Determination of CYP activity in liver organoids with LC-MS

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Introduction

The use of animal testing in pharmaceutical drug development comes with biological and ethical issues that need to be approached. New and better methods and models are needed. Microsomes are the golden standard for drug metabolism studies, and especially liver microsomes because the liver is the main site for drug metabolism. Unfortunately, they lack organ complexity. A new model with the potential for patient specific drug development and treatment, are stem cell derived 3D tissue models called organoids. They have the complexity of an organ but organoid development is still in its infancy, thus there are need for characterisation.

Liver

- Main site for drug metabolism.
- Most drugs are metabolized by CYP enzymes.

Microsomes

- The golden standard in drug metabolism studies.
- Extracted from homogenous liver tissue by centrifugation and fractionation.
- Vesicles that contain CYP enzymes.
- Lack of organ complexity [1].

Organoids

- Potential new in vitro model [2].
- More complex 3D tissue model, mini-organ.
- Derived from patient stem cells.
- Potential for patient specific drug development.
- Need for characterisation.

Aim

Develop a methodology for measuring drugs and metabolites that are telltale for CYP activity in liver organoids.

Method

A method has been established for the determination of CYP activity in liver microsomes by measuring the concentration of three well studied drugs and their metabolites after incubation. A phase 1 metabolism reaction that converts the hydrophobic drug into a more polar metabolite occurs by introducing or exposing a functional group (e.g. -OH). This reaction is done by CYP enzymes and catalysed by NADPH.

1. Sample solution

NADPH regenerating system, drugs, and microsomes were added to a phosphate buffer with a pH of 7.4.

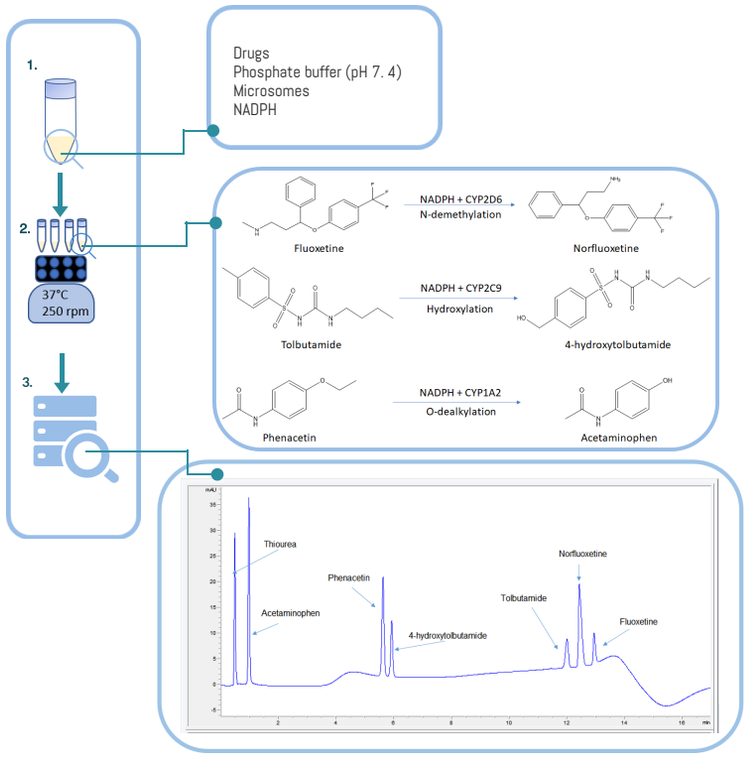
2. Incubation

The microsome samples were incubated in a thermoshaker for 0-4 hours, 37°C, and 250 rpm, to achieve metabolism.

3. HPLC-UV/HPLC-MS

A HPLC-UV system with a reversed phase C18 column (2.1x50 mm) have been used to determine the drug and metabolite concentrations, before a triple quadrupole MS are to be used as a detector in future analyses.

Drug	Metabolite	Group
Fluoxetine	Norfluoxetine	Antidepressant
Tolbutamide	4-hydroxytolbutamide	Blod sugar regulant
Phenacetin	Acetaminophen (Paracetamol)	Antiinflammatory



Results & future work

Metabolism of all three drugs, and hence CYP activity was found using microsomes. The LC-UV method will be further developed using MS, and will be used to determine CYP activity in liver organoids.

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