XenoTech
A BioIVT Company

XenoTech's reaction phenotyping services provide insight into drug elimination pathways.

Knowing how a drug candidate is eliminated by the human body is important in understanding the potential for drug-drug interactions with co-administered compounds. Compounds with a single route of elimination have a high victim potential, which is why the FDA requires reaction phenotyping studies. Regulatory agencies suggest elucidation of enzymes involved in the metabolism of a drug when a clearance pathway constitutes larger than 25% of the total clearance of a drug. These studies generally involve multiple approaches: correlation analysis, antibody or chemical inhibition and metabolism by recombinant human enzymes. Each approach has its advantages and disadvantages, and a combination of approaches is highly recommended.

Reaction phenotyping may be performed based on metabolite formation (quantify the formation of the metabolite) or based on substrate depletion (quantify the substance disappearance). XenoTech currently offers reaction phenotyping studies for both CYP & UGT enzymes.

Test System

Microsomes are the test system of choice for CYP & UGT reaction phenotyping studies. Reactions catalyzed by CYP enzymes such as hydroxylation and/or dehydrogenation require NADPH as a cofactor while reactions catalyzed by UGT enzymes require UDPGA as a cofactor.

Analytical Procedure

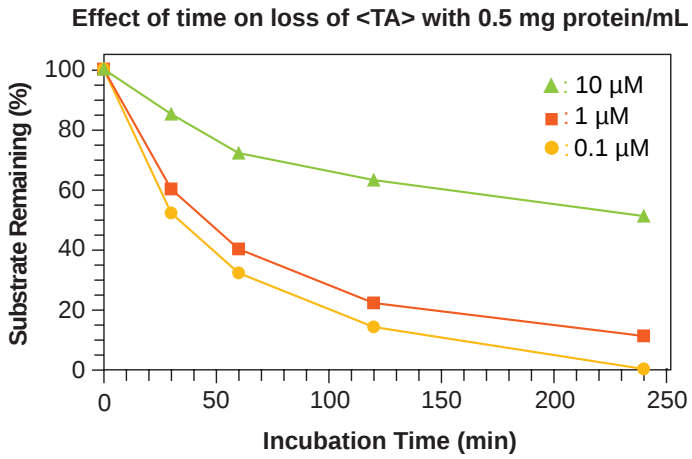
XenoTech develops an analytical procedure to measure the metabolite formation rate &/or the rate of substrate disappearance. Conditions are optimized to maxmize the detection of the analyte.

Time, Protein and Substrate Concentration (Preliminary Evaluation)

Before proceeding with reaction phenotyping of the compound, an experiment is conducted to evaluate the effect of incubation time, protein concentration and substrate concentration on the metabolism of the compound. The experiment establishes whether the metabolite formation or the substrate depletion is proportional to incubation time and protein concentration and helps to establish initial rate condition.

Figure 1

Effect of incubation time and protein concentration on the loss of <TA> (0.1, 1 and 10 µM) from incubations with NADPH-fortified human liver microsomes



<TA> = Test Article

Kinetic Values

After the establishment of initial rate conditions (conditions under which metabolite formation is proportional to incubation time and protein concentration, and the metabolism of the substrate does not exceed 20%, ideally <10%), an experiment is performed to determine Michaelis-Menten kinetic constants, namely K_m and V_{max}. These constants are determined by incubating microsomes with a range of substrate concentrations, typically 0.1 to 10 times the crude K_m value estimated in the preliminary evaluation. K_m and V_{max} provide useful information to assess the importance of specific metabolic pathways of the compound.

Correlation Analysis

Correlation analysis involves determining the rate of drug metabolism, followed by correlating reaction rates with the sample-to-sample variation of CYP (11 CYPs) or UGT (7 UGTs) enzyme activity in individual samples. This approach is successful when the variation in CYP or UGT enzyme activity from sample-to-sample varies greatly and independently from one enzyme to the other.

