

## Introduction

Metabolic stability plays a major role in drug clearance, with the liver being the primary site for drug biotransformation via two major enzymatic reactions:

- Phase I (modifications to the molecular structure itself) and
- Phase II reactions (conjugation reactions)

The metabolic stability of compounds is commonly investigated early on in the drug discovery process using various *in vitro* test systems, such as liver microsomes, S9 fraction and hepatocytes.

Among these *in vitro* test systems hepatocytes are considered to be the golden standard for investigating metabolic stability as they represent an independent *in vitro* cellular test system, containing both Phase I and Phase II enzymes, as well as all cofactors and drug transporters.

However, the use of hepatocytes can be quite laborious, and both time and money consuming.

## Objectives

The aim was to develop a quick and high-throughput approach for metabolic stability screening in hepatocytes.

## Materials and Methods

A total of 600 compounds (200 compounds per species) were tested in human, rat and mouse hepatocytes.

All tested compounds were incubated for 3h at 37°C in the hepatocytes resuspended at a density of  $0.5 \times 10^6$  cells/mL in modified Krebs–Henseleit buffer. Aliquots were taken at several different time points (0, 30, 60, 90, 120, 150 and 180 min) and reaction was terminated by addition of acetonitrile/methanol (2:1) mixture, containing internal standard. After centrifugation (at 4000 rpm, at 4°C, for 10 min) of collected aliquots, the resulting supernatants were subjected to LC-MS/MS analysis.

The *in vitro* half-life ( $t_{1/2}$ ) was in GraphPadPrism software from % remaining vs. time regression using non-linear regression fit. The hepatic *in vitro* intrinsic clearance (mL/min/g liver) was determined from the *in vitro* half-life ( $t_{1/2}$ ), normalized to the cell concentration in the incubation mixture and assuming hepatocellularity number of  $120 \times 10^6$  cells/g of liver.

This value was further scaled to predicted *in vivo* hepatic clearance and % of liver blood flow, enabling classification of all compounds as either low, moderate or highly stable drugs.

A correlation analysis of the percent remaining at 3 different time-points (45, 90 and 120min) was conducted, in order to evaluate the time-point with best predictivity of *in vitro* clearance and predicted *in vivo* hepatic clearance.

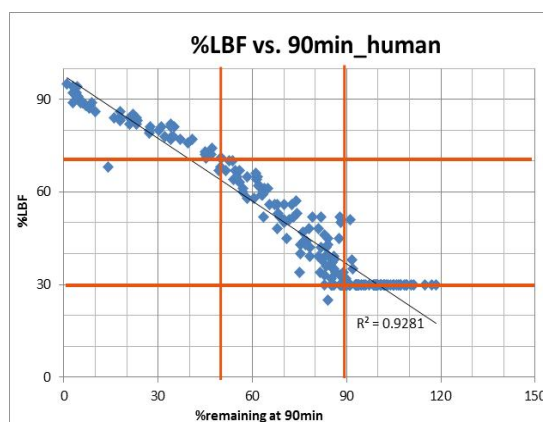
## Results

Among the three different time-points investigated, our results suggest that the 90 min time-point gives the best predictivity ( $R^2 > 0.9$ ) in all three tested species, i.e. the most accurate ranking of tested compounds.

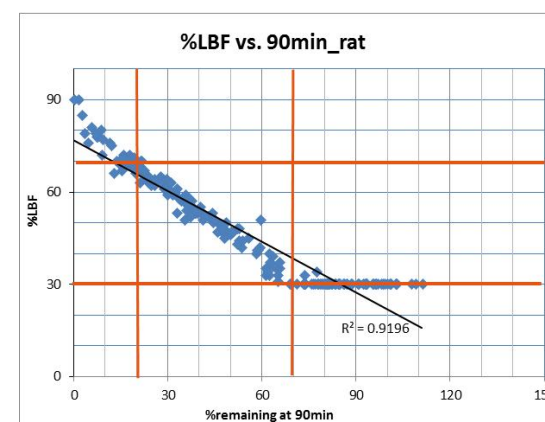
Very similar results were obtained for the 120 min time-point, while overlaps were seen at the 45 min time-point.

Due to species differences, a different range of % remaining values was determined for each species at the 90min time-point, corresponding to the low, moderate and high stability classification group.

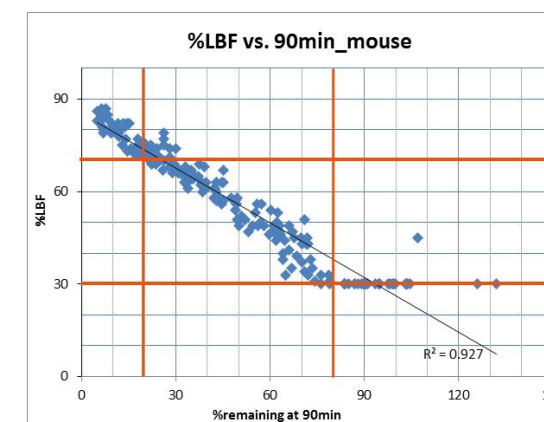
### Human hepatocytes



### Rat hepatocytes



### Mouse hepatocytes



Species	%remaining at 90min	Classification
HUMAN	<50	LOW
	50-90	MODERATE
	>90	HIGH
RAT	<20	LOW
	20-70	MODERATE
	>70	HIGH
MOUSE	<20	LOW
	20-80	MODERATE
	>80	HIGH

## Conclusion

A good correlation found for the 90 minute time point and an accurate ranking of the majority of tested compounds favors the routine use of the two time-point approach for high-throughput ADME screening of hepatic stability of new compounds.

In addition, the high-throughput approach presented here is both time- and cost effective as the following improvements have been achieved:

- a significantly higher number of compounds can be tested per vial of hepatocytes
- the analytical time per compound is shortened
- an overall decrease of assay cost per compound

## Acknowledgement

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## References

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- 2) Riley RJ and Girme K, Drug Discovery Today: Technologies, 2004, 1:365-372

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