TCG Lifesciences | Chembiotek

In Vitro ADME

At TCG Lifesciences (TCGLS), our *in vitro* ADME services provide rapid, robust, and reproducible data to support drug discovery and development programmes. Combining multiple assay platforms, diverse species coverage, and deep scientific expertise, we help sponsors understand the drug-like properties of the compounds early in development. From solubility profiling to complex transporter studies, we deliver quality data for lead optimisation, candidate selection, and risk assessment—reducing attrition and accelerating project progress.

Besides performing the studies, our experienced team also interprets the data, provides input in SAR and further proposes customised assays to meet project requirements.

We can perform the ADME studies for small molecules, depsipeptides, macrolides, and other types of compound.

Our Core Capabilities

Matrices available for ADME studies

TCGLS has wide range of biological and bio-relevant matrices		
Hepatocytes	:	Human, Monkey, Dog, Minipig, Rat, Mouse, Rabbit
Liver Cytosol	:	Human, Monkey, Dog, Rat, Mouse
Liver S9	:	Human, Dog, Chicken, Rat, Mouse
Liver Microsomes	:	Human, Cattle, Monkey, Pig, Dog, Cat, Minipig, Guinea pig, Rabbit, Hamster, Gerbil, Chicken, Rat, Mouse
Intestinal S9	:	Human, Dog, Rat, Mouse
Blood	:	Human, Rat, Mouse
Plasma	:	Human, Cattle, Monkey, Dog, Cat, Minipig, Guinea pig, Rabbit, Hamster, Rat, Mouse
Tissue	:	Brain, Spleen, Kidney of Rat and Mouse
rCYP	:	Human rCYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5
Cell-line	:	Caco-2, MDCK-II, MDCK-MDR1, HEK-293, CHO-K1 (Transporter qualified)
Vesicle	:	MRP2, MRP4, BSEP, BCRP
Buffer	:	PBS, Citrate, Phosphate, JP-2, 0.05(N) HCl, HBSS, Sea-water, Fresh-water, Tris-HCl, KHB, Other buffers as needed
Biorelevant	:	SGF, FaSGF, SIF, FaSSIF, FeSSIF, FaSSIF-V2, FeSSIF-V2, Canine FaSSIF/FaSSGF

1. Physicochemical Profiling

Evaluation of physicochemical properties of compounds helps in understanding their behaviour in biological environments and also in formulation preparation.

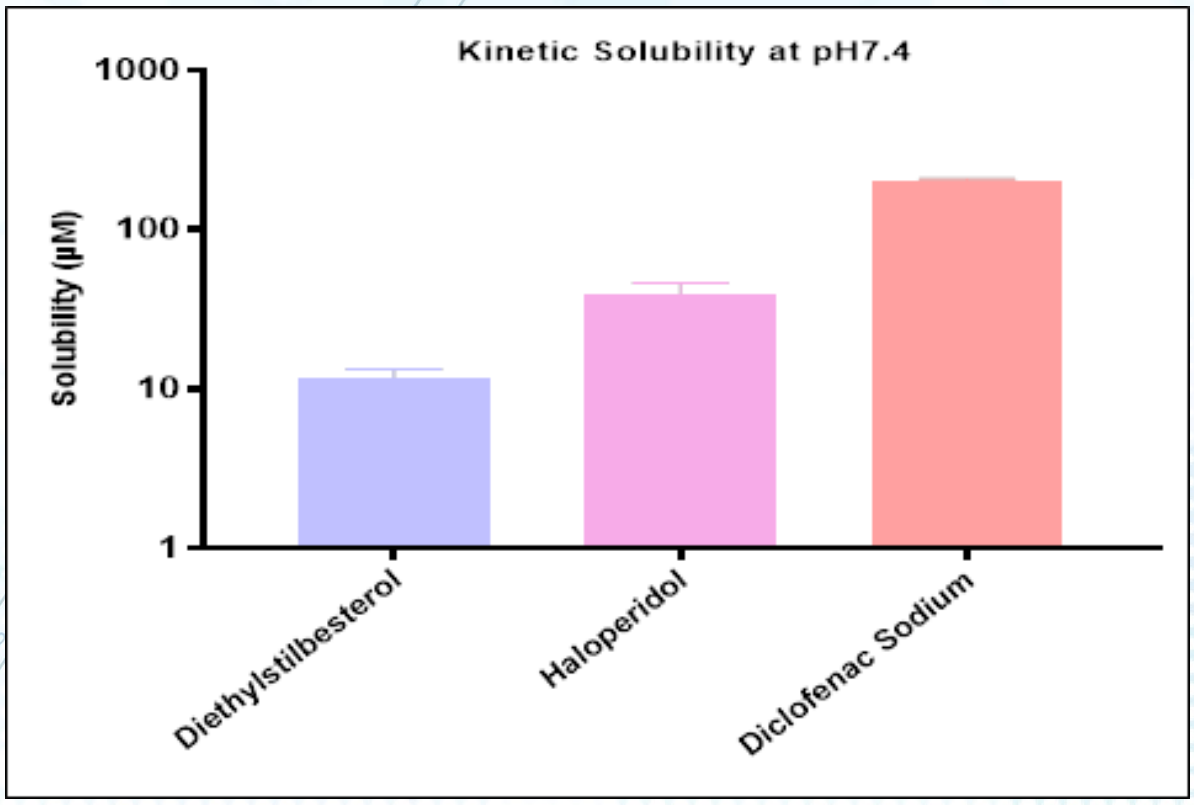
At TCGLS, we offer a comprehensive range of studies to assess solubility, lipophilicity, and ionisation properties — the critical parameters that influence absorption, distribution, metabolism, and excretion (ADME).

- Solubility: Kinetic, Thermodynamic, pH dependent, Biorelevant medium solubility
- pKa determination
- LogP
- LogD

1.1. Solubility

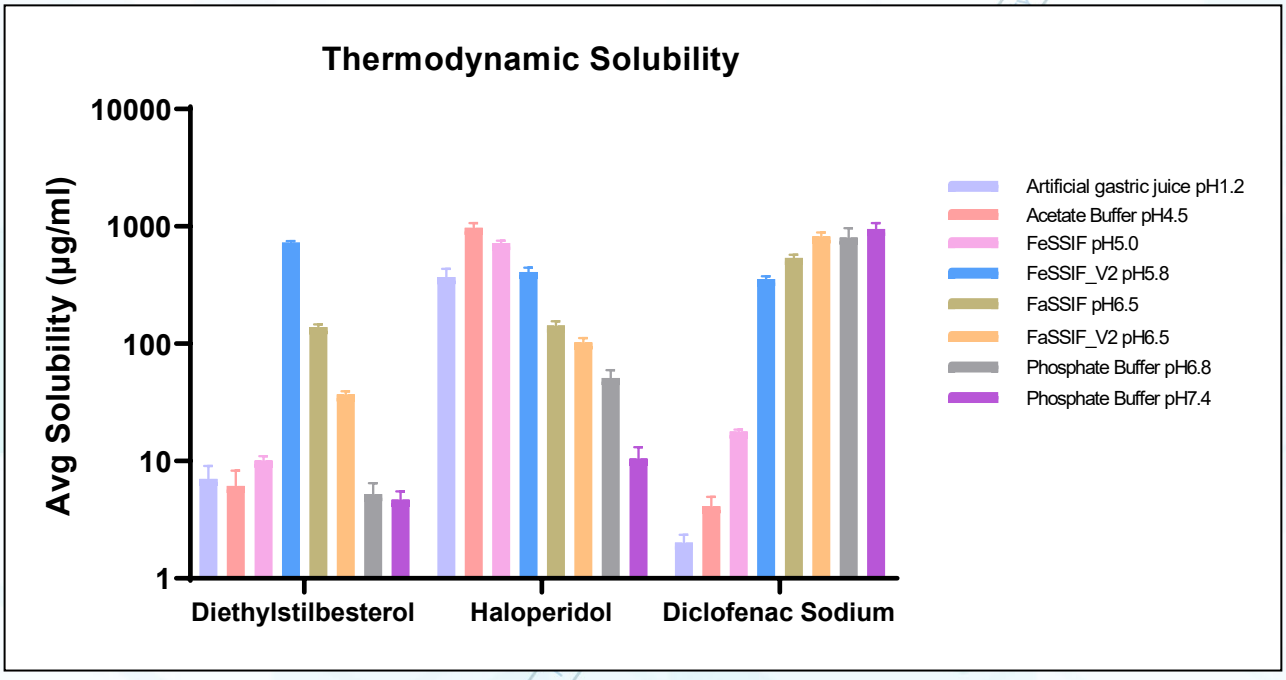
Aqueous solubility is an important physicochemical parameter that influences oral absorption, bioavailability, and other pharmacokinetic performances of a compound. Also, estimation of aqueous solubility at an early stage helps in identifying false positives in other biological assays.

Test system	Solubility in PBS, pH 7.4
Test concentration	200µM (DMSO: 1%); n=2
Test condition	25°C, 2h shaking
Solubility range	2.50 – 200µM
Linearity curve	5 points (2.50, 25, 75, 150 and 200µM), n=1
Detection	Spectrophotometer or LC-MS/MS



Thermodynamic solubility

Test system	Thermodynamic solubility in Buffer pH 7.4/6.8/4.5/1.2, FaSSIF pH 6.5, FeSSIF pH 5.0, FaSSIF-V2 pH 6.5, FeSSIF-V2 pH 5.8
Test concentration	1mg/ml, n=2
Incubation	Continuous shaking at 25°C for 24h
Reference standard	Diethylstilbesterol, Haloperidol, Diclofenac sodium
Detection	LC-MS/MS

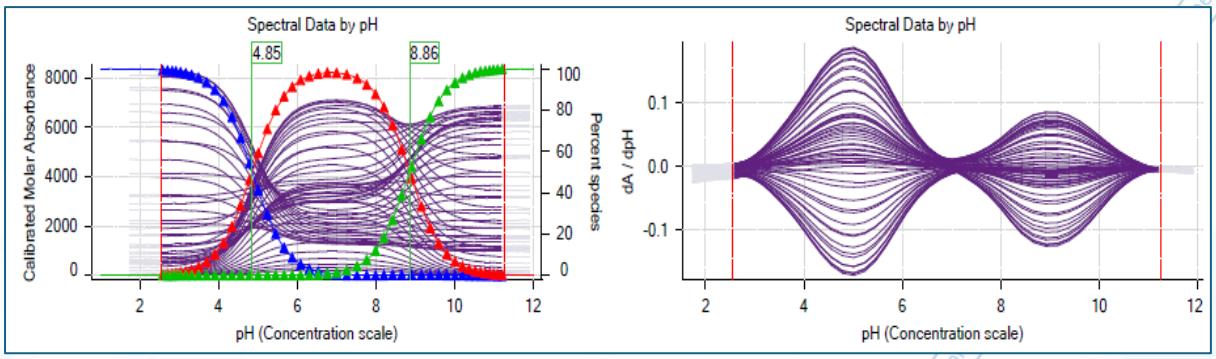


1.2. pKa determination (Ionisation constant)

pKa is critical for drug development as it dictates a molecule's ionisation state at a given pH. This parameter is useful for formulation preparation, and for understanding dissolution and site of absorption. We perform pKa determination using Sirius T3 (Pion) via UV–Visible detection as well as potentiometric titration across the pH range of 2 to 12. Cosolvent-based titration is also performed to cover a broad range of compounds.

