

Cloe Screen Cytochrome P450 Reaction Phenotyping

cyprotexexperts in **ADME**

Background Information



'If suitable *in vitro* studies at therapeutic concentrations indicate that CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A enzyme systems do not metabolize an investigational drug, then clinical studies to evaluate the effect of CYP2D6 inhibitors or CYP1A2, CYP2C8, CYP2C9, CYP2C19, or CYP3A inhibitors / inducers on the elimination of the investigational drug will not be needed.'

FDA Draft Guidance for Industry - Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling (September 2006).

- Cloe Screen Cytochrome P450 Reaction Phenotyping assay uses expressed enzymes to identify which of the main cytochromes P450 (CYP450) drug metabolising isoforms are responsible for the metabolism of a test compound.
- Certain CYP450s can be induced or exhibit polymorphisms which can greatly affect plasma levels *in vivo* therefore it is important to identify if this is expected to be a problem at an early stage.
- By identifying the CYP450 responsible for the metabolism, it provides direction in identifying potential drug-drug interactions.

Protocol

Test Compound Concentration
5 μ M (different concentrations available)

CYP Isoforms
CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4
Bactosomes or Supersomes

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

Number of Replicates
n = 1 per time point

Negative Controls
Without NADPH (45 minutes only)
Control Bactosomes/Supersomes (no CYP450 enzyme present)

Positive Control
Known probe substrate

Compound Requirements
250 μ L of a 10 mM solution

Analysis method
LC-MS/MS

Data Delivery
Parent compound remaining at each time point for each isoform
Half life
Standard error of half life

Understanding which cytochrome P450 isoform is 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.



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7 known substrates for the respective cytochrome P450 isoforms were screened in the Cloe Screen Cytochrome P450 Reaction Phenotyping assay. For the validation, the substrates were incubated with Bactosomes™ (bacterial membranes containing human cytochrome P450s coexpressed with human NADPH-cytochrome P450 reductase) in the presence of NADPH.

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.

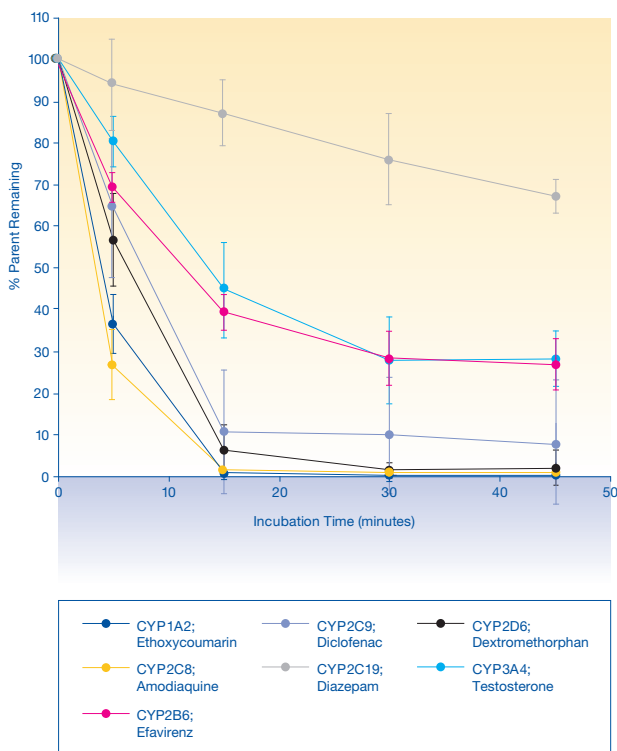


Figure 2

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.

