

# Low Clearance Hepatocyte Stability Assay

## Background Information



'Optimization of clearance is one of the more significant challenges for a drug discovery project. Identification of the rate in preclinical species and optimization in human are major goals in most projects.'

<sup>1</sup>Grime KH, Barton P & McGinnity DF (2013) *Mol Pharm* **10**; 1191-1206

- Reducing the metabolic clearance of new chemical entities is a common goal in drug discovery projects in order to reduce dose, improve exposure and prolong the half-life. However, accurately predicting the clearance of stable compounds is challenging using standard *in vitro* suspension methods.
- Prolonged incubation times are restricted using suspended primary hepatocytes due to activity and viability issues. This can lead to inaccuracies in the intrinsic clearance values.
- New methods are being developed to address this concern through extension of the incubation time, which in turn is able to provide a more accurate estimation of the intrinsic clearance.
- Through its parent company, Evotec, Cyprotex are able to offer a low clearance method which utilises plated primary human hepatocytes, and matrix overlay to extend the time course for up to 5 days.

### Protocol

#### Cells

Primary human hepatocytes

#### Test Compound Concentration

1  $\mu$ M (different concentrations available)

#### Overlay Matrix

Geltrex<sup>®</sup>

#### Incubation Time

0, 1, 2, 4, 8, 22, 26, 30 h

#### Replicates

n=2

#### Compounds Requirements

20  $\mu$ L of 10 mM solution

#### Analysis Method

LC-MS/MS quantification

#### Assay Controls

Disopyramide (low clearance)  
Metoprolol (moderate clearance)  
Sildenafil (high clearance)

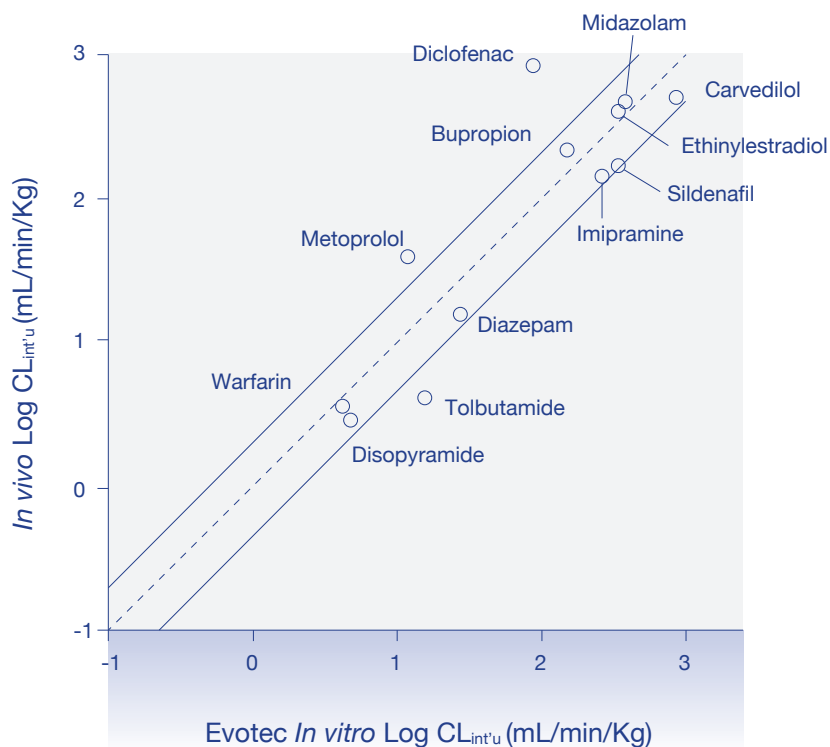
#### Data Delivery

Intrinsic clearance  
Half life

**Table 1**

Comparison of human *in vitro* intrinsic clearance data generated by Evotec (Cyprotex's parent company) and a publication by Bonn *et al* 2016<sup>2</sup> where plated human hepatocytes and a co-culture model were used.

	Ion Class	Major Drug Metabolising Enzyme	Bonn <i>et al.</i> , 2016 PHH CL <sub>int</sub> (μL/min/10 <sup>6</sup> cells)	Bonn <i>et al.</i> , 2016 Hurel CL <sub>int</sub> (μL/min/10 <sup>6</sup> cells)	Evotec CL <sub>int</sub> (μL/min/10 <sup>6</sup> cells)
<b>Bupropion</b>	Base	CYP2B6, CYP1A2, CYP2A6, CYP3A4, CYP2E1	Not reported	Not reported	5.4
<b>Carvedilol</b>	Base	CYP2D6, CYP2C9	26.3	34.2	14.5
<b>Diazepam</b>	Neutral	CYP2C19, CYP3A4	0.8	1.3	0.7
<b>Diclofenac</b>	Acid	CYP2C9, UGT2B7	Not reported	Not reported	4.7
<b>Disopyramide</b>	Base	CYP3A4	0.2	0.4	0.1
<b>Ethinylestradiol</b>	Acid	UGT1A1, CYP3A4	Not reported	Not reported	3.3
<b>Imipramine</b>	Base	CYP1A2, CYP2C19, CYP2D6	8.6	1.7	8.5
<b>Metoprolol</b>	Base	CYP2D6, CYP3A4	2.2	0.8	0.9
<b>Midazolam</b>	Neutral	CYP3A4	Not reported	Not reported	5.1
<b>Sildenafil</b>	Base	CYP3A4, CYP2C9, CYP2C19	7.0	6.2	9.0
<b>Tolbutamide</b>	Acid	CYP2C9	Not reported	Not reported	0.8
<b>Warfarin</b>	Neutral	CYP2C9, CYP3A4	BLQ	0.7	0.3

**Figure 1**

Correlation of scaled *in vitro* human intrinsic clearance (using Evotec's low clearance model) with *in vivo* human intrinsic clearance for a set of 12 known drugs.

The data generated by Evotec is consistent to those reported by Bonn *et al.*, 2016 as illustrated in Table 1. Further, the scaled *in vitro* human intrinsic clearance data from the Evotec model demonstrates a strong correlation with *in vivo* human intrinsic clearance showing the advantages of this approach as illustrated in Figure 1.

**References**

- Grime KH *et al.*, (2013) Application of *in silico*, *in vitro* and preclinical pharmacokinetic data for the effective and efficient prediction of human pharmacokinetics. *Mol Pharm* **10**(4); 1191-1206
- Bonn B *et al.*, (2016) Determination of human hepatocyte intrinsic clearance for slowly metabolised compounds: Comparison of a primary hepatocyte/stromal cell co-culture with plated primary hepatocytes and HepaRG. *Drug Metab Dispos* **44**: 527-533