cyprotex AN EVOTEC COMPANY

In vitro **ADME & PK**

Reaction Phenotyping

Background Information

'If glucuronidation is a predominant pathway of drug elimination, *in vitro* studies to determine whether the drug is a substrate of UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, or 2B15 are recommended.'

[1]FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (February 2012)

- Cyprotex's Reaction Phenotyping assay uses expressed enzymes to identify which drug metabolising isoforms are responsible for the metabolism of a test compound.
- Certain enzymes (e.g. CYP450s) can be induced or exhibit polymorphisms which can greatly affect plasma drug levels *in vivo*. This evaluation should be conducted in the early stages of the drug development process to avoid costly late-stage attrition.
- Identification of the enzyme(s) responsible for drug metabolism provides insight into potential drug-drug interactions.

Protocol

Typical Test Article Concentration
5 µM (different concentrations available)

CYP Isoforms
CYP1A2,CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and UGT2B15 (others available on request)

Time Points
0, 5, 15, 30, 45 minutes

Number of Replicates
n = 1 per time point

Negative Controls
Without NADPH (45 minutes only)

cDNA expressed control preparation (no CYP450 or UGT enzyme present)

Positive Control
Dependent on number of isoforms requested

Analysis Method
LC-MS/MS

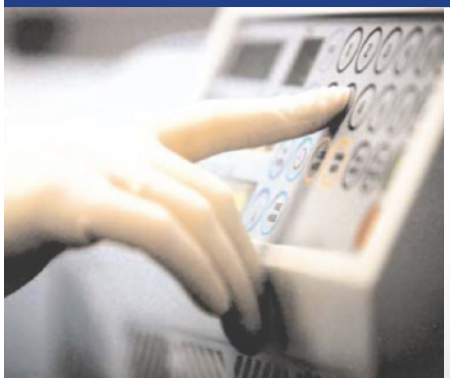
Data Delivery
Parent compound remaining at each time point for each isoform

Half life

Standard error of half life

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

Understanding which of the cytochrome P450 and uridine diphosphate glucuronosyl transferase enzymes are involved in the metabolism of a drug is important in predicting the propensity towards inter individual variability due to polymorphisms in enzyme expression and the tendency for drug-drug interactions.



Reaction Phenotyping

Known substrates for the respective cytochrome P450 and uridine diphosphate glucuronosyl transferase enzyme were screened in Cyprotex's Reaction Phenotyping Assay. For the validation, the substrates were incubated with recombinant enzyme (in the presence of NADPH for the CYP450 isoforms).

Figure 1

The graph shows the percentage of parent compound remaining after incubation of probe substrates with individual cytochrome P450 isoforms. The error bars represent the standard deviation from 4 separate experiments.

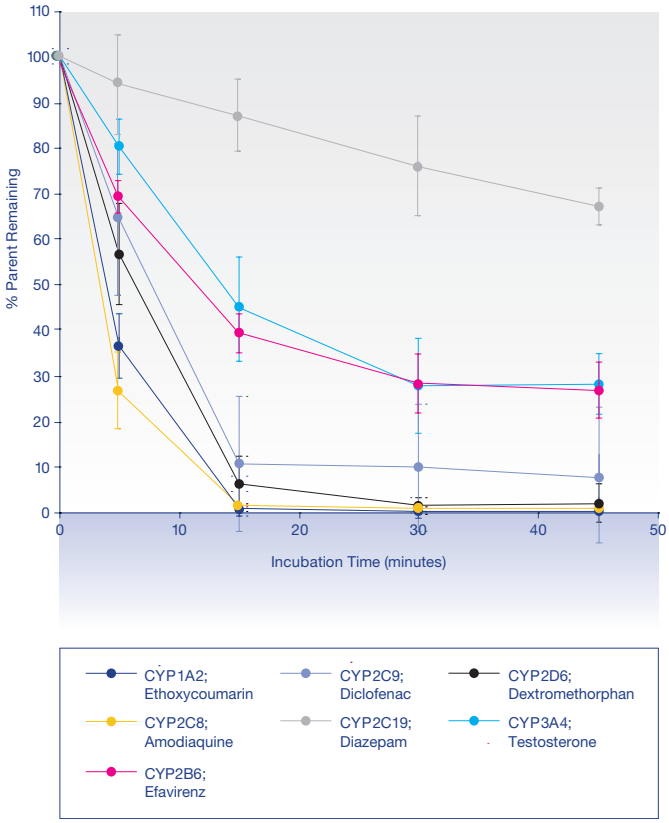
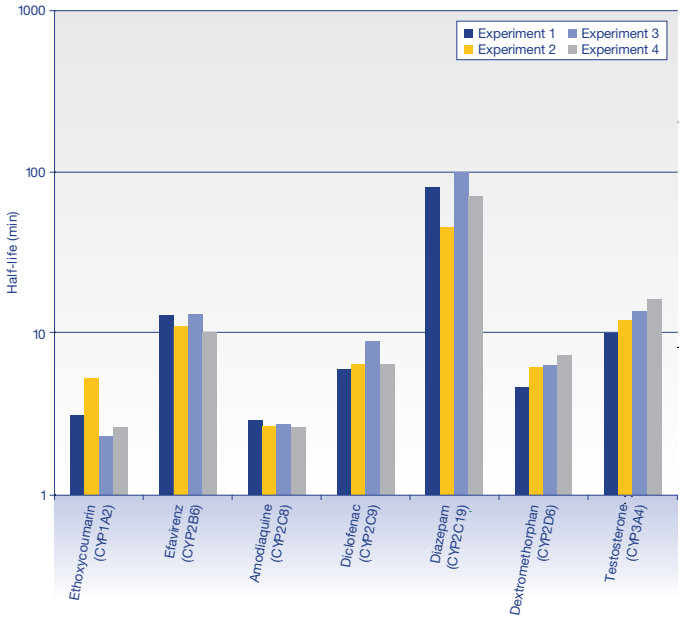


Figure 2

Reproducibility of half life determination for the positive control compounds. The graph shows the half life determination after incubation of probe substrates with individual cytochrome P450 isoforms over 4 separate experiments.



References

[1] FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations (February 2012)