LC-MS/MS based Cytochrome P450 Inhibition Assay (Panel of 5 or 7 CYP450)

Background: Cytochrome P450 (CYP) enzymes represent a heme containing protein superfamily metabolizing a broad variety of xenobiotics, including drugs and toxic chemicals. 11 CYP families are expressed in a human liver and gastrointestinal tract (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9/18/19, CYP2D6, CYP2E1, and CYP3A4/5). Five major isoforms (CYPs 1A2, 3A4, 2C9, 2C19, and 2D6) are involved in about 95% of the known drug metabolism. Recent studies showed the increasing role of CYP2C8 and CYP2B6 in metabolism of numerous drugs and important endogenous compounds. Cytochrome P450s are of critical importance due to the two of the most significant problems in clinical pharmacology: metabolism-mediated drug-drug interactions (DDI) and individual variability in drug metabolism. It is important to evaluate the potential inhibition of a new drug candidate for the most clinically relevant CYP450 enzymes. CYP450 inhibition may potentially lead to elevated in vivo plasma levels of a co-administered drug metabolized by the inhibited enzyme, and, consequently, to adverse drug reactions and toxicity. Assessment of the following CYP450 enzymes inhibition by a new drug candidate is recommended by FDA[1] and EMA[2-3]: CYP3A4, CYP2C9, CYP2C19, CYP2D6, CYP1A2, CYP2C8 and CYP2B6.

Service Details: The potential inhibition of 7 major cytochromes CYP1A2, CYP3A4, CYP2C9, CYP2C8, CYP2C19, CYP2D6, and CYP2B6 is assessed using LC-MS/MS based assay, in which biotransformations of the CYP450 specific substrates are used as markers to quantify the enzymatic activity. The FDA recommended CYP-selective substrates are used in the assay, which represent well characterized currently or previously marketed drugs. Quantification of a decrease in the formation of a metabolite in the presence of an inhibitor compared to the vehicle control is used to determine the CYP450 inhibition. Due to high specificity of the substrates to CYP450 isoforms not only individually expressed human cytochromes can be used in the assay but also human liver microsomes comprising a full set of CYP450 enzymes. The human liver microsomes assay is more comparable to the in vivo processes occurred in the liver and it is considered as ‘‘gold standard’’ for in vitro drug-drug interaction evaluation. This testing system is accepted for regulatory in vitro DDI studies.

CYP450	Substrate	Metabolite	Reference Inhibitor
1A2	Phenacetin	Acetaminophen	Furafylline
3A4	Testosterone	6β-Hydroxytestosterone	Ketoconazole
2C9	Diclofenac	4’-Hydroxydiclofenac	Sulphaphenazole
2C19	S-Mephenytoin	S-4-Hydroxymephenytoin	Tranylcypromine
2D6	Dextromethorphan	Dextrorphan	Quinidine
2B6	Efavirenz	8-Hydroxyefavirenz	Ticlopidine
2C8	Amodiaquine	Desethylamodiaquine	Montelukast

Advantages of LC-MS/MS method over fluorogenic assay:

• LC-MS/MS analysis method is sensitive and specific, therefore human liver microsomes can be used, the fluorescent probes are not isoform-specific and should be used only with individual recombinant cytochromes • interference can occur from the test compound exhibiting fluorescence or fluorescence quenching and lead to false results

The service is available both in panel of 5, comprising the most clinically important CYPs (1A2, 3A4, 2C9, 2C19, and 2D6), and more expanded panel of 7 (1A2, 3A4, 2C9, 2C19, 2D6, 2B6, and 2C8) formats. Both individual recombinant cytochromes and human liver microsomes can be used. For the rough estimate, single point assays are typically performed for each compound at 10 μM concentration or another concentration stipulated by the customer. All test points are performed in duplicates. If a noticeable inhibition is detected, the IC50 values (test compound concentration which produces 50% inhibition) can be determined upon request. For this purpose, doseresponse inhibition curves (8 points, 3-fold serial dilution) of the test compound and reference inhibitor are built starting at 100 μM concentration. The IC50 values are calculated using Microsoft Excel and GraphPad Prism software. Reference inhibitors specific for each CYP enzyme are used to assess inhibition in the control experiments for every batch of tested compounds. Test concentrations of the reference compounds correspond to approximately 5x fold of IC50 values for the corresponding cytochromes P450, which is expected to produce 80-100% inhibition in the properly performing assay.

Deliverable: Either single point assay data for each compound at 10 μM concentration or IC50 values for the tested compounds based on 8-point, 3-fold serial dilution dose-response inhibition curves (upon request). Full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (~1 mg or 2 μmol) or 100 μL of 10-20 mM stock DMSO solution is required for this assay.