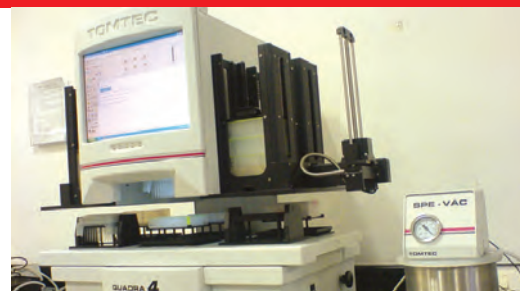


ADME SERVICES



Our *In-vitro* ADME screening service offers a portfolio of assays for investigating: metabolism, distribution and toxicity, permeability, solubility & physicochemical properties, GVK BIO delivers consistent, accurate compound data with cost-efficiency.

In-vitro ADME Capabilities

Physicochemical studies

- Log D/Log P
- Solubility(Kinetic/Equilibrium)
- Chemical stability
- Biological matrix stability (serum/ plasma/ microsomes/ blood /hepatocytes/tissue homogenates)

Absorption/Distribution Assays

- Caco2 and PAMPA permeability assay
- Pgp substrate / inhibitor assay
- Protein binding
- Blood/Plasma Partitioning Ratio

Metabolism/Excretion

- Half life/clearance determination using microsomes/Hepatocytes /S9 fractions/Cyp across species (Human/Rat/Mouse/Dog/ monkey)
- CYP Inhibition (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4)
- Pathway determination (Phase I and Phase II)
- Metabolite identification using Microsomes / Hepatocytes and
- Characterization of potential metabolites using microsomes and Hepatocytes across species (Human/Rat/Mouse/Dog/ monkey)

Log D/Log P

Log D is determined by the shake-flask method, by dissolving some of the solute in a volume of octanol and water/buffer and measure the concentration of the solute in each solvent. Log D is determined by HPLC-UV with confirmation by mass.

Solubility

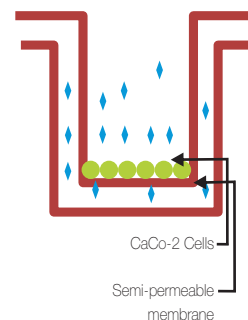
Solubility is one of the most important physicochemical properties. We determine equilibrium solubility by dried DMSO method. We can also determine solubility in aqueous buffer (pH 1-9), organic solvents (DMSO, Ethanol, etc), formulations and excipients by pION/Multiscreen/HPLC/UV/MS/MSMS methods.

Protein Binding

In-vitro binding studies with plasma have proven to be a valuable tool for predicting *In-vivo* protein binding. We determine the protein binding by both ultra filtration and rapid equilibrium dialysis. Using RED device we determine protein binding in microsomes. Plasma and tissue homogenate across various species (Rat/mice/human &dog).

Caco2 Permeability Assay

Caco2 cells are the most frequently used *In-vitro* models to assess intestinal permeability. Permeability across Caco2 cell monolayer is used to predict human permeability of drug candidates, to perform in-depth mechanistic and absorption studies, to study the effects of transporters on permeability. We can determine apparent permeability (P_{app}) / efflux ratio / Unidirectional / Bidirectional by LCMS.



PAMPA

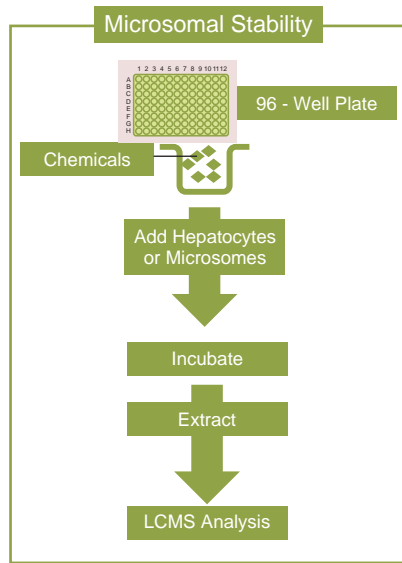
(Parallel artificial membrane permeability assay)

The Parallel Artificial Membrane Permeability Assay (PAMPA) assay is used as an *in-vitro* model of passive, transcellular permeability. As well as for the prediction of oral absorption and brain penetration. Effective permeability ($\log P_e$) may be measured by pION/Multiscreen/LCMS.

