

Metabolic Stability in Rat or Human Hepatocytes

Purpose

The metabolic stability of a drug candidate is an important parameter for its use as a therapeutic agent especially for drugs given perorally. Perorally applied drug undergo first-pass metabolism which influences the pharmacokinetic properties such as the clearance rate, the half-life or the bioavailability. To determine, whether a compound is metabolized by hepatic drug metabolizing enzymes, Pharmacelsus uses freshly isolated rat hepatocytes, cryopreserved rat hepatocytes or cryopreserved human hepatocytes in culture as test models.

Isolation of rat hepatocytes

Hepatocytes are isolated from male rats by an *in situ* enzymatic perfusion. After the collagenase perfusion, the liver is transferred into medium and the liver cells are freed of connective-vascular tissue. To achieve high purity, the cell suspension is centrifuged and the supernatant with non-parenchymal dead cells and residual erythrocytes is discarded. The pellet is resuspended in growth medium and the cells are seeded on collagen-coated well plates. The cell viability is determined by trypan blue exclusion staining.

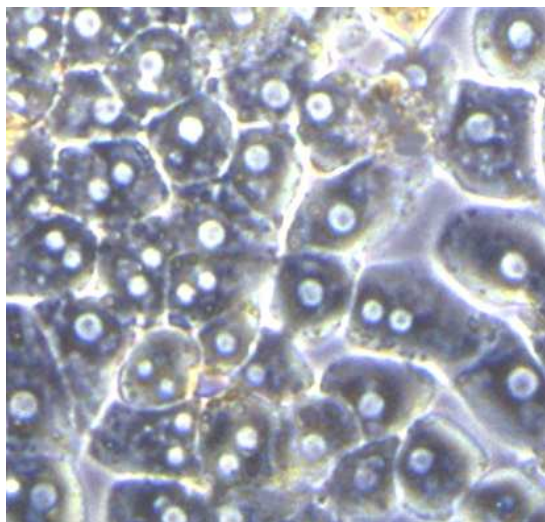


Figure 1: Isolated rat hepatocytes in culture

Assay protocol

Hepatocytes (rat or human) are incubated with the test compound for an appropriate time period. At different time points, aliquots of the culture medium are removed and precipitated with acetonitril. After centrifugation, the disappearance of the parent compound is determined by LC-MS/MS.

- **METABOLIC STABILITY**
Metabolic stability is determined by the concentration of the parent compound in the medium over time. long
- **DRUG UPTAKE**
The rate of drug uptake is given by the levels of the compound measured in the cellular fraction. The potency of drug uptake inhibitors can also be assessed by using this model.
- **HEPATOTOXIC EFFECT OF DRUGS**
Damage of hepatocytes in the presence of drugs can be determined by light microscopy, trypan blue exclusion test or by the lactate dehydrogenase leakage which indicates damages of membrane integrity. Viability can be determined using MTT/XTT or by the determination of the oxygen uptake of the cells using oxygen-sensor plates.

Metabolic Stability in Rat or Human Hepatocytes

Model validation

Metabolic stability of one test compound incubated for 24h on cultivated rat hepatocytes.

Calculated half-life values	100 µg/ml	$t_{1/2} = 6.1\text{h}$
	50 µg/ml	$t_{1/2} = 6.0\text{h}$
	20 µg/ml	$t_{1/2} = 6.7\text{h}$

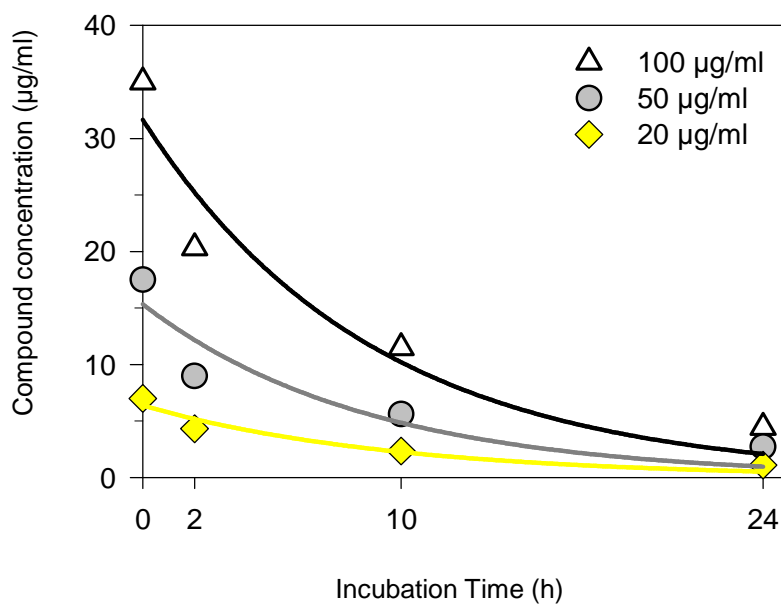


Figure 2: Metabolic stability of test compound at different concentrations.

Please don't hesitate to contact us for a customized quotation

Dr. Ursula Mueller-Vieira
Head of ADME & *in vitro* Pharmacology
Tel: +49 681 3946-7521
mueller@pharmacelsus.de
www.pharmacelsus.de