Detection Platform	Pion Sirius T3
Assay Method	Fast UV-metric titration
	UV-metric psKa determination using cosolvent
	Potentiometric (pH-metric) titration
	Potentiometric (pH-metric) titration for psKa using cosolvent
pH range	2-12
Cosolvents	Methanol, Dioxane, MDM mix or as suggested
Data type	pKa value

pKa of Pyridoxine

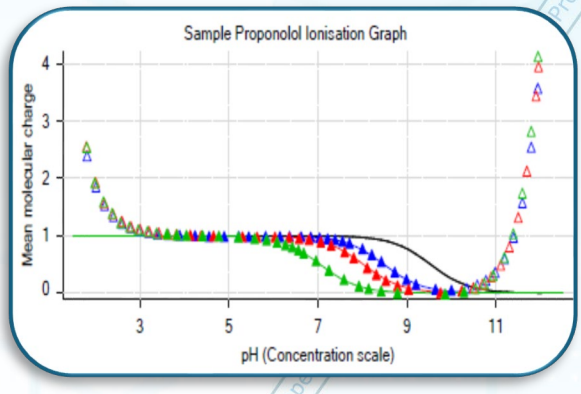


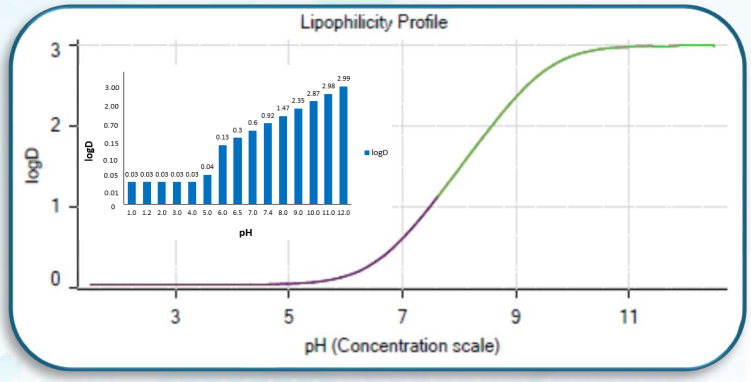
1.3. LogP

LogP (Partition Coefficient) is defined as the partitioning between aqueous and lipophilic phases when in the unionised form. This parameter helps in understanding the lipophilicity and the type of formulation required at the later stage of discovery.

Detection Platform	Pion Sirius T3
Assay Method	Potentiometric (pH-metric) titration
pH range	2-12
Partition solvents type	Pre-saturated Octanol [Saturated with ionic-strength adjusted water (0.15M KCl)]
Data type	LogP and LogD values

***Note**: pKa value must be provided



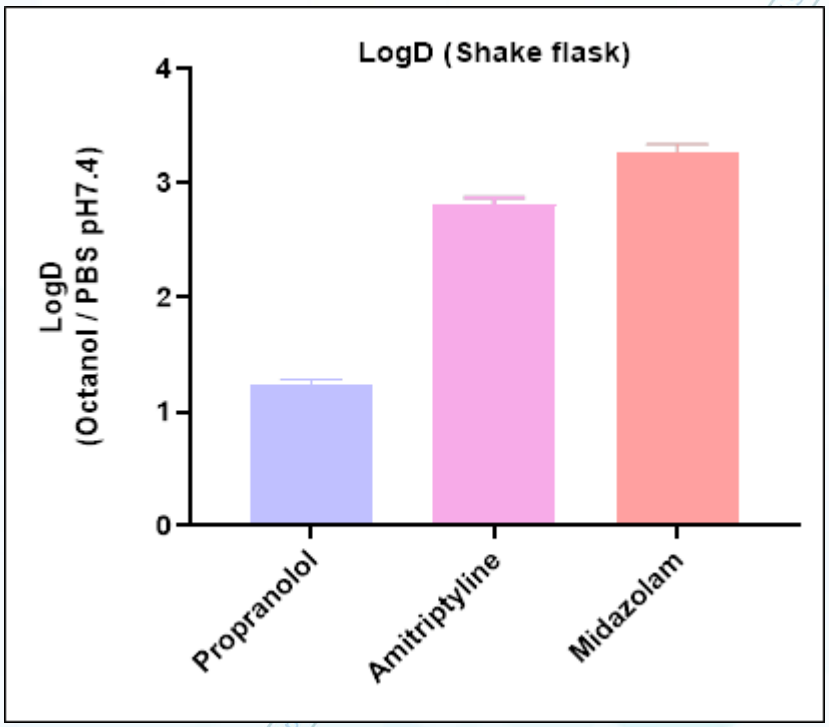


Log P of Propranolol

1.4. Log D

Lipophilicity indirectly governs the solubility and permeability of compounds. LogD values serve as an indication of a compound's oral absorption. We perform miniaturised shake-flask method to determine LogD at various pH values.

Test system	LogD pH7.4
Organic/Aqueous phase	Octanol/PBS pH7.4
Test condition	75µM compound (n=2) in 1:1 Octanol:buffer volume, phase mixing for 2 hr @25°C followed by phase separation
Quantitation	Compound in both octanol and PBS layer
Reference standard	Propranolol, Amitriptyline, Midazolam
Detection	LC-MS/MS

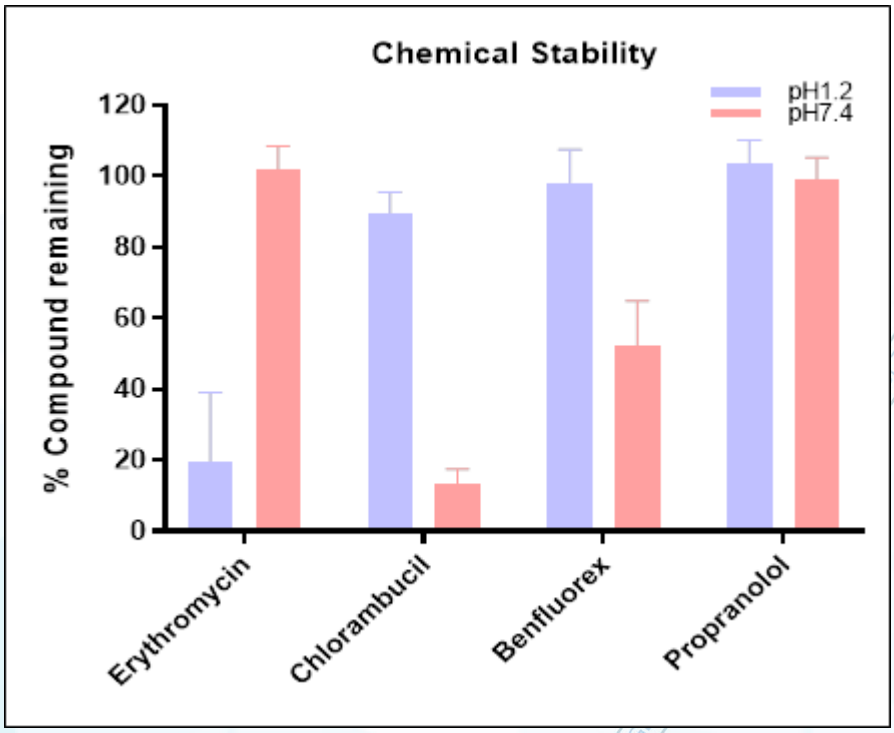


1.5. Chemical stability

Chemical stability refers to a compound's ability to maintain its chemical composition in a biological environment. While stability at physiological pH is widely useful, understanding stability across the varied pH values along the gastrointestinal tract provides better insights and serves as useful information for formulation development.

We perform chemical stability studies in different buffers as well as in biorelevant media.

Test system:	Chemical Stability in 0.1(N) HCl pH 1.2 and PBS pH 7.4
Test Concentration:	1µM (DMSO: 1%); n=2
Incubation Period:	25°C with shaking for up to 2h (0, 60 and 120min)
Reference standard:	Propranolol, Chlorambucil, Benfluorex, Erythromycin, Amoxicillin
Detection Method:	LC-MS/MS



2. Permeability & Transporter Studies

Understanding membrane permeability and transporter interactions early in drug development is vital for selecting candidates with favourable ADME and safety profiles. At TCGLS, we offer rapid *in vitro* assays, both non–cell-based and cell-based bidirectional permeability studies, using well-established cell lines such as Caco-2, MDCK (W/T), and MDCK-MDR1, as well as vesicle-based transporter assays.

Our Permeability & Transporter Services Include

PAMPA (GIT / BBB)

Caco-2 / MDCK-WT / MDCK-MDR1 Permeability

- Caco-2 cells mimic intestinal epithelium for oral absorption prediction.
- MDCK-WT provides baseline/intrinsic permeability data.
- MDCK-MDR1 (overexpresses human P-gp) is ideal for assessing P-gp-mediated efflux transport

Substrate & Inhibitor Assessment of Transporters using Cell Lines

- P-gp (MDR1)
- BCRP
- MRP2
- OATP1B1
- OATP1B3

Substrate & Inhibitor assessment of Transporters using Vesicles

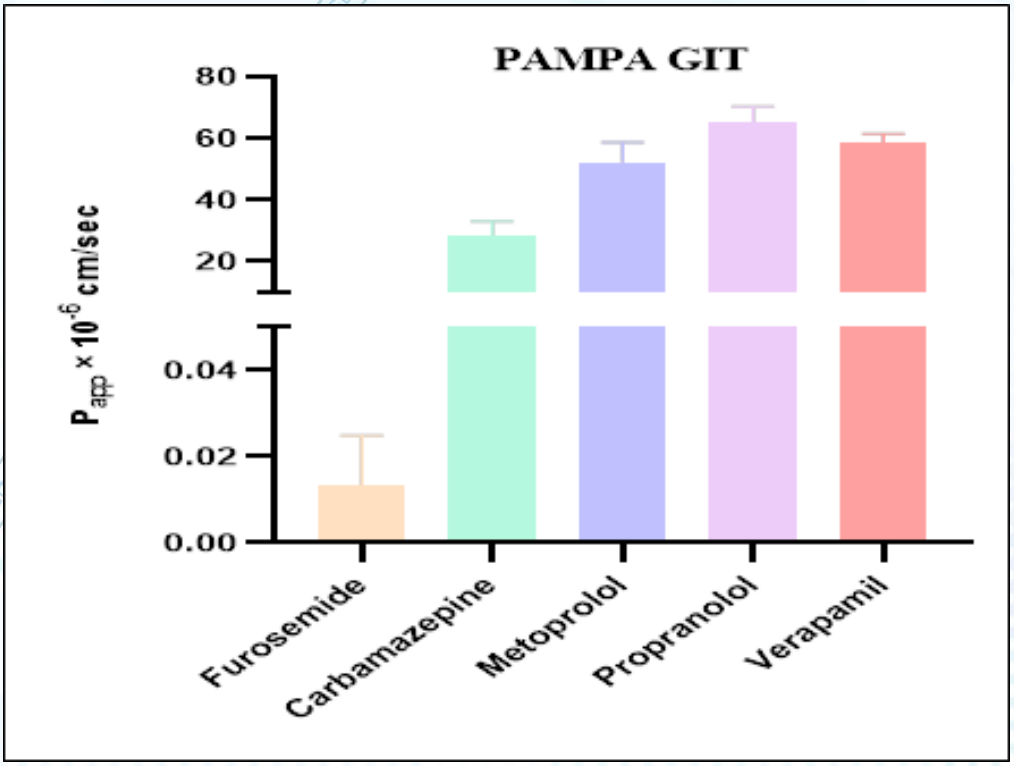
- P-gp
- BCRP
- BSEP
- MRP2
- MRP4

2.1. PAMPA-GIT

The Parallel Artificial Membrane Permeability Assay (PAMPA) predicts a drug's passive membrane permeability. Apparent permeability (Papp) is calculated to rank compounds for absorption across barriers such as the intestinal tract or blood–brain barrier.

