Welfare, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
- 3 Department of Zoology, Fisheries, Hydrobiology and Apiculture, Faculty of Agronomy, Mendel University, Brno, Czech Republic

Correspondence to: Mgr. Zdenka Soukupova-Markova
Department of Ecology and Diseases of Game, Fish and Bees
Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences Brno
Palackeho tr. 1/3, 612 42, Brno, Czech Republic.
TEL: +420-721696997; FAX: +420-221951283
E-MAIL: soukupova.zdenka@centrum.cz

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Abstract

OBJECTIVES: The aim of this study was to evaluate and compare the rate of degradation and elimination of praziquantel and fenbendazole antiparasitics following oral administration to salmonids. In addition, we determine whether the length of the legal withdrawal period is sufficient for complete elimination of antiparasitic residue from the body. The use of these drugs in fish is currently considered off-label and data on degradation are not available for rainbow trout.

METHODS: The model species for this experiment was the rainbow trout (*Oncorhynchus mykiss*) and praziquantel and fenbendazole were chosen for experimental therapy. Both drugs were administered into the gastrointestinal tract using a stomach tube. Concentrations of fenbendazole and praziquantel were established through high performance liquid chromatography-tandem mass spectrometry.

RESULTS: Our results show that concentrations of praziquantel and fenbendazole reach their maximum in the body within 24 hours of administration, with concentrations dropping sharply over the following 24 hours. With one exception, when trace amounts of both substances were found in blood plasma, the drugs were completely degraded and eliminated from the body by the end of the experiment (corresponding to 497.6 degree days).

CONCLUSIONS: Praziquantel and fenbendazole both show a high rate of degradation and elimination from fish. As both substances were eliminated from the body within the required withdrawal period (i.e. within 500 degree days) they can be safely used based on current knowledge of their therapeutic effect for treating helminth infections.

Abbreviations

D°	- day degree
HESI-II	- heated electrospray ionisation
LC-MS/MS	- liquid chromatography coupled with triple quadrupole tandem mass spectrometry
MRL	- maximal residual limit
QA/QC	- Quality Assurance versus Quality Control
S/N	- signal versus noise value
SPE	- solid phase extraction
WHP	- withdrawal period

INTRODUCTION

Fish can be exposed to natural toxins (Kopp *et al.* 2014), anthropogenic pollutants (Hostovsky *et al.* 2014; Plhalova *et al.* 2014) and infectious agents. Parasitic worms (helminthiasis) are a common cause of infections. While some parasitic species undergo simple development, others undergo a complex development cycle involving the participation of intermediate hosts. Fish, along with waterfowl and other aquatic animals, can play the role of either the principal or intermediate parasite host. Due to their complex mode of development, therapeutic control and prevention of helminths can sometimes be problematic. According to the Czech Act on Veterinary Care (No. 166/1999 Coll., as amended), a fish breeder is obliged to monitor the health status of the fish reared and provide them with timely specialised veterinary care in justified cases. The same act stipulates that veterinary therapeutic preparations can be dispensed only upon the veterinary physician's prescription and administered to animals only upon the veterinarian's consent and directions. Due to the difficulties of administration and economic costs, interventional treatments at fish breeding stations tend to be an emergency measure assigned for cases of inefficient prevention.

Fish breeding is usually divided into hobby breeding (ornamental and aquarium fish) or production breeding, which falls under the category of food animals. In food animals, medication may not be administered *lege artis* if containing substances that have not been classified in terms of the maximal residual limit (MRL). Based on the MRL and the results of depletion studies in edible tissue (i.e. fish muscle and skin), a withholding period (WHP) has been determined for registered fish veterinary preparations, expressed in degree days (D°). Unless the WHP is established by the drug manufacturer and the medication administered within the so-called cascade (off-label, described in § 3 of Regulation No. 344/2008 on the use, prescription and dispensing of medicinal products in veterinary care), the preparation in question is assigned a WHP as stipulated by Act No. 166/1999 Coll. on Veterinary Care. For fish, at least 500 D° are used. At present, there is no registered antiparasitic medicinal product designed for fish in use in the Czech Republic; hence, it is necessary to proceed according to the above-mentioned cascade for elimination of parasites during fish breeding. With this in mind,

two drugs offer potential for treatment: praziquantel and fenbendazole. The efficacy of one of these (praziquantel) has already been tested against a range of fish parasites in fish (Sudová *et al.* 2009; Sudová *et al.* 2010). Fenbendazole is used to treat non-encapsulated nematodes in the gastrointestinal tract of fish (Noga, 2010).