ADME SERVICES

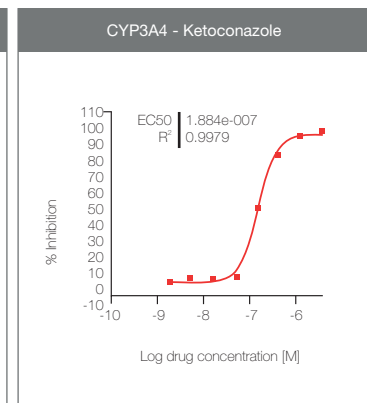
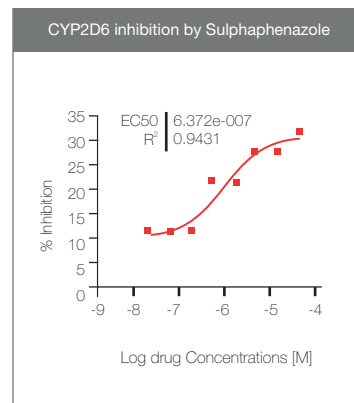
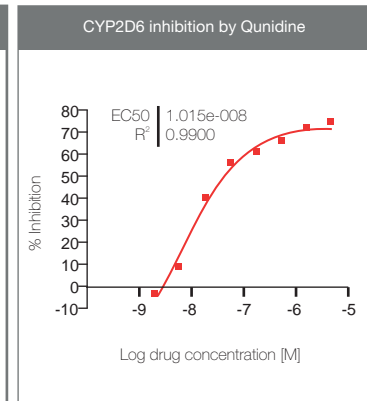
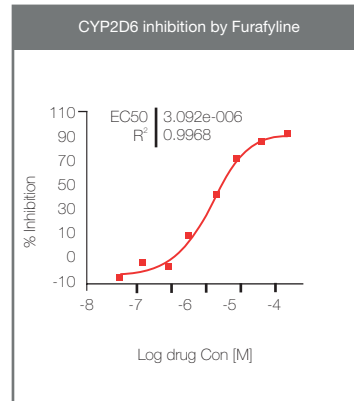
Microsomal Stability

Metabolic stability plays an important role in the success of drug candidates. First pass metabolism is one of the major causes of poor oral bioavailability and short half life and the study influences both oral bioavailability and half life. The half life / clearance / % metabolized can be determined in microsomes / S9 fractions / hepatocytes.

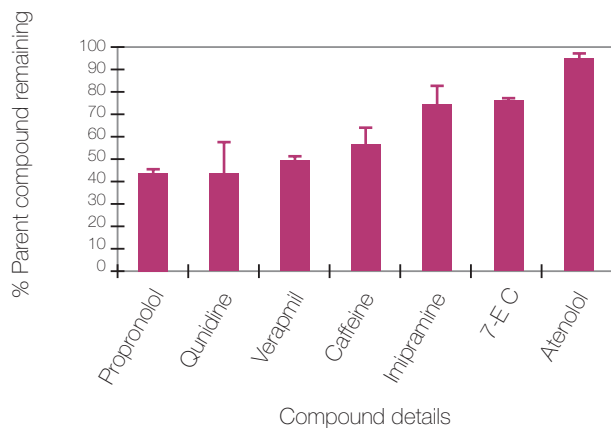


Hepatocyte metabolic stability

Per orally administered drug may undergo first-pass metabolism which influences the pharmacokinetic properties such as the clearance, the half-life or the bioavailability. Cryopreserved hepatocytes are used to calculate half life/clearance/% parent compound remaining.



Human hepatocytes metabolic stability



Metabolite identification using Light Sight Software

Metabolic stability and the identification of formed metabolites has become an important tool. *In-vitro* metabolite identification & characterization of the test compounds is determined using Q trap with light sight software.

Bioanalysis

LC-MS/MS

- 3200 QTRAP
- API3200
- API4000

HPLC

- Agilent 1200 RRLLC with PDA
- Shimadzu Prominence with UV
- Agilent 1200 RRLLC with HTS PAL

Others

- Spectramax (Molecular Devices)
- BMG Polarstar
- Qudra4 Liquid Handler (Tomtec)

CYP Inhibition

CYP inhibition occurs either as reversible inhibition, quasi-irreversible inhibition or irreversible inhibition. CYP inhibition is a fluorescent based/LCMS assay. Specific isoforms of CYP (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) are used to determine the inhibition (IC₅₀).