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

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Working

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Betty San Martín[1], Lisette Lapierre[1], Marcela Fresno[1], Javiera Cornejo[1]

[1]Universidad de Chile

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Lisette Lapierre

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Martín BS, Fresno M, Cornejo J, Godoy M, Ibarra R, Vidal R, Araneda M, Anadón A, Lapierre L (2019) Optimization of florfenicol dose against *Piscirickettsia salmonis* in *Salmo salar* through PK/PD studies. PLoS ONE 14(5): e0215174. doi: 10.1371/journal.pone.0215174

STEPS MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Water				
Acetone				
Dichloromethane	DC3600.SIZE.1L	Bio Basic Inc.		
Water				
Acetoacetic acid				
Water				
Acetoacetic acid				
Water				
Methanol				

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

and **2 ml**

Acetone

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
by 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

) 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^{TM} 4 µm fusion RP 80Â 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

in

Water

; and a mobile phase solvent B: 0.1% of

Acetoacetic acid

in

Water

/

Methanol

1:9 ratio; with a gradient flow of **350 µl** 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 °C**.

14 **Parameters of the MS/MS detector**

Ionization	Electrospray (ESI)
Scan type	MRM
Source temperature (TEM)	550ºC
Nebulizer (GS1)	60 psi
Turbo ion (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 (CCα), detection capability (CCβ), and linearity.