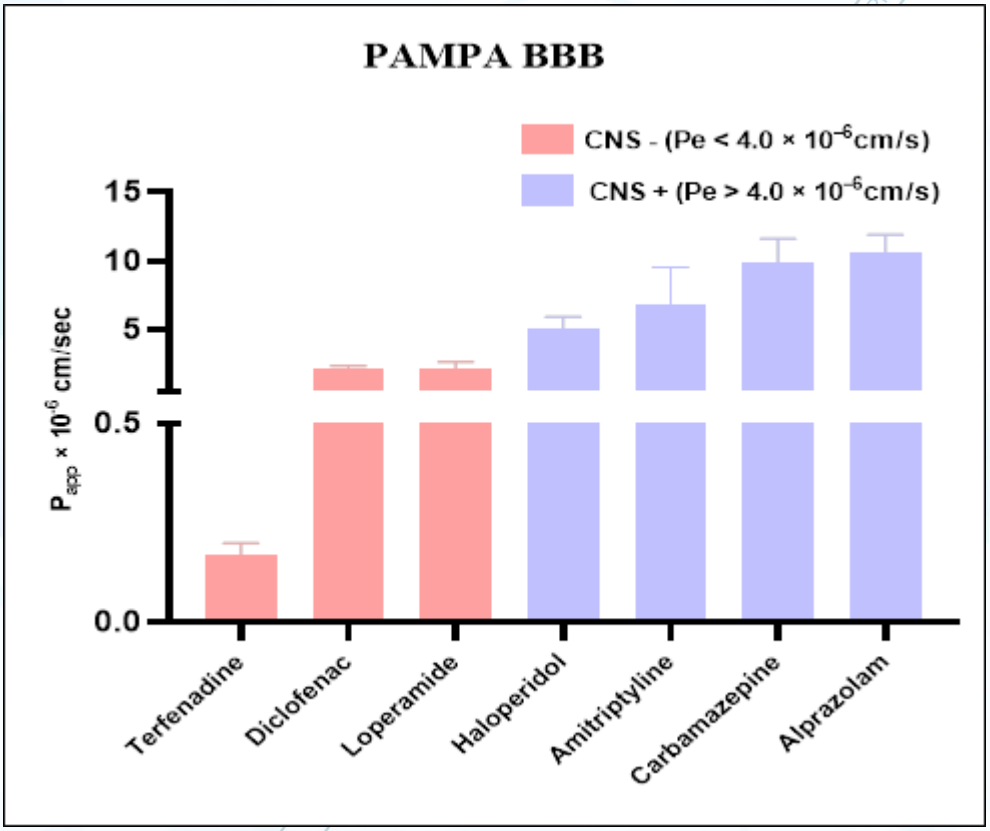
Test concentration:	10µM (5% DMSO), n=2
Basal volume:	200µl (Buffer pH 7.4 with test compound)
Apical volume:	200µl (Buffer with 5% DMSO)
Artificial membrane:	Precoated plates from commercial vendors
Incubation:	6h at room temperature
Sample analysis:	Acceptor, donor and initial donor samples
Standard compound:	Furosemide, Metoprolol, Propranolol, Verapamil, Carbamazepine
Analysis method:	LC-MS/MS

- ***PAMPA using kit from Pion is under development***



PAMPA-BBB

Test concentration:	10µM (5% DMSO), n=2
Basal volume:	200µl (SS pH 7.4 with compound which gives 5%DMSO)
Apical volume:	200µl (BSB with 5% DMSO)
Artificial membrane:	Plates obtained from Pion Inc
Incubation:	6h at room temperature
Sample analysis:	Acceptor, donor and initial donor samples
Standard compound:	Terfenadine, Diclofenac, Loperamide, Haloperidol, Amitriptyline, Carbamazepine, Alprazolam
Analysis method:	LC-MS/MS



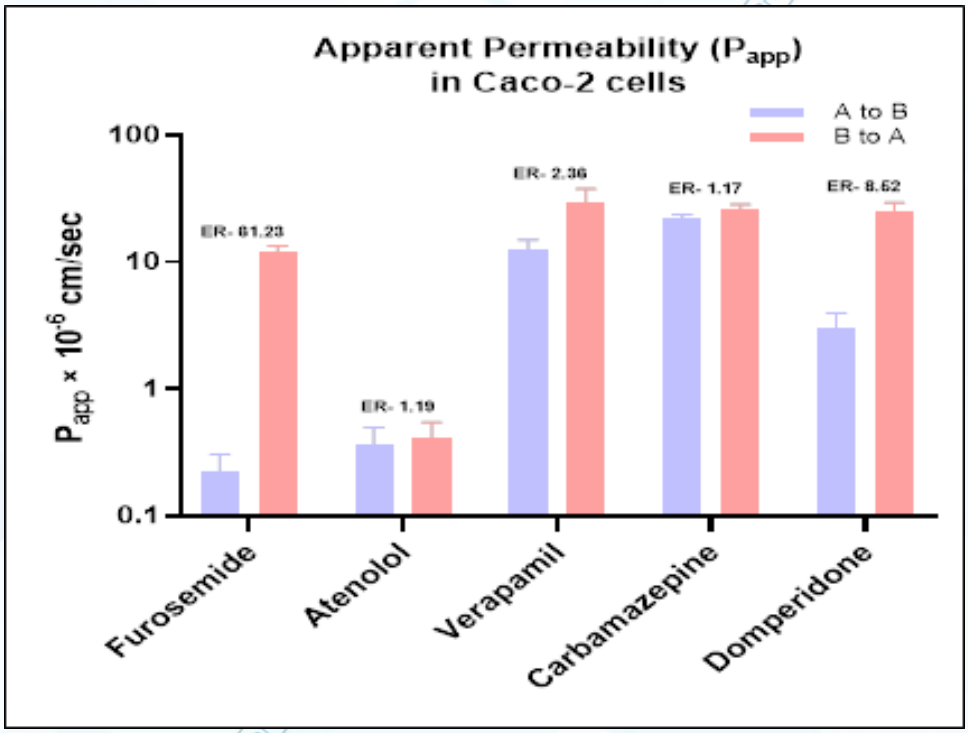
2.2. Caco-2 Permeability

Permeability is a critical parameter for predicting oral bioavailability and identifying potential efflux liabilities. A drug's ability to traverse biological membranes is governed by both passive diffusion and active transport mechanisms, the latter being mediated by membrane-bound transporter proteins such as P-glycoprotein (P-gp), BCRP, and MRP2, etc.

Our Caco-2 permeability study enables the binning of compounds with respect to their gastrointestinal permeability. We run this study in two different modes:

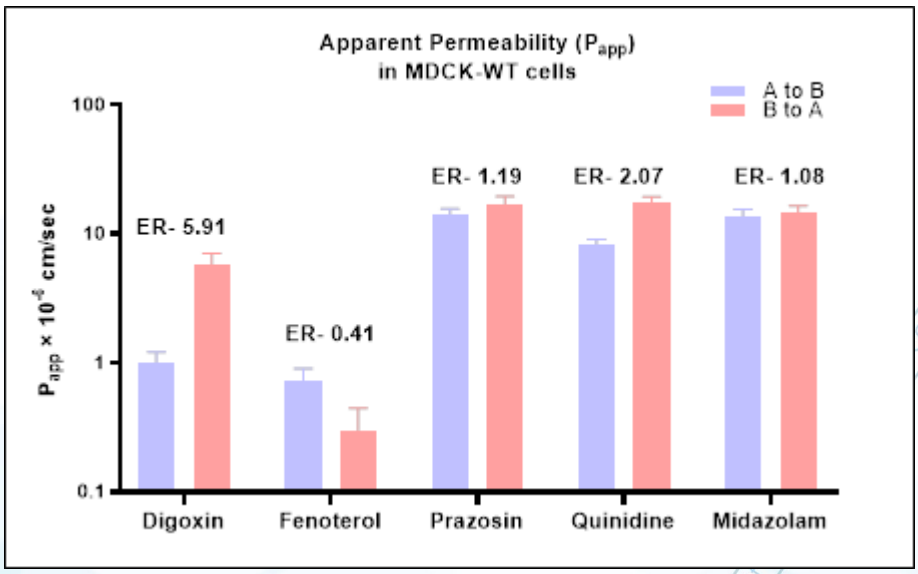
a) Gold-standard 21-day growth protocol
b) Accelerated 10-day growth protocol: Cost-effective and with quicker turnaround time

Test concentration	2µM (DMSO: 1%) in duplicate
Seeding density	Cells seeded onto the growth plates as per in-house protocol
Buffer	HBSS containing 10mM HEPES
Apical/basal pH	7.4/7.4
Apical/basal volume	75/250µl
Incubation	2.5hr at 37°C under 5% CO2 & 95% RH
Reference standard	Furosemide, Atenolol, Verapamil, Carbamazepine, Domperidone
Membrane integrity	Lucifer Yellow permeability (Fluorimetry)



