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APPLICATION NOTE

LC-MS measurement of whole-body uptake of pharmaceuticals in zebrafish using the Bioruptor® Plus for sample preparation

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Introduction

The laboratory for Pharmaceutical Analysis at KU Leuven is developing innovative separation and detection techniques in pharmaceutical applications. Within the Department of Pharmaceutical and Pharmacological Sciences, the research group of Professor Deirdre Cabooter is specifically working on the implementation of different liquid chromatography (LC) based techniques for pharmaceutical and bio-analytical purposes. These techniques include 2D-LC and capillary LC hyphenated with mass spectrometry. Another emphasis is on the fundamental investigation of novel chromatographic supports and the development of generic separation strategies for complex samples.

Aim

Development of an analytical procedure to measure the whole-body uptake of pharmaceuticals in zebrafish (*Danio rerio*) for its further investigation as a potential ADME model.

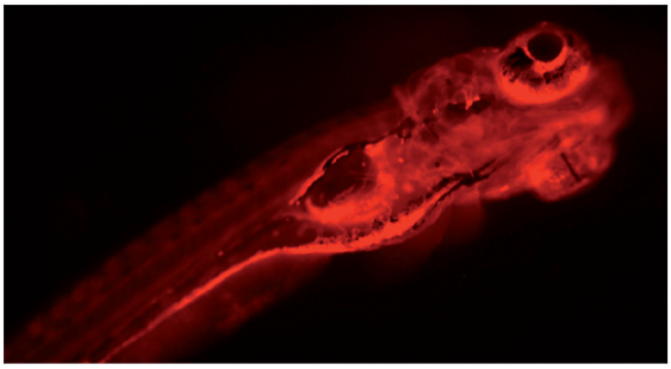

Figure 1: Zebrafish (*Danio rerio*) under fluorescence microscope.

Procedure

- Place 1 fish previously incubated with the compound of interest in a 1.5 ml tube.
- Add 270 µl of solvent with a composition of 1:2 (v/v) water:methanol together with 0.1% formic acid (extraction medium).
- Sonicate 6 samples at the same time using the 1.5 ml tube holder and the Bioruptor Plus for 30 cycles (30'' ON / 30'' OFF) to ensure the best homogenization of samples (high power input setting).
- Centrifuge samples for 15 min at 14.1 x 1000g.
- Remove 200 µl of supernatant and transfer it into a clean Eppendorf tube.

- Evaporate supernatant until dry in a vacuum oven for 45 min at 45°C.
- Reconstitute the evaporated sample in water containing a low percentage of acetonitrile.
- Analyze the obtained samples using ultra-high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC – MS/MS).[1]

The sample preparation procedure requires less than 3h to process 6 zebrafish samples making the whole process amenable to high-throughput screening.

Results

A correlation between compound absorption (µg compound absorbed/g dry body weight) and its lipophilicity (log D at pH= 7.6) was clearly observed in 10-day old zebrafish. While significant uptake was measured, low RSD-values (each experiment was repeated separately in three zebrafish) indicated good reproducibility of the method used.

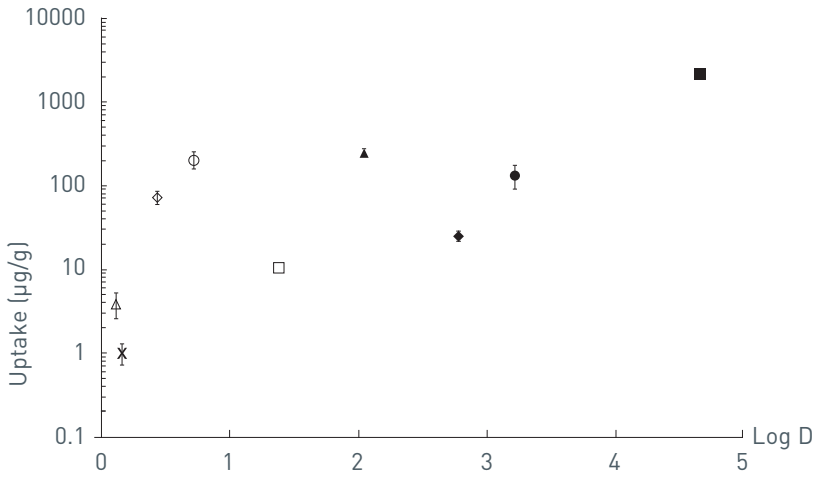

Figure 2: Dependency of the whole body uptake for 9 pharmaceutical compounds on their lipophilicity (Log D).

Conclusion

The obtained results demonstrate that immersion experiments performed on a single zebrafish processed with the Bioruptor Plus enable a high-throughput screening of compound uptake and a method to utilize zebrafish as a model for other ADME studies.

Publications

[1] Kislyuk, S.; Kroonen, J.; Adams, E.; Augustijns P.; de Witte P.; Cabooter D. **Development of a sensitive and quantitative UHPLC-MS/MS method to study the whole-body uptake of pharmaceuticals in zebrafish**, *Talanta 174 (2017) 780-788*. https://doi.org/10.1016/j.talanta.2017.06.075.