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Determination of florfenicol and florfenicol amine in fish plasma (*Salmo salar*) through HPLC MS/MS

PLOS One

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MATERIALS

STEP MATERIALS

- ⊗ Water **Contributed by users**
- ⊗ Acetone **Contributed by users**
- ⊗ Dichloromethane **Bio Basic Inc. Catalog #DC3600.SIZE.1L**
- ⊗ Water **Contributed by users**
- ⊗ Acetoacetic acid **Contributed by users**
- ⊗ Water **Contributed by users**
- ⊗ Acetoacetic acid **Contributed by users**
- ⊗ Water **Contributed by users**
- ⊗ Methanol **Contributed by users**

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Sample preparation

- 1 Extraction was carried out according to the method described by Li *et al.* (2006), with modifications.
- 2 250 µL of each plasma sample were placed in 15 mL Falcon tubes and 500 µL Water **Contributed by users** and 2 mL Acetone **Contributed by users** were added.
- 3 The tubes were shaken for 00:10:00, sonicated for 00:10:00 and centrifuged at 5,000 rpm for 00:05:00
- 4 Subsequently, the resulting supernatant was transferred to another 15 mL Falcon tube containing 2 mL Dichloromethane **Bio Basic Inc. Catalog #DC3600.SIZE.1L**, and then shaken, sonicated and centrifuged at 5,000 rpm for the same amount of time as before
- 5 Then, the upper phase (Water **Contributed by users**) was discarded and the remaining phase evaporated at 40 °C to 50 °C under nitrogen flow.

- 6 Once completely dry, 1 mL of mobile phase and 1 mL hexane were used to reconstitute each sample, which then was shaken, sonicated and centrifuged at 5,000 rpm.
- 7 Finally, 300 µL of the lower phase were transferred to a vial to be injected into the .
- 8 The limit of detection (LOD) and the limit of quantification (LOQ) established for both analytes in plasma were 0.1 Parts per Million (PPM) and 0.2 Parts per Million (PPM) , respectively.

Instrumental Analysis

- 9 For instrumental analysis, an LC (Agilent, 1290 infinity series) coupled to a triple quadrupole mass spectrometer (API 5500, ABSCIEX) was used.
- 10 A Synergi™ 4 µm fusion RP 80Å 50 x 2.0 mm analytic column was used.
- 11 Analyst 1.6.3 and Multiquant 3.0 software was used for equipment management and integration, respectively.
- 12 Chromatographic separation was performed through a mobile phase using solvent A: 0.1% of Acetoacetic acid Contributed by users in Water Contributed by users ; and a mobile phase solvent B: 0.1% of Acetoacetic acid Contributed by users in

Water **Contributed by users**

/

Methanol **Contributed by users**

1:9 ratio; with a gradient flow of 350 $\mu\text{L min}^{-1}$ and a gradient elution from 32% up to 68% solvent A in 00:03:00 of 35% phase solvent A, and 75% phase solvent B.

13 Injection volume was 2 μL ; column oven temperature was set at 37 $^{\circ}\text{C}$.

14 Parameters of the MS/MS detector

Ionization	Electrospray (ESI)
Scan type	MRM
Source temperature (TEM)	550 $^{\circ}\text{C}$
Nebulizer (GS1)	60 psi
Turboion (GS2)	80 psi
Curtain gas (CUR)	20 psi
Collision gas (CAD)	10 psi
Ion-spray voltage (IS)	4500 V

15 Monitored ion masses

Analyte	Precursor ion (Q1 mass) (Da)	Fragment ion (Q3 mass) (Da)	Time (ms)	EP (V)	CE (V)	CXP (V)
FF1	356.0	336.0	100.0	-5,000	-15,000	-8,000
FF2	356.0	185.0	100.0	-5,000	-17,000	-12,000
FFA1	248.0	230.0	200.0	5,000	22,000	25,000
FFA2	248.0	130.0	200.0	2,000	30,000	10,000
CAF-d5 (IS)	326.0	157.0	100.0	10,000	-25,000	-20,000

Validation of analytical method

- 16 Prior to determining concentrations in plasma, the analytical method was validated by HPLC MS/MS, according to instructions from the European Commission Decision 2002/657/EC (2002).
- 17 Precursor ions and the two product ions were identified for florfenicol and florfenicol amine, respectively.
- 18 Values for essential parameters were estimated for the validation of the analytical method on plasma: specificity, recovery, repeatability, intralaboratory reproducibility, decision limit ($CC\alpha$), detection capability ($CC\beta$), and linearity.