METHOD DEVELOPMENT FOR THE ANALYSIS OF METFORMIN AND ITS METABOLITE GUANYLUREA IN BIOTA

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With more than 600 million defined daily doses the antidiabetic drug metformin is one of the most commonly prescribed pharmaceutical worldwide. In wastewater treatment plants (WWTPs) metformin is partially metabolized to guanylurea. Both compounds are often detected in WWTPs effluents and surface waters in the low µg/L range. Interestingly, metformin as well as guanylurea are still poorly investigated in terms of their ecological effects and uptake by aquatic organisms. In this study, an analytical methodology for the determination of the uptake of these pharmaceuticals in zebrafish embryos (*Danio rerio*) and brown trouts *(Salmo trutta f. fario*) was developed.

As metformin and guanylurea are strong bases and highly polar, routine analytical methods suffer from poor recovery during solid-phase extraction and insufficient retention during chromatographic separation. Thus, it was decided to use a method based on capillary electrophoresis coupled to mass spectrometry (CE-MS). The advantage of CE-MS is that the analytes' physico-chemical properties with a high charge already at intermediate pH can be utilized to achieve extreme selectivity of the method without the need for further sample clean-up steps.

For the developed method an extensive matrix effect evaluation was performed in order to achieve high selectivity and robustness with regard to the complex matrices present in the fish samples. Matrix problems were accounted via optimization of the background electrolyte (BGE). Robust analysis was achieved using a bare fused silica capillary with an applied voltage of 30 kV in a non-aqueous BGE. The extraction procedure was only based on sonication with methanol as extraction solvent. The absolute recovery was 95 % determined by using deuterated metformin as internal standard.

The method was validated with respect to specificity, linearity (10 nmol/L-10 µmol/L), limit of detection (6-8 nmol/L), accuracy and precision. Afterwards, the method was successfully applied to zebrafish embryos and brown trouts.