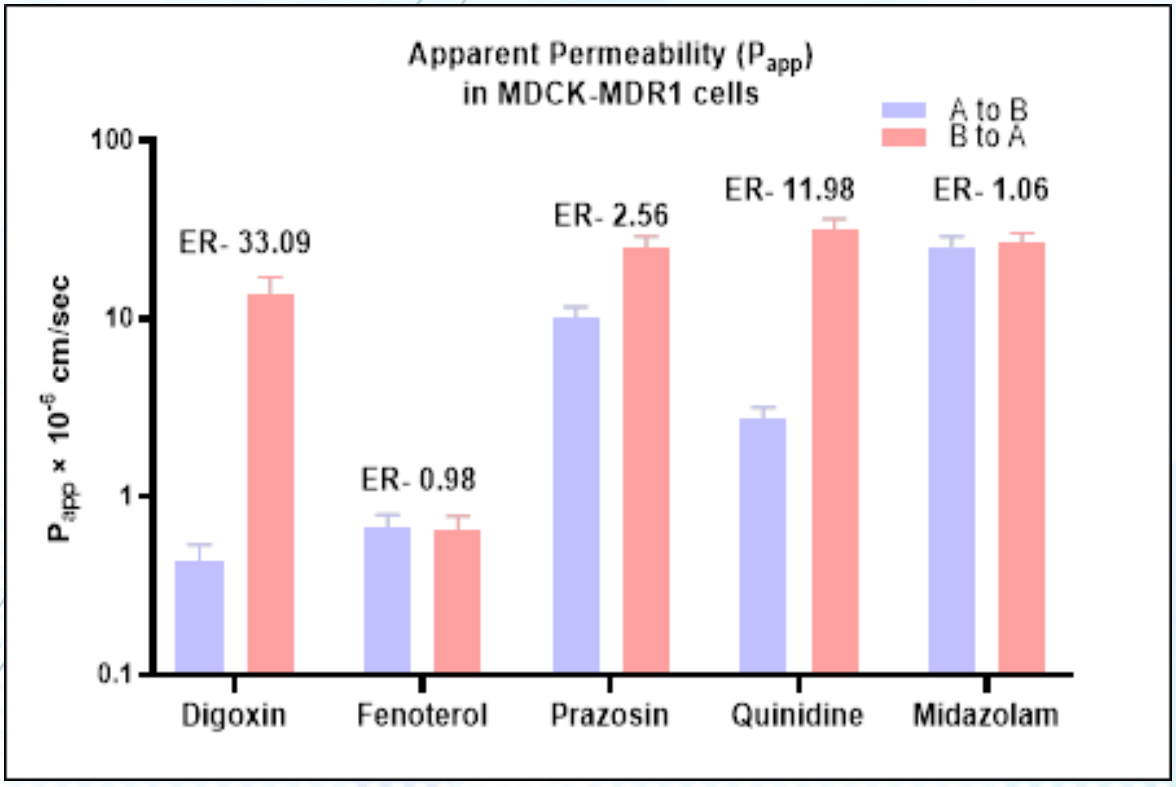
MCDK (W/T) Permeability

Test concentration	2µM in duplicate (1% DMSO content)
Seeding density	Cells seeded onto the growth plates as per in-house protocol
Buffer	HBSS containing 10mM HEPES
Apical/basal pH:	pH7.4/7.4
Apical/basal volume:	75/250µl
Incubation	2.5hr at 37°C (no shaking) under 5% CO_2 & 95% RH)
Reference standard	Digoxin, Fenoterol, Prazosin, Quinidine, Midazolam
Analysis method	LC-MS/MS quantification of test compound
Membrane integrity test	Lucifer Yellow permeability assessment (by Fluorimetry)



MCDK-MDR1 Permeability

Test concentration	2µM in duplicate (1% DMSO content)
Seeding density	Cells seeded onto the growth plates as per in-house protocol
Buffer	HBSS containing 10mM HEPES
Apical/basal pH:	pH7.4/7.4
Apical/basal volume:	75/250µl
Incubation	2.5hr at 37°C (no shaking) under 5% CO_2 & 95% RH)
Reference standard	Digoxin, Fenoterol, Prazosin, Quinidine, Midazolam
Analysis method	LC-MS/MS quantification of test compound
Membrane integrity test	Lucifer Yellow permeability assessment (by Fluorimetry)

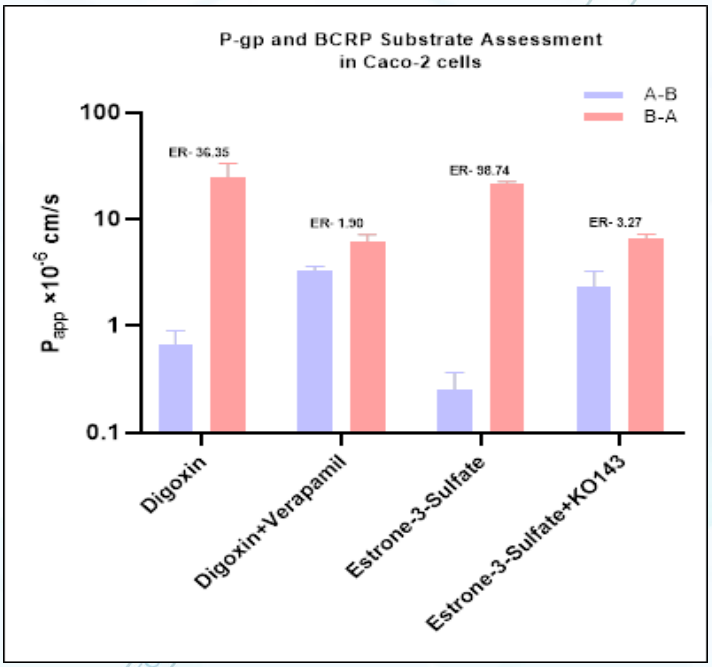


2.3. P-gp and BCRP Substrate Assessment using Caco-2 Cell Line

To evaluate whether a test compound is a substrate of efflux transporters, the assay is performed in the presence and absence of specific inhibitors. A significant reduction in the efflux ratio upon inhibitor treatment indicates that the compound is likely to be a substrate of the respective transporter.

When utilising Caco-2-based permeability assays, we typically use Digoxin (5 µM) and Estrone-3-sulfate (5 µM) as substrates for P-gp and BCRP, respectively. Standard inhibitors are Elacridar (10 µM) or Verapamil (50 µM) for P-gp, and Ko143 (10 µM) for BCRP.

Likewise, MDCK-MDR1 is used for P-gp substrate assessment.



Our experienced team also provides inhibitor assessment using all these validated platforms. The team also provides both substrate and inhibitor assessment through the use of membrane vesicles.

Please reach out to us for further information.

3. Distribution Studies

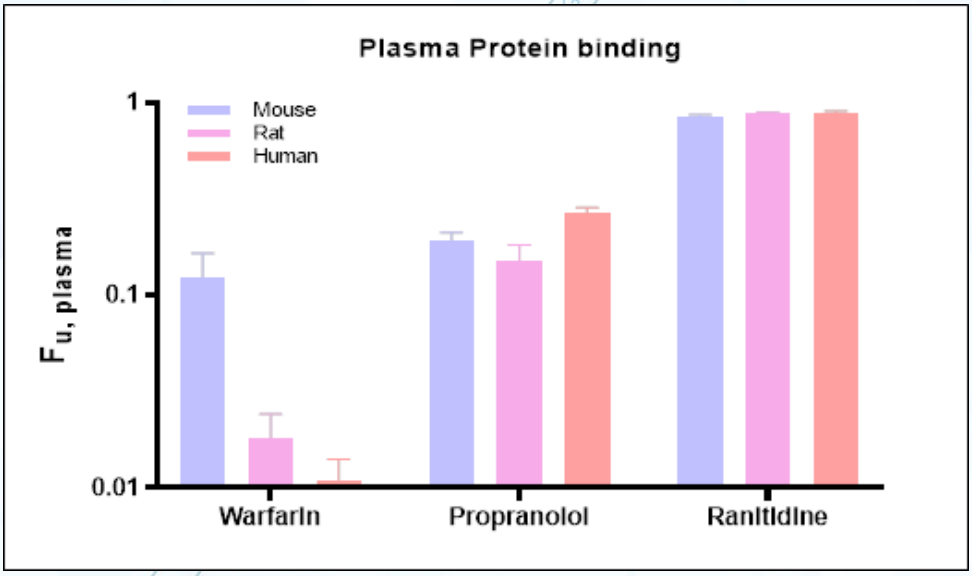
Understanding a compound's distribution profile is crucial for optimising its pharmacokinetic and pharmacodynamic (PK/PD) properties. Plasma protein binding (PPB), brain tissue binding (BTB), and blood-to-plasma (B/P) ratio help predict how a drug behaves in the body, including its metabolism, clearance, and tissue targeting. We provide comprehensive binding studies across multiple species.

- Protein Binding – Equilibrium Dialysis
- Microsomal Binding
- Hepatocytes Binding
- Brain Tissue Binding
- Blood-to-Plasma (B/P) Ratio

3.1. Plasma Protein Binding - Equilibrium Dialysis

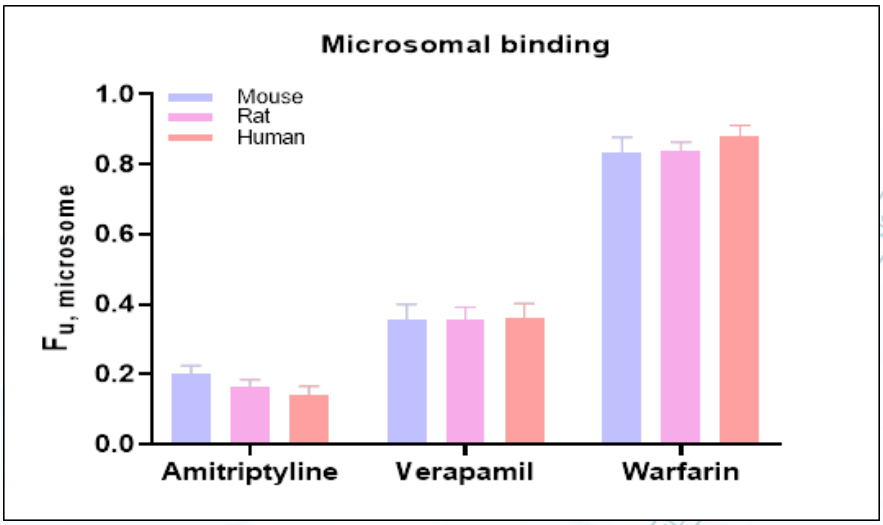
Test system	*In vitro* plasma protein binding
Species:	Human/rat/mouse/other species
Test compound concentration:	1µM, n=2
Method	Equilibrium dialysis
Incubation Period:	6h at 37°C, 5% CO2, 95% RH, shaking
Organic solvent:	≤1%
Reference standard:	Ranitidine, Propranolol, Warfarin or ketoconazole
Detection Method:	LC-MS/MS

We also conduct PPB studies using diluted plasma



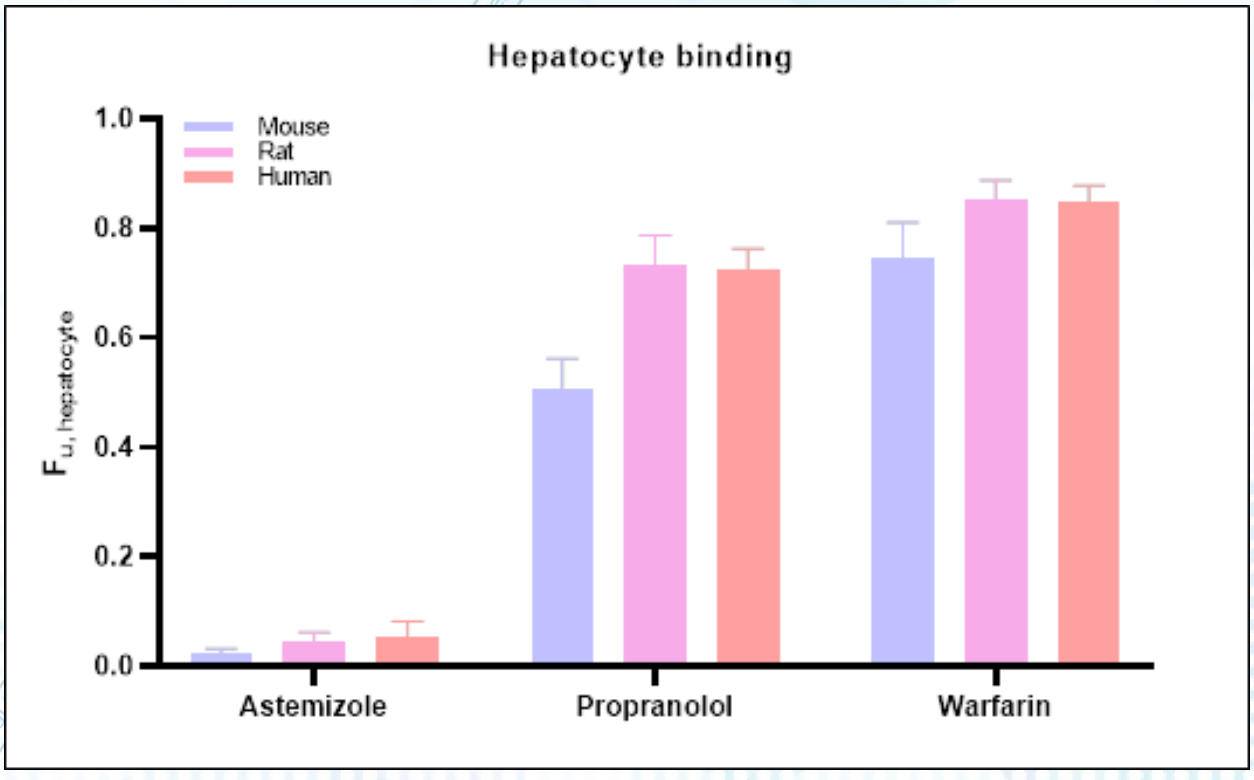
Microsomal Binding - Equilibrium Dialysis

