Human in vitro liver metabolism using HLM, HLCYT and Liquid Chromatography coupled to High-Resolution Mass Spectrometry

Created on: 04-10-2019 - Last modified on: 08-11-2019

Contact person

Philippe Vervliet

Organisation

Name of the organisation University of Antwerp (UAntwerpen)

Department Department of Pharmaceutical Sciences

Country Belgium

Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Environment, Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	Human Liver Microsomes and Human Liver Cytosol

DESCRIPTION

Method keywords

HLM

HLCYT

Liquid chromatography

mass spectrometry

Metabolism

liver

in vitro

Scientific area keywords

Toxicology

analytical chemistry

liver metabolism

Drug metabolism

Drug discovery

Method description

A compound of interest (e.g. new psychoactive substance, endocrine disrupting compound, ...) is incubated with human liver microsomes and liver cytosolic fractions to generate both Phase I and II metabolites. Samples are prepared for analysis using a simple method in order to avoid possible losses of biotransformation products. The extracts are analysed using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Identification of the biotransformation products is performed using complementary screening workflows. These include a suspect screening based on *in silico* predictions and non-targeted screening using either vendor-specific or in-house developed open-source software protocols.

Lab equipment

- Warm water bath (37°C) ;
- Temperature-controlled nitrogen evaporator ;
- Centrifuge ;
- LC coupled to high-resolution mass spectrometry (for identification).

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Optimized assay with different timepoints, negative and positive controls and method blanks ;
- Tested for a variety of substrates (NPSs, EDCs, ...) resulting in multiple publications ;
- Custom data analysis possible, according to research question ;
- Besides analytical equipment (LC-HRMS) no need for expensive equipment.

Challenges

- Possible over or underestimation of *in vivo* biotransformation ;
- Suspect screening dependent on strength of *in silico* predictions.

Modifications

- No further optimizations are planned for the near future.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Associated documents

2018 - Vervliet Mortele et al - DTA - 5Cl-THJ-018.pdf

2019 - Vervliet - Toxicology - HLM DEMO.pdf