AN HPLC-UV METHOD FOR FLORFENICOL BIOAVAILABILITY ASSESSMENT AFTER ORAL ADMINISTRATION IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

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Introduction/Aim: Aquaculture production has notably increased in a global level the last decades, mainly due to intensive fish farming. Inevitably, high density conditions, as those found in caged farming, trigger the spreading of microbial fish diseases. Thus, the demand for the use of effective veterinary drugs to combat bacterial pathogens has been increased. Florfenicol, a fluorinated derivative of thiamphenicol, is a broad-spectrum antibiotic used in veterinary medicine. This compound acts by preventing the peptide bond formation and is generally considered to be bacteriostatic antibiotic. Florfenicol has activity against a wide range of fish-pathogenic bacteria (Feng et al. 2008). Furthermore, it has been proven to be bioavailable and clinically effective in controlling fish pathogens for a variety of fish species including Atlantic salmon, rainbow trout, gilthead sea bream, cod, tilapia, etc (Feng et al. 2008). European sea bass is one of the most important warm-water farmed fish in the Mediterranean region. In order to design effective dosing schedules of florfenicol as a therapeutic in European sea bass, information on its pharmacokinetics in the particular species is needed. However, such information is not available in the pertinent literature for European sea bass. The aim of the present study was therefore to develop a reliable extraction procedure and an HPLC detection method to obtain information about the florfenicol kinetics in European sea bass tissues after oral administration.

Methods: Blood from healthy European sea bass was removed from the caudal vein. After plasma isolation, standard addition of florfenicol to isolates was followed. A multi-step extraction procedure was carried out to isolate the drug and an HPLC-UV method was developed to determine florfenicol levels in spiked-plasma samples. Briefly, 4.5mL of ethyl acetate-acetonitrile-ammonium hydroxide (49:49:2 v/v) were added to each plasma sample to precipitate proteins. The supernatant was removed and the extraction procedure was repeated at least three times. The extracts were evaporated to dryness, dissolved in a water-based solution, defatted, filtered and injected automatically on the HPLC column (C18, 4.6mm x 250mm, 5µm particle size). Quantification was carried out by constructing reference curves for florfenicol by means of standard solution.

Results: The standard curve for florfenicol determination was found to be linear over the range studied. These preliminary results indicate that orally administrated florfenicol is bioavailable in sea bass.

Main Conclusions: This study is the first attempt to obtain information on the pharmacokinetics of florfenicol administered orally to sea bass. The extraction and the chromatographic separation method are still in progress.

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References:

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