DEVELOPMENT OF SPE-HPLC/MS METHOD FOR AQUACULTURE ANTIBIOTICS DETERMINATION IN FISH FARMS

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INTRODUCTION
Antibiotics are widely used in fish farms to control bacterial infectious diseases and to limit fish mortality. The use of antibiotics in animal food production in general and also in aquaculture is one of the major concerns because there is evidence that their use may cause bacterial resistance. The common introduction of drug supplemented feeds and the lack of effluent treatments in fish farms provide opportunities for drugs to enter the environment. Some authors consider that the over prescription and the misuse of antimicrobials are the major cause of bacterial resistance in the aquaculture context and that the systematic treatment of fish farms effluents should be considered.

This work focuses on the application of SPE-HPLC-MS analysis of aquaculture antibiotics in fish farm.

HPLC-ESI(+)–MS
Determination of target analytes was performed on a HPLC interfaced with a mass spectrometer with atmosphere pressure electrospray ionization in positive mode (ESI+) (1200 LC/MS, Varian, USA).

Column: C18 embedded Synergy Fusion 4 µm, 150x2.0 mm (Phenomenex, USA)
Gradient elution: 0.01% formic acid in water (A) – 0.01% formic acid in acetonitrile (B)
Column temperature: 25 °C
Capillary voltage: 30 V

APPLICATION TO REAL WATER SAMPLE
The developed method was applied to the determination of target analytes in water of fish farm basen Crna Mlaka, Croatia. Before analysis, water samples were filtered through Buckner funnel, Black Whatman filtering paper and 0.45 µm nylon filter and acidified to pH 4 with HCl. The studied pharmaceuticals were extracted from water samples and pre-concentrated on 500 mg/3 mL Strata-X (Phenomenex, USA). Sample volume of 100 mL was applied to cartridges. The analytes retained were eluted with 2x5 mL of methanol. Filtrates were evaporated to dryness, dissolved in 1 mL of methanol and injected to HPLC system.

CONCLUSION
HPLC coupled to MS with ESI interface, operating in positive ionization mode, allowed simultaneous separation of seven pharmaceuticals often used on fish farms in food production. In fish farm water Crna Mlaka four of seven pharmaceuticals were detected: ciprofloxacin 2.17 µg/L; dexamethasone 2.96 µg/L; febantel 0.16 µg/L and oxytetracycline 0.86 µg/L.

ACKNOWLEDGEMENT
This work has been supported by Croatian Ministry of Science, Education and Sports Projects: 125-1253008-1350 Advanced analytical methods for pharmaceuticals determination in the environment and 125-2120898-3148 Croatian nomenclature in analytical chemistry and UKF: Reduction of environmental risks posed by pharmaceuticals and their degradation products in process wastewaters, through RO/NF membrane treatment (REPHAD).