cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

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.'

[1]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 µM (different concentrations available)

Overlay Matrix
Geltrex®

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

Replicates
n=2

Compounds Requirements
20 µL 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

To find out more contact **enquiries@cyprotex.com**

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[2] where plated human hepatocytes and a co-culture model were used.

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

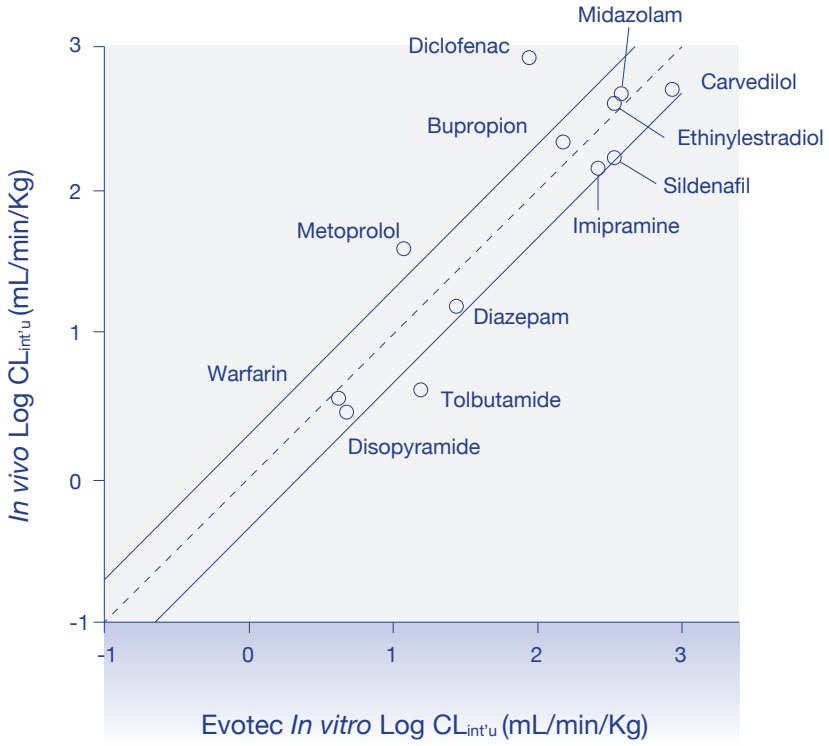

Figure 1

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

[1] 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

[2] 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