**A comprehensive LC-HRMS screening method to investigate biotransformation of polar chemicals in zebrafish embryos (*Danio rerio*) -clofibric acid as an example**

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The zebrafish embryo (ZFE) became a popular test organism in ecotoxicological studies. In these observed effects are often correlated to the (external) exposure concentrations. In order to better understand the extent of toxic effects of test compound it can be important to investigate the internal concentration e.g. the actual dose at the site of action.

For sensitivity reasons the determination of the test compound in the exposure solution as well as inside the ZFE is usually performed using triple-quadrupole-instruments [1]. However, the knowledge of the concentration-time profile of the parent compound is only one part of the story. Besides that the purity of the test compound in the exposure medium and its stability during the test have to be evaluated. Moreover, the metabolic transformation of the test compound inside the test organism has to be considered, as this would influence its internal concentration and the toxic effects on the ZFE.

Therefore we established screening method based on LC coupled to high-resolution mass spectrometry using state-of-the-art data evaluation to support ecotoxicological studies. This approach is exemplarily shown for clofibric acid in ZFE. Our investigations show that the phase I and II metabolism is already active at this early life-stage of the ZFE. Targeted and non-target screening approaches are compared.

Using this approach known and previously unknown phase I and phase II metabolites were found for clofibric acid in ZFE. Identification of biotransformation products is based on the exact masses, specific fragmentation and the isotopic pattern. Two of the numerous metabolites of clofibric acid that were detected and identified with this approach are especially interesting as these phase I metabolites have never been reported before in other biological or technical systems.

[1] Brox S, Ritter AP, Kuster E, Reemtsma T, *Anal Bioanal Chem* 406, **2014**, 4831-4840