The aim of this study was to evaluate and compare the rate of degradation and elimination of the antiparasitic preparations praziquantel and fenbendazole following oral administration to salmonids. In addition, we determine how the period sufficient for complete elimination of these substances from the fish's body.

MATERIAL AND METHODS

The experiment was conducted in the spring season (April, May) on rainbow trout (*Oncorhynchus mykiss*) aged 6–12 months and weighing 100–200 g in salmonid breeding. For the experiment 150 fish were used but because of the deaths during the experiment, a total of 115 fish were used for analysis. Praziquantel EP 100 g in powder form (active ingredient praziquantel, batch no. C-PQ 2011003, exp. 10/26/2014) was imported with special permission of the State Veterinary Institute, and Panacur 25 mg.L⁻¹, *p.o.* suspension (active ingredient fenbendazole reg. no. 10/014/76 – F/C, batch no. E590A04, exp. 02/2017) were used for experimental therapy. Three groups of fish were designated as a control, the fenbendazole-treated group and the praziquantel-treated group. Both medications were administered into the gastrointestinal tract using a stomach tube at a single dose of 50 mg.kg⁻¹ live weight at time t0 (water temperature: 10.5°C). For monitoring of drug degradation, six fish were sampled (if it was possible) at time t24 (9.7°C), t48 (9.5°C), t120 (9.8°C), t312 (10.9°C), t480 (13.9°C), t648 (10.9°C), t816 (12.9°C) and t1008 (hours after drug administration) (13.6°C). Saturation with oxygen did not fall below 80%. Blood samples were also taken from the experimental fish. Thereafter, the animals were euthanised *lege artis* by bleeding after having been stunned by a blow to the head. Following euthanasia, each fish was subjected to a parasitological examination and the inner organs (liver and muscle without skin) were removed for further analysis.

Fenbendazole and praziquantel measurement was performed using liquid chromatography coupled with triple quadrupole tandem mass spectrometry (LC-MS/MS). Homogenised tissue (0.5 g) or blood plasma (0.5 mL) was placed in a 10 mL glass tube and extracted for 5 min with 3 mL of methanol. The samples were then centrifuged at 800 × *g* for 10 min at 20°C, following which the extraction step was repeated with 2 mL of methanol. The combined supernatants were diluted with water (1/1; v/v) and subjected to solid phase extraction (SPE) using SPEC C₁₈ AR cartridges (3 mL, 30 mg; Varian Inc., Palo Alto, CA). Eight millilitres of diluted supernatant were passed through a preconditioned cartridge (500 µL methanol and 500 µL

water) and the analyte was eluted with 1 mL of 90% acetonitrile. This was used for LC-MS/MS analysis. The Thermo Scientific UHPLC Accela 1250 system was connected to the Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Instrument (Thermo, San Jose, CA, USA) equipped with a heated electrospray ionisation (HESI-II) probe. The Thermo Scientific Hypersil C₁₈ (2.1 mm × 50 mm, 1.9 μm) column was used at a constant flow rate of 300 μL.min⁻¹, along with a mobile phase consisting of water containing 0.1% formic acid (v/v) (solvent A) and acetonitrile (solvent B). We used a gradient of 0–5.5 min linear gradient from 20 to 100% B; 5.5–9.2 min held at 100% B; 9.2–9.7 min from 100 to 200% B and 9.7–10 min held at 20% B in order to allow the column to re-equilibrate before the next injection. The full loop injection volume of the sample was set at 10 μL. The heated electrospray ionisation was operated in positive mode under the following conditions: capillary temperature 325.0 °C; vaporiser temperature 300.0 °C; sheath gas pressure 35.0 psi; auxiliary (drying) gas 10 a.u.; and spray voltage 3,300 V. Standards were purchased from Sigma-Aldrich (St. Louis, MO). All solvents were analysed for residual purity (Chromservis s.r.o., Czech Republic). For our QA/QC program, the instrument was calibrated daily with multi-level calibration curves. In addition, a procedural blank and solvent blank were analysed for every set of 10 samples. Spiked recoveries for fenbendazole were 92% and 97% for praziquantel. Note that concentrations are reported after corrections based on the recoveries. Coefficients of variation for between-series were 7.6% for fenbendazole and 9.7% for praziquantel. Limits of detection, determined as 3:1 signal versus noise value (S/N), were 20 ng.kg⁻¹ for fenbendazole and 45 ng.kg⁻¹ for praziquantel.