Test system	*In vitro* microsomal protein binding
Source of LM:	Human/ Rat/Mouse and other species
Test compound conc:	1 µM, n=2
LM conc:	0.5mg/ml
Reference standard:	Amitriptyline, Verapamil, Warfarin
Detection Method:	LC-MS/MS



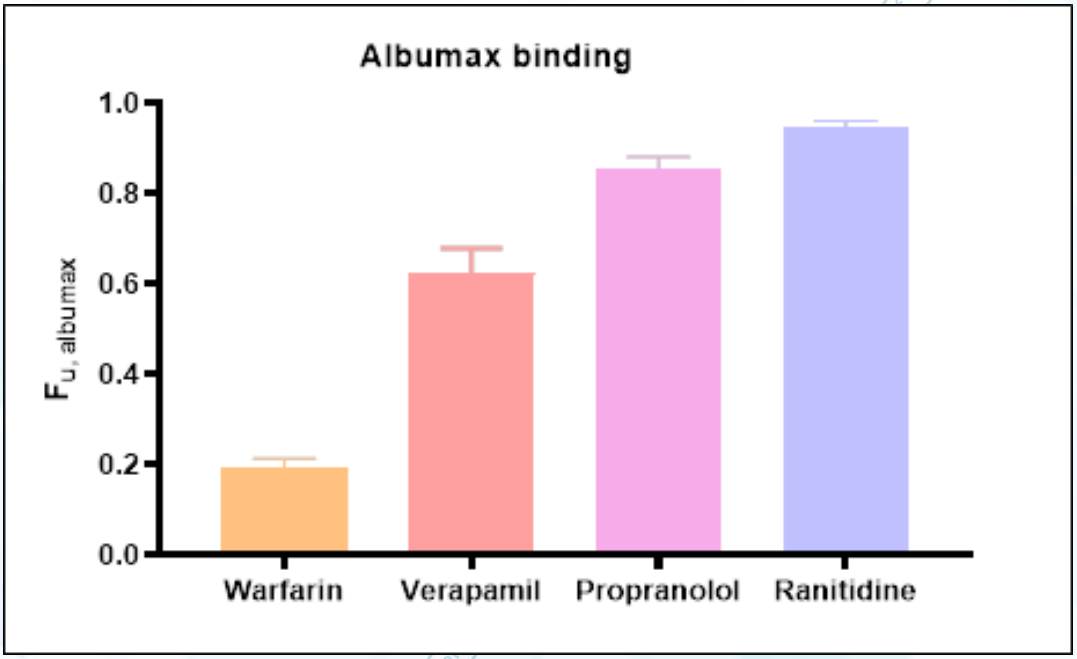
Hepatocytes Binding - Equilibrium Dialysis

Test system:	*In vitro* binding to Hepatocyte cells
Species:	Human/rat/mouse/dog/monkey and other species
Cell number	1 million dead hepatocytes/ml (human/dog/monkey) and 0.5 million dead hepatocytes/ml (rat & mouse)
Test conc	1 µM, n=2
Reference standard:	Propranolol, Warfarin, Astemizole
Detection Method:	LC-MS/MS



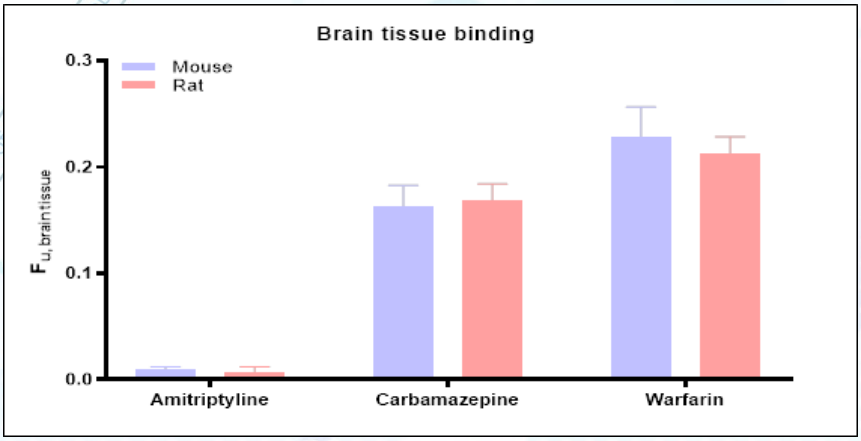
Albumax binding - Equilibrium Dialysis

Test system:	*In vitro* binding to Albumax
Protein:	0.5% Albumax in RPMI media
Organic solvent:	≤1%
Test compound concentration:	1µg/ml, n=2
Reference standard:	Ranitidine, Propranolol, Verapamil, Warfarin
Detection Method:	LC-MS/MS



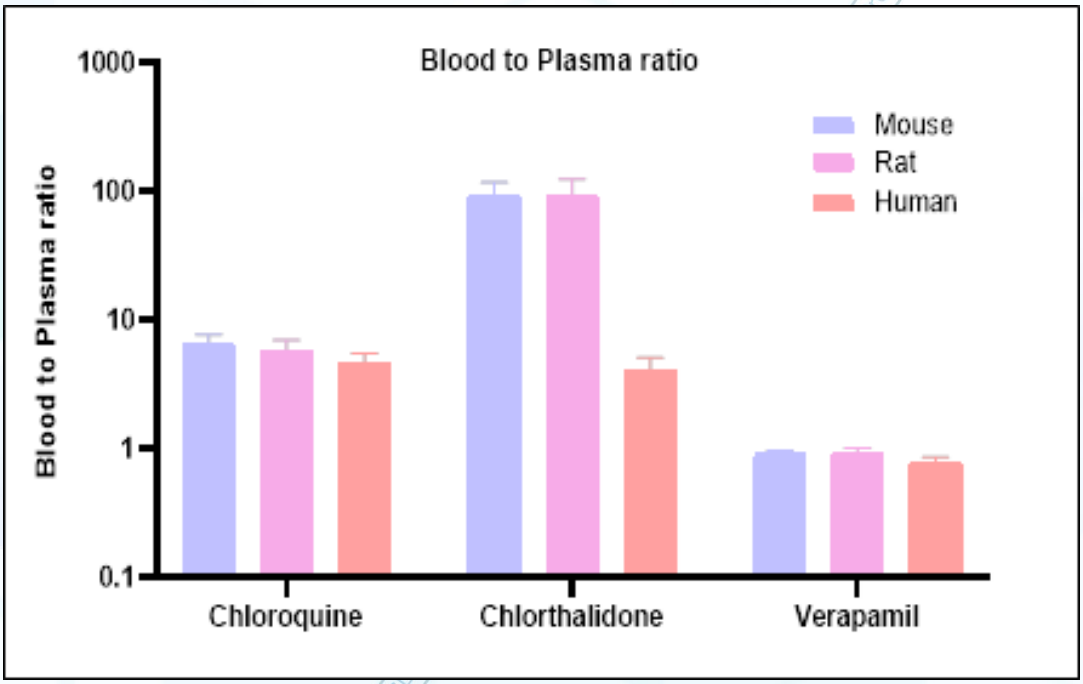
Brain tissue binding - Equilibrium Dialysis

Test system:	*In vitro* brain tissue binding
Biomatrix:	Rat / Mouse brain homogenate
Organic solvent:	≤1%
Test compound concentration	2µM, n=2
Reference standard:	Amitriptyline, Carbamazepine, Warfarin
Detection Method:	LC-MS/MS



3.2. Blood to plasma ratio

Test system:	*In vitro* Blood to plasma ratio
Species:	Human, rat, mouse blood
Anticoagulant:	Heparin sodium (20U/ml)
Testing Compound Concentration:	2.5 µM, n=2
Incubation Period:	30minutes at 37 °C
Reference standard:	Chloroquine, Chlorthalidone, Verapamil
Detection Method:	LC-MS/MS
Quantitation:	Compound in plasma and whole blood



4. Metabolic Stability

Metabolic stability is a key ADME parameter that assesses how quickly a compound is metabolised, or in other words, how good its stability is. To become a successful drug candidate, the compound should have good or optimum stability in the central circulatory system. This information is pivotal in determining a compound's clearance, bioavailability, and dosing frequency.

We assess metabolic clearance using both cytochrome P450 and non-P450 pathways.
Our experienced team specialises in metabolic stability testing across species using a range of *in vitro* systems, including:

- Liver microsomes
- Hepatocytes
- S9 fractions

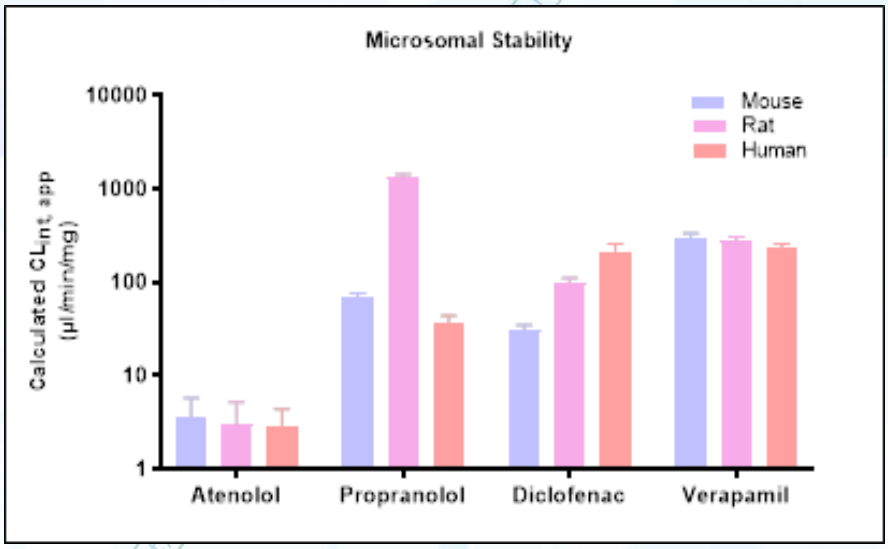
- Cytosol
- Recombinant cytochrome P450 enzymes (rCYPs)

4.1. Liver Microsomal Stability

The liver is rich in metabolic enzymes, making it the most important organ for metabolism. Liver microsomes contain abundant amounts of cytochrome P450s (CYPs) and other metabolic enzymes.