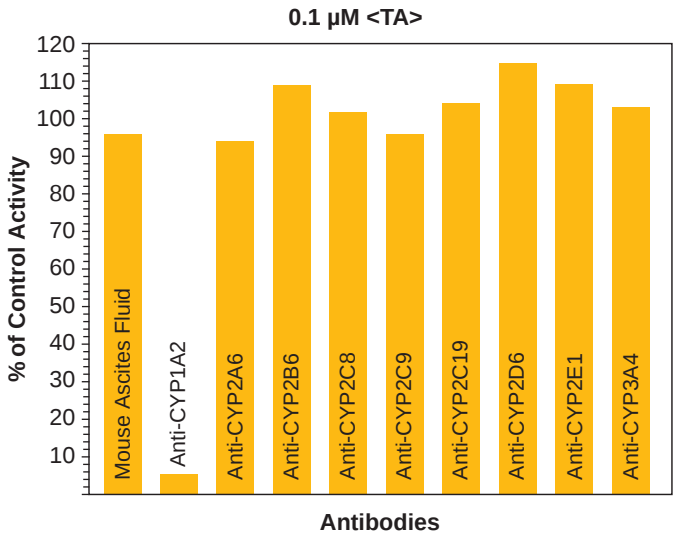
Antibody & Chemical Inhibition

Antibody and chemical inhibition experiments involve an evaluation of the effect of selective CYP & UGT inhibition on the metabolism of a drug in pooled human liver microsomes. Chemical inhibitors can be non-selective when used at inappropriate concentrations; XenoTech has extensive experience designing these experiments and avoiding such problems. The concern arising with chemical inhibitors is not a factor with those antibodies that have shown to be selective CYP inhibitors.

**Antibodies are currently available for CYP enzymes but not for UGT enzymes*

Figure 2

Effect of monoclonal antibodies on the disappearance of <TA> (0.1 and 1 µM) with human liver microsomes



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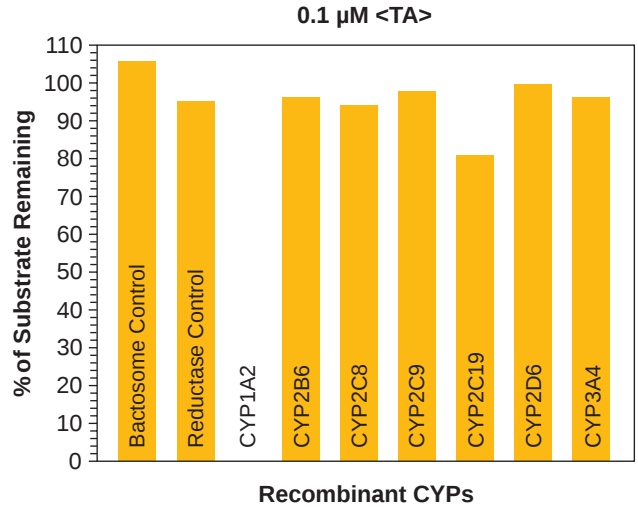
Recombinant Human Enzymes

Recombinant human CYP / UGT enzymes are incubated individually with a test compound to assess the ability of a particular CYP / UGT enzyme to metabolize it. Additional experiments may be carried out to allow determination of K_m and V_{max} for each CYP / UGT enzyme that contributes to the reaction.

Figure 3

Metabolism of <TA> in incubation with a panel of recombinant human CYP enzymes



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UGT Reaction Phenotyping

The approaches outlined above are applicable to UGT Reaction phenotyping studies. Metabolism can be carried out by recombinant human UGT enzymes and chemical inhibition (selective chemical inhibitors are not known for certain UGT enzymes). Correlation analysis is also available upon request.

XenoTech offers the below UGT's in their reaction phenotyping services:

Recombinant UGTs

UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15, 2B17

XenoTech's Reaction Phenotyping Kit is characterized for the above UGT enzyme activities (Product ID: H0500)

Contact us to learn more at **www.xenotech.com** or call **913.438.7450**.