RESULTS AND DISCUSSION

Concentrations of both praziquantel and fenbendazole reached their maximum within 24 h after administration in all samples tested, and dropped sharply over the subsequent 24 h (Table 1). Both antiparasitics were largely eliminated from plasma, liver and muscle in 30, 60 and 223 D°, respectively (Table 1). Praziquantel is known to be quickly absorbed and distributed in body tissue, with peak serum concentration in humans (but also in monkeys and rats) being achieved within 1–2 h after administering 50 mg.kg⁻¹ live weight (Leopold *et al.* 1978; Pearson & Guerrant, 1983). In fish, both praziquantel and fenbendazole are subject to intensive liver metabolism and they are largely metabolised and eliminated through the kidneys and bile, as in other vertebrates. The half-life of the unaltered substances has been calculated as around 1.5 hours, and around 4–6 hours for the metabolites (Pearson & Guerrant, 1983, King & Mahmoud, 1989). Fenbendazole is eliminated unaltered in mammals with the excrement. Cioli *et al.* (1995) reported that approximately 80% of total praziquantel was eliminated from a treated rat within 24 hours, and no traces of the preparation were found after 4–5 days. The fact that we still detected both substances in blood plasma after several days, and even in very small amounts (0.06 ng.kg⁻¹) at the end of experiment, may be explained by the experiment being conducted in recirculating tanks with relatively low water exchange. This probably caused a certain degree of circulation of the eliminated substances and their subsequent reabsorption by the organism.

Using of fenbendazole in rainbow trout was in both *in vivo* and *in vitro* studies without any apparent signs of toxicity (Tojo *et al.* 1992). Animal testing has shown very low praziquantel toxicity in both acute and long-term experiments (Frohberg, 1984), while mutagenic studies on bacterial, yeast and mammal systems have demonstrated no genotoxicity hazard (Cioli & Picamattocia, 2003). This is especially important for evaluat-

Tab. 1. Concentration of praziquantel (Praz) and fenbendazole (Fen) in liver, blood plasma and muscle tissue of rainbow trout (mean ± SD) in relation to the related degree days (D°) and time since start of experiment; (n = numbers of examined fish).

D°	Time [h]	Liver		Blood plasma		Muscle		n
		Praz [μg.kg ⁻¹]	Fen [μg.kg ⁻¹]	Praz [ng.mL ⁻¹]	Fen [ng.mL ⁻¹]	Praz [μg.kg ⁻¹]	Fen [μg.kg ⁻¹]	
20.24	t24	416.42±337.23	45.27±74.64	22.67±24.78	0.82±0.72	3.49±3.66	8.05±0.16	18
29.71	t48	9.11±2.09	7.64±0.70	0.15±0.16	0.71±0.36	0.88±0.12	1.82±0.58	18
59.37	t120	0.18±0.06	0.16±0.01	0±0.00	0±0.00	0.84±0.39	0.91±0.94	18
132.89	t312	0±0.00	0.07±0.05	0±0.00	0±0.00	0.48±0.10	0.58±0.03	18
223.79	t480	0±0.00	0.03±0.00	0±0.00	0.43±0.02	0±0.00	0.13±0.03	10
309.95	t648	0±0.00	0.10±0.03	0±0.00	0.09±0.06	0±0.00	0±0.00	8
400.77	t816	0±0.00	0±0.00	0±0.00	0±0.00	0.36±0.00	0±0.00	10
497.6	t1008	0±0.00	0±0.00	0.06±0.00	0.06±0.03	0±0.00	0±0.00	15

ing the occurrence of potential residues in food animals. Apart from the one exception where trace amounts of both substances were still found in blood plasma after t1008 (Table 1), both substances were completely degraded and eliminated from the body. By the end of the experiment, therefore, we had achieved a WHP corresponding to 497.6 D° (Table 1) and we can confirm that the legal WHP for market fish was met in this case.

Our use of highly sensitive LC equipment for the analysis of drug residues allowed us to detect even very small amounts of the xenobiotics in body fluids and tissue. The results confirm that both praziquantel and fenbendazole were quickly eliminated from the fish; hence, based on our present knowledge of their therapeutic effects, both substances appear suitable for therapeutic treatment of helminthoses as they both meet the requirements for the WHP as per Czech Act No. 166/1999 Coll. on Veterinary Care.

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