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Efficacy of five peroral therapeutics against *Ichthyophthirius multifiliis* infestation in salmonids

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Electronic Supplementary Material (ESM)

Preparation of fish tissue samples for liquid chromatography-mass spectrometry

Briefly, after thawing at room temperature, a 0.5 g portion of the homogenised sample was mixed with 20 µl of methacycline working solution (internal standard for DOX and PZQ, approx. 10 ppm) and 2.1 ml of HPLC-grade water. The sample was vortexed for 5 min at 2 500 rpm. Subsequently, 2.5 ml of extraction solution (1% formic acid in acetonitrile) was added and vortexed again (1 min at 2 500 rpm). Then, 0.3 g of NaCl and 1.0 g of $MgSO_4$ were added, followed by vigorous vortexing (1 min at 2 500 rpm) and centrifugation in a sealed tube (4 min at 1 500 × *g*). After centrifugation, 200 µl of the upper acetonitrile layer was transferred to a microvial for analysis. Each sample was prepared once and analysed in duplicate.

Water samples were thawed at room temperature, filtered through a 0.45 µm syringe filter, transferred to a microvial, and analysed directly. Detection was performed at a wavelength of 350 nm.

The calibration standards of doxycycline hyclate (DOX, purity > 95%) and praziquantel (PZQ, purity > 95%) were prepared at five calibration levels, and each calibration point was prepared once and analysed twice. Tissue samples (whole fish homogenate) from untreated fish were spiked with a reference standard solution of DOX and PZQ at the same final concentration of 50, 200, 500, 1 000 and 2 000 µg kg^{-1} (limit of detection, LOQ < 50 µg kg^{-1}). Water samples from aquaria of untreated fish were spiked with a standard solution of DOX and PZQ to yield concentrations of 2, 10, 50, 100, 200 µg l^{-1} (LOQ < 2 µg l^{-1}). Samples were prepared and analysed as described above.