We routinely utilise liver microsomes from different species for studying Phase-I metabolism. *In vitro* liver microsomal (LM) stability study data help to predict *in vivo* clearance, bioavailability, and overall pharmacokinetic behaviour of molecules. We offer high-quality LM stability studies across a variety of species (human, rat, mouse, dog, monkey, pig, guinea pig, rabbit, cat, gerbil, bovine) with LC–MS/MS analysis.

Test system:	Metabolic stability using liver microsomes
Species:	Mouse/Rat/Human/dog/monkey/others
Test Compound:	1µM, n=2
Liver Microsomes conc:	0.4mg/ml
Incubation Period:	0,5,10,20,30 & 60min at 37°C, 5% CO2, 95%RH
Reference standard:	Atenolol, Propranolol, Diclofenac, Verapamil
Detection Method:	LC-MS/MS

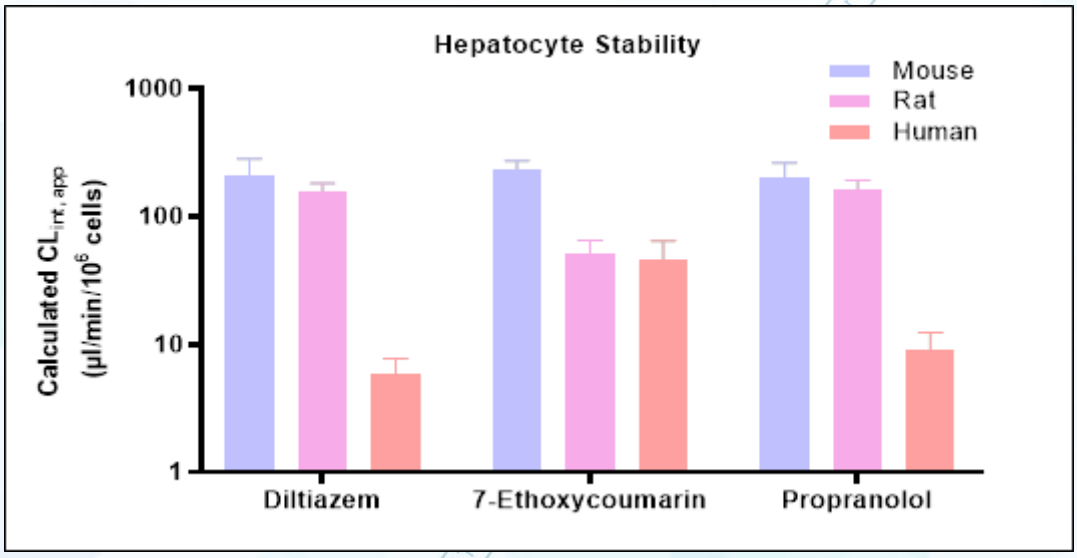


Please reach out to us for details of UGT, AO, XO, and FMO-based metabolism assays.

4.2. Hepatocytes Stability

Hepatocyte stability studies use intact liver cells to assess how stable a compound is in the liver. This method provides a comprehensive assessment of metabolism involving both Phase I and Phase II enzymes and is helpful in predicting hepatic clearance. We offer hepatocyte stability studies across multiple species with LC–MS/MS-based analysis.

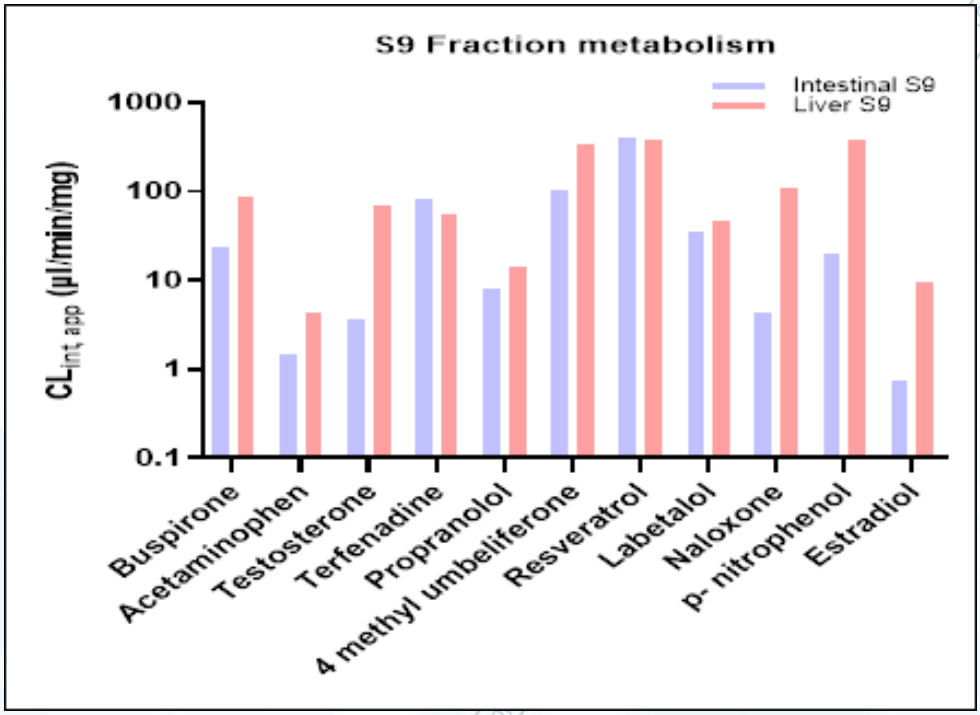
Test system:	Metabolic stability using cryopreserved hepatocytes
Species:	Human/Rat/Mouse/dog/monkey and other hepatocytes
Test Concentration:	1µM, n=2
Cell number:	1×10^6 live cells/ml(Human/dog/monkey) and 0.5×10^6 live cells/ml (rat & mouse)
Incubation Period:	0, 20, 40, 60, 90 & 120min (human/dog/monkey); 0, 15, 30, 45, 60 and 75min (rat & mouse) at 37°C, 5% CO2, 95%RH
Reference standard:	Diltiazem, 7-Ethoxy coumarin, Propranolol
Detection Method:	LC-MS/MS



4.3. Metabolic Stability in S9 Fraction

Liver S9 fraction and intestinal S9 fraction are used to assess both microsomal- and cytosolic enzyme-mediated metabolism. TCGLS offers liver and intestinal S9 fraction-based metabolism studies for human and other species, with LC–MS/MS-based analysis.

Test system	Metabolic stability study using S9 Fraction
S9 Fractions	Human/Rat/Mouse S9 Fraction (1mg/ml)
Test concentration	2µM, n=2
Cofactors	1.3mM NADPH, 8mM UDPGA, 0.5mM PAPS, 3.3mM MgCl2
Other components	Alamethicin (25µg/mg protein)
Incubation	0, 5, 10, 20, 30 and 60 minutes with NRS and 60min No-Cofactor (60NCF) at 37°C
Reference standard	Acetaminophen, 4-Methy Umbelliferon, Terfenadine, Propranolol, p- nitrophenol

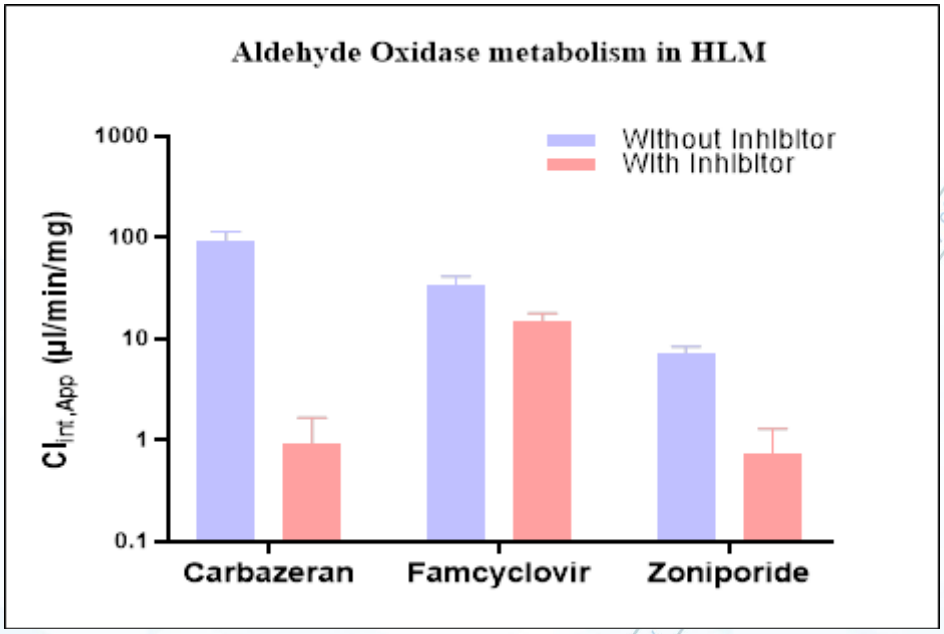


4.4. Aldehyde Oxidase (AO)-Based Metabolism

Aldehyde Oxidase (AO) metabolism is performed using liver cytosol as the source of AO. This study is critical for identifying potential stability issues via non-microsomal enzymes.

We provide cytosol-based stability assays across various species.

Test system:	*In vitro* AO-based metabolic stability
Test concentration:	1µM, n=2
Liver Microsomes:	Human/rat liver cytosol (1mg/ml)
Incubation:	37°C for 0, 10, 20, 40, 60, 90 and 120min
Reference standard:	Carbazeran, Zoniporide, Famciclovir
Standard inhibitor:	Hydralazine (50µM)
Detection:	LC-MS/MS

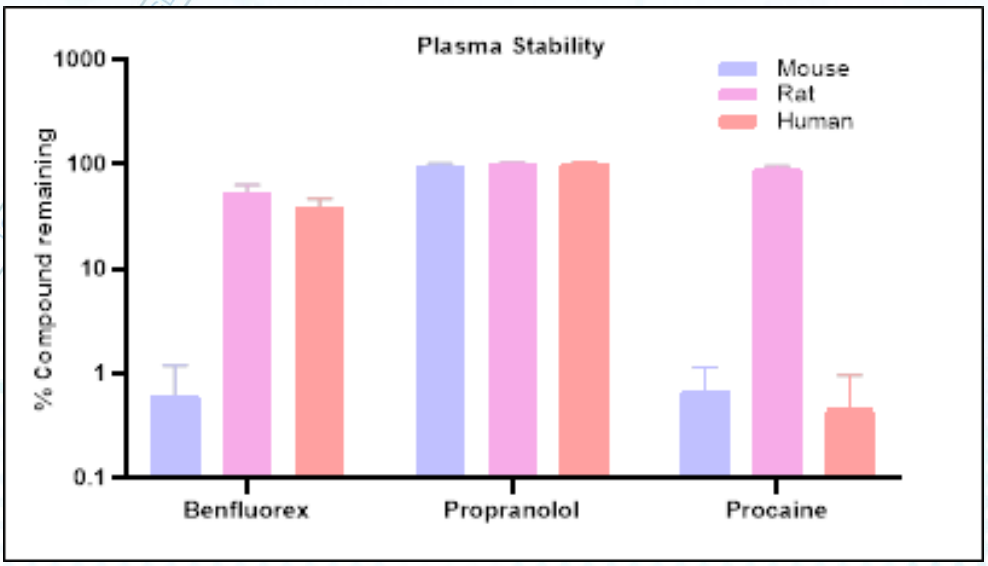


4.5. Plasma Stability

Plasma stability studies assess how drug molecules degrade in plasma due to esterases and other enzymes, providing insight into their potential *in vivo* behaviour. These study data are useful for evaluating a compound's overall half-life, bioavailability, and formulation design.

