

Safety Assessment of Compounds after In Vitro Metabolic Conversion Using Zebrafish Eleuthero Embryos

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SCOPE OF THE METHOD

The Method relates to	Other: Safety Assessment of Compounds after In Vitro Metabolic Conversion
The Method is situated in	Translational - Applied Research
Type of method	In vivo
Used species	Zebrafish
Targeted organ system or type of research	General toxicity

DESCRIPTION

Method keywords

microsomes
zebrafish eleuthero embryos

Scientific area keywords

Drug discovery
toxicity
microsomes
metabolism

Method description

Zebrafish-based platforms have recently emerged as a useful tool for toxicity testing as they combine the advantages of *in vitro* and *in vivo* methodologies. Nevertheless, the capacity to metabolically convert xenobiotics by zebrafish eleuthero embryos is supposedly low. To circumvent this concern, a comprehensive methodology was developed wherein test compounds were first exposed *in vitro* to rat liver microsomes

(RLM) for 1 h at 37 °C. After adding methanol, the mixture was ultrasonicated, placed for 2 h at 20 °C, centrifuged and the supernatant evaporated. The pellet was resuspended in water for the quantification of the metabolic conversion and the detection of the presence of metabolites using ultra high performance liquid chromatography-Ultraviolet-Mass (UHPLC-UV-MS). Next, three days post fertilization (dpf) zebrafish eleuthero embryos were exposed to the metabolic mix diluted in Danieau's medium for 48 h at 28 °C, followed by a stereomicroscopic examination of the adverse effects induced, if any. The novelty of our method relies in the possibility to quantify the rate of the *in vitro* metabolism of the parent compound and to co-incubate three dpf larvae and the diluted metabolic mix for 48 h without inducing major toxic effects.

Lab equipment

- UHPLC system,
- Mass spectrometer,
- Stereomicroscope.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

It is uncertain whether zebrafish eleuthero embryos have a sufficient capacity to metabolically convert xenobiotics in an adequate way, and little is known regarding the identity of the metabolites formed. Since metabolism might increase or reduce the toxic profile of compounds dramatically, the use of eleuthero embryos might result in false negative or positive results. We successfully developed a platform combining the high-throughput of an *in vivo* zebrafish-based toxicity test with a mammalian pre-metabolism step.

Challenges

Although validating the platform with many more compounds is necessary to further confirm its applicability, we believe that the comprehensive methodology developed herein is promising in identifying compounds that are converted into toxic/non-toxic metabolites via phase I metabolism, thereby increasing the possibility to detect harmful chemicals in an early drug discovery phase.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

<https://doi.org/10.3390/ijms20071712>

Associated documents

[Safety Assessment of Compounds after In Vitro Metabolic Conversion Using Zebrafish Eleuthero Embryos.pdf](#)

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