Holistic approach for comprehensive xeno-metabolome coverage of Zebrafish embryos exposed to benzotriazoles, combining orthogonal chromatographic modes and Trapped- Ion-Mobility QTOF

ASMS 2020 WP 149

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Methods

The zebrafish embryo (ZFE) xeneicity assay was used to calculate the LC50 of BTs, as well as for phenotypic evaluation of their toxicity. A holistic analytical platform was developed combining orthogonal chromatographic modes (HILIC and RPLC), trapped ion mobility (TIMS) and HRMS for the analysis of parent BTs and the identification of their xenobiotics. ZFEs were extracted utilizing a 2-step extraction protocol with organic solvent mixtures (Methanol/Water and Dichloromethane/Water). Analysis of ZFE extracts was performed on UHPLC-QTOF and UHPLC-TIMS-QTOF for the chromatographic separation, an RPLC and a HILIC column were used. A “biotransformation oriented” data treatment workflow, consisting of suspect and non-target screening approaches, was developed.

Results

Bio-TPs arising from both oxidative (phase I) and conjugative (phase II) metabolic reactions were identified. Overall, 26 bio-TPs were identified through suspect and non-target screening workflows, while 22 are reported for the first time. The main categories are hydroxylated bio-TPs, sulfate conjugated and glucuronic acid conjugated bio-TPs. 4-MeBT demonstrated the highest toxicity potential and was more extensively biotransformed, compared to BT and 5-MeBT. Interestingly, bio-TPs were also detected in the exposure medium, highlighting the relevance of bio-TPs assessment in the aquatic environment. Finally, it was demonstrated that biotransformation data could be used complementary to the Cint of the parent BTs to interpret the induced toxicity.

Conclusions

➢ The power of combining different orthogonal separation modes (RPLC, HILIC, TIMS) with HRMS for the identification of unknown bio-TPs had been highlighted.
➢ A powerful biotransformation-oriented data processing workflow was developed.
➢ TIMS proved to be a very promising technique for the separation of positional isomers.
➢ A comprehensive xenometabolome coverage of aquatic organisms requires the use of holistic approaches that provide extensive analytical evidence and high-throughput identification.

Fig. 1: A. Sample Preparation Protocol

Fig. 2: Bio-transformation oriented data processing workflow (Suspect & Non-Target screening) for the identification of tentative bio-TPs.