We perform this study using plasma from humans and multiple other species.

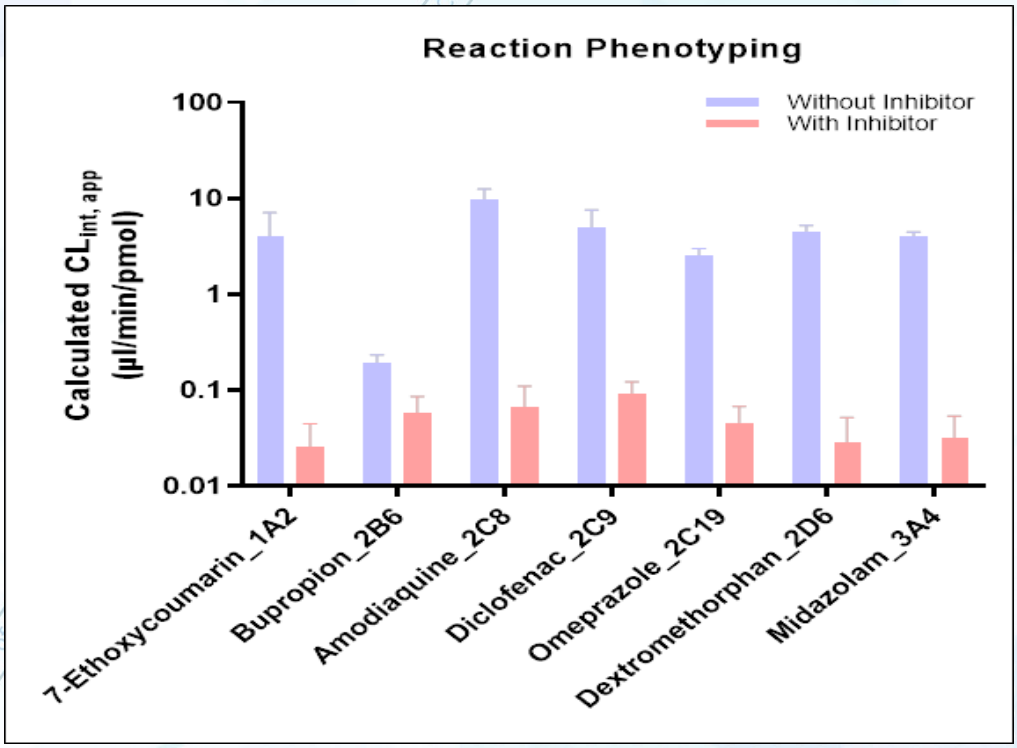
Test system:	*In vitro* plasma stability assay
Species:	Human/ rat/mouse/other species plasma
Organic solvent:	≤1%
Test concentration:	1µM, n=2
Incubation Period:	37°C for up to 2h (0, 60 and 120min)
Reference standard:	Benfluorex, Propranolol, Procaine
Detection Method:	LC-MS/MS



4.6. Reaction Phenotyping

Reaction phenotyping helps to identify which drug-metabolising enzymes are involved in the metabolism of a compound, revealing its metabolic pathway. This information is also useful in drug–drug interaction risk assessment and regulatory submissions. We use several human recombinant cytochrome P450 enzymes (rCYPs) and CYP inhibitors to identify and confirm the contribution of each CYP towards the overall metabolism of the compound.

Test system	*In vitro* rCYP reaction phenotyping
Source of CYPs	Recombinant Human, EasyCyp, Cypex, 50pmol/ml
Test concentration	1µM, n=2
Incubation	0, 5, 10, 20, 30, 45 minutes with NRS and 45min No-Cofactor (60NCF) at 37°C
Cofactor	NADPH (1.2mM)
Inhibitor Concentration	20µM
Reference standard	7-Ethoxy Coumarin / Tacrine (CYP1A2LR), Dextromethorphan (CYP2D6LR), Diclofenac (CYP2C9BR), Midazolam / Testosterone (CYP3A4LR), Bupropion / Efavirenz (CYP2B6LR + B5), Amodiaquine / Rosiglitazone (CYP2C8LR), S-Mephenytoin / Omeprazole (CYP2C19BLR)
Reference Inhibitors	Alpha Naphthoflavone (CYP1A2LR), Quinidine (CYP2D6LR), Sulfaphenazole (CYP2C9BR), Ketoconazole (CYP3A4LR), Miconazole (CYP2B6LR + B5), Montelukast (CYP2C8LR), Miconazole (CYP2C19BLR)



5. Drug–Drug Interaction (DDI) Assessment

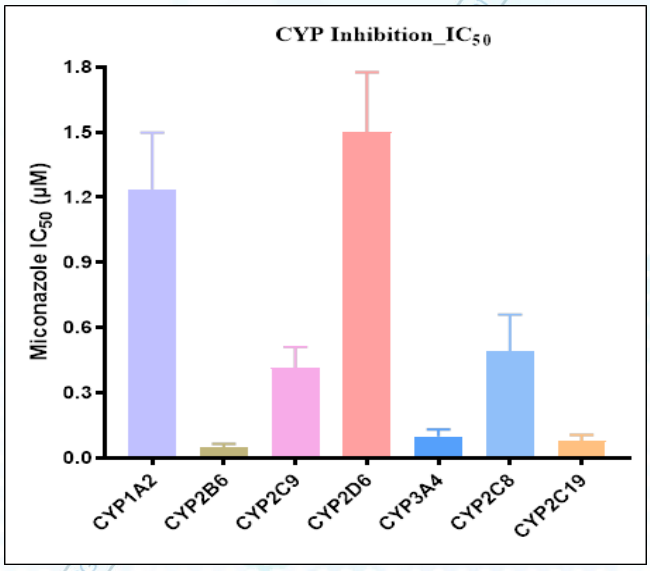
Drug–drug interactions (DDIs) occur when one drug (perpetrator drug) alters the ADME behaviour of another drug (victim drug). Our expertise includes evaluating potential liability through CYP-mediated metabolism, whether via CYP P450 inhibition or induction, supporting regulatory submissions.

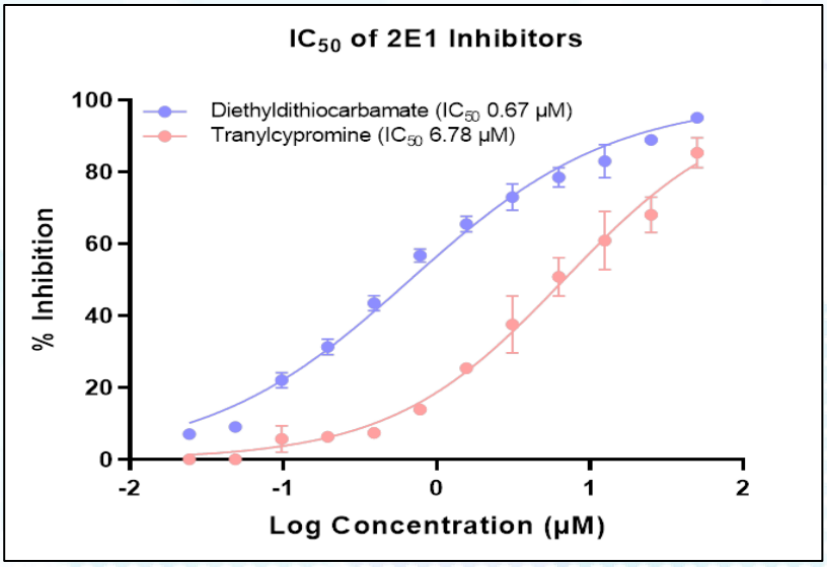
We offer the following services:

- Reversible CYP inhibition (IC_{50} / 2 concentration mode)
- Time dependent inhibition (TDI)
- K_{inact}
- K_I
- CYP induction: CYP1A2, 2B6, 3A4 [HepaRG]

5.1. CYP Inhibition Assay using Human Liver Microsomes

Test system	*In vitro* CYP inhibition assay
CYP Isoforms	CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4
CYP Source	Human liver microsomes (0.02 / 0.1 / 0.2mg mg/ml depending on CYP isoform)
Test conc	8 or 10 concentrations (IC_{50}) or single (3µM) or 2 concentrations (1 and 10µM); n=2
Substrates	CYP1A2: Tacrine (2µM); CYP2B6: Bupropion (20µM); CYP2C8: Amodiaquine (2µM); CYP2C9: Diclofenac (8µM); 2C19: S-Mephenytoin(80µM); CYP2D6: Dextromethorphan (2µM); CYP2E1: Chlorzoxazone (20µM); CYP3A4: Midazolam (2µM) and Testosterone (50µM)
Cofactor	NADPH (1.2mM)
Incubation	5/10/30 min based on CYP isoform
Standard Inhibitor:	Miconazole (Pan inhibitor) or specific inhibitors





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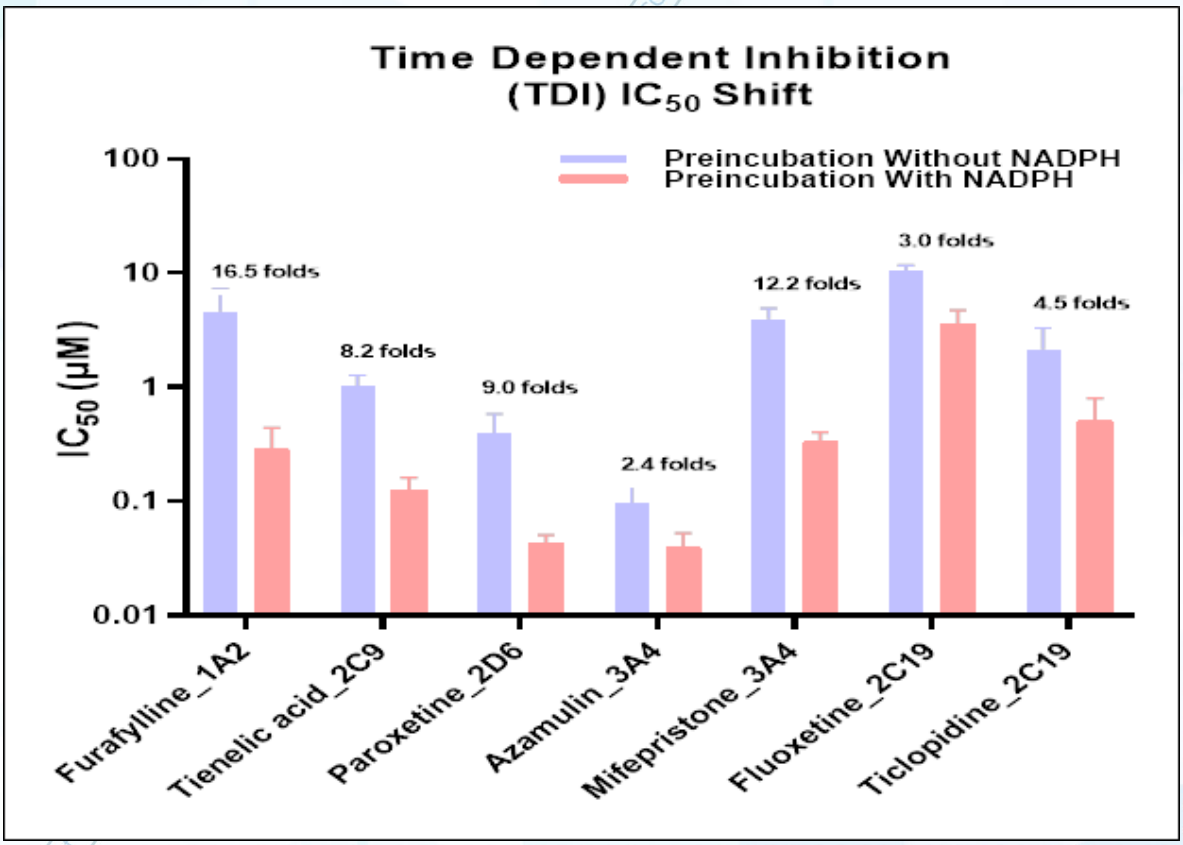
Time-Dependent CYP Inhibition Assay Using Human Liver Microsomes (hLM)

Cytochrome P450 (CYP) enzymes play a central role in drug metabolism, and inhibition of these enzymes can lead to clinically significant drug–drug interactions. Time-dependent inhibition (TDI) refers to irreversible or quasi-irreversible inactivation of CYP enzymes, where the inhibitory effect increases with pre-incubation of the test compound with hLM prior to addition of the substrate.

