Zebrafish as a small-animal model for ADME studies: analytical challenges

Deirdre Cabooter, Stanislav Kislyuk, Wannes Van den Bosch, Erwin Adams, Peter de Witte, Patrick Augustijns

University of Leuven (KU Leuven), Department of Pharmaceutical and Pharmacological Sciences, Herestraat 49, 3000 Leuven, Belgium

The present study explores the potential of 10-day old zebrafish (*Danio rerio*) as a predictive model for ADME studies. For this purpose, an analytical method to measure the whole-body uptake of pharmaceuticals in zebrafish using state-of-the-art equipment is developed. 10-day old zebrafish are incubated with pharmaceuticals displaying a variety in physicochemical properties via the route of immersion at the maximum tolerated concentration, after which the zebrafish are homogenized and extracted using a powerful batch sonicator. Samples are then analyzed using ultra-high performance liquid chromatography (UHPLC) on a reversed-phase 2.1 mm I.D. column, coupled to a state-of-the-art Waters Xevo TQ-S mass spectrometer. Recovery, matrix effects, linearity and sensitivity are investigated for all compounds. It is demonstrated that the lower limits of quantification of the analytical method are so good, that a single zebrafish can be used to study the whole-body uptake of a particular drug. A clear correlation between lipophilicity and absorption of the drugs is observed in zebrafish using this methodology.

Subsequently, a similar methodology is used to study the uptake of pharmaceuticals in the brain of zebrafish and hence explore the potential of zebrafish as a predictive blood-brain-barrier model. For this purpose, a brain extraction procedure allowing to isolate the intact brain from the head of zebrafish larvae is developed. This brain extraction procedure is established for a zebrafish strain exhibiting red fluorescence of the brain, allowing to control the integrity of the extracted parts. To improve the sensitivity of the analysis further, the diameter of the analytical column is reduced to 300 µm I.D and quantitative experiments are carried out on pooled samples of six zebrafish (n=6). The selective semi-permeable nature of the blood-brain-barrier is demonstrated by measuring the uptake in the brain and trunk separately and the obtained results are discussed with regards to brain-to-plasma ratios typically obtained for more traditional murine models.