Our time-dependent inhibition assays evaluate the inactivation of major human CYP isoforms, including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4, by a chemically reactive metabolite of the test compound. Using probe substrates and industry-standard protocols, we deliver high-quality, reproducible data to support regulatory submissions. This assay enables differentiation among compounds that cause reversible, irreversible, or both reversible and irreversible inhibition.

Format of studies:

Quick and cost-effective: Single test concentration
Robust and extensive: IC_{50} (10 concentrations) and IC_{50} shift



K_{Inact} /K_I Determination Using Human Liver Microsomes

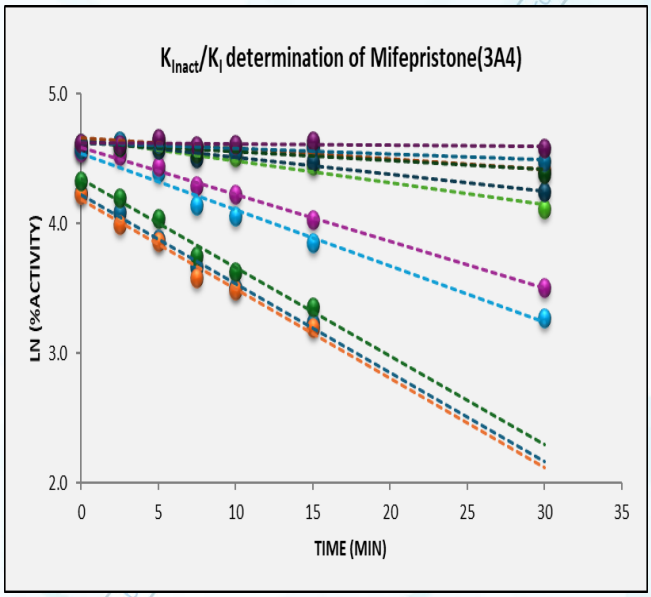
Understanding CYP-mediated drug metabolism is crucial for predicting and managing drug–drug interactions (DDIs) throughout the drug development lifecycle. Our tiered approach to CYP inhibition assessment encompasses:

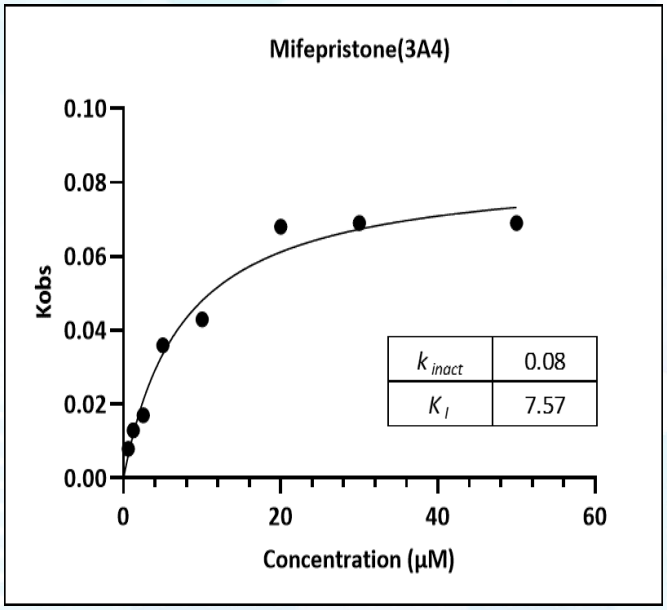
- **Tier I:** Reversible inhibition studies using IC_{50} or two concentrations
- **Tier II:** Time-dependent inhibition (TDI) evaluation using IC_{50} shift or single concentrations
- **Tier III:** K_{Inact} and K_I kinetic characterisation for five CYP isoforms (CYP1A2, 2C9, 2D6, 3A4, and 2C19)

These parameters— K_{Inact} reflecting the rate of enzyme inactivation, and K_I indicating inhibitor potency—are crucial for estimating the risk of clinically relevant DDIs. A high K_{Inact} / K_I ratio signals strong potential for time-dependent inhibition, impacting both safety and dosage strategies.

Our integrated platform supports preclinical *in vitro* assays, enabling robust, mechanism-based risk assessment and adding value to the discovery process.

Test system	KInact/KI Determination assay for CYP1A2, 2C9, 2D6, 3A4, 2C19 (cocktail of substrates) and CYP2C19 (single substrate)
CYP Source	Human liver microsomes
Cofactor	NADPH
Pre-incubation	30min with and without NADPH
Standard compound	Furafylline (1A2), Tienilic acid (2C9), Paroxetine (2D6), Azamulin (3A4), Mifepristone (3A4), Fluoxetine(2C19), Ticlopidine (2C19)
Data type	KInact/KI Value
Detection Method	LC-MS/MS (detection of metabolites)





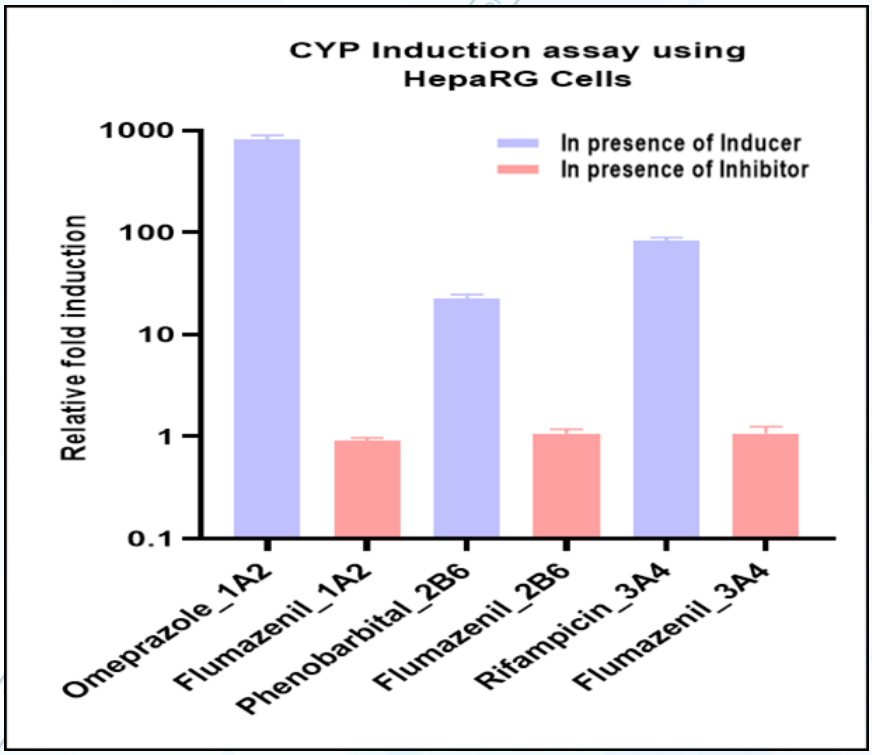
5.2. CYP Induction using HepaRG

Along with CYP inhibition, induction of CYP enzymes plays a crucial role in metabolism-based drug–drug interactions. Any compound with the potential to cause CYP induction may pose a risk to co-administered drugs that undergo metabolism via the same CYPs.

At TCGLS, we perform CYP1A2, CYP2B6, and CYP3A4 induction assays using HepaRG cells, a robust alternative to primary hepatocytes. Cells are treated with test compounds, and CYP mRNA levels are quantified by RT-qPCR, normalised to the housekeeping gene GAPDH.

Results are reported as fold induction versus vehicle control and compared with known inducers such as rifampicin, omeprazole, or phenobarbital.

Assay format	96-Well
Target CYPs	1A2(AhR), 2B6(CAR), 3A4(PXR)
Cell line	Cryopreserved HepaRG Cells
Reference inducer	Omeprazole (1A2), Phenobarbital (2B6), Rifampicin (3A4)
Negative control	Flumazenil
Detection	Taqman RT-qPCR



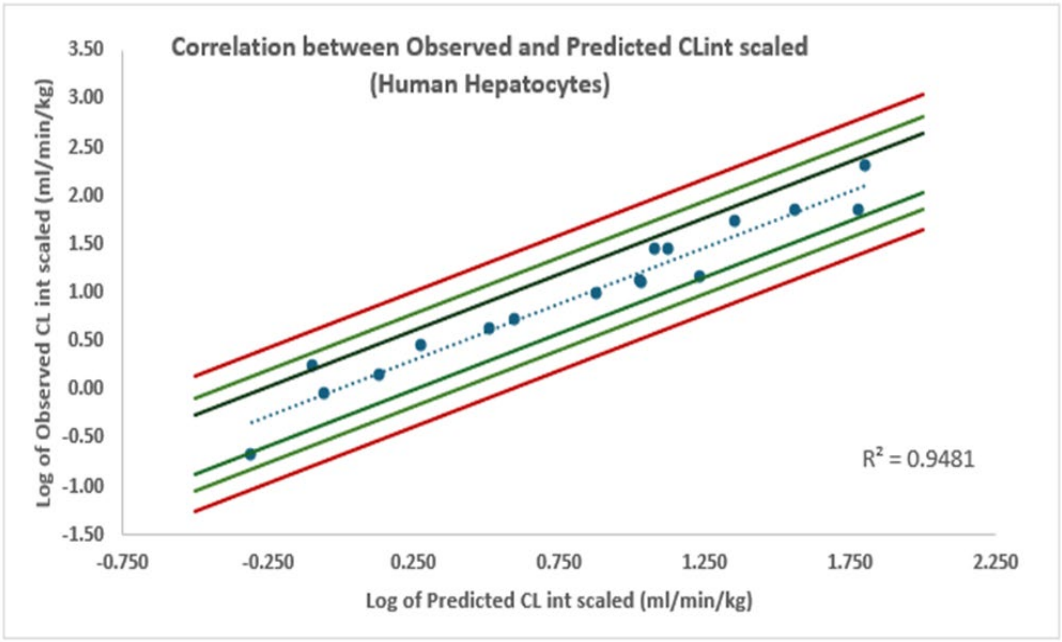
6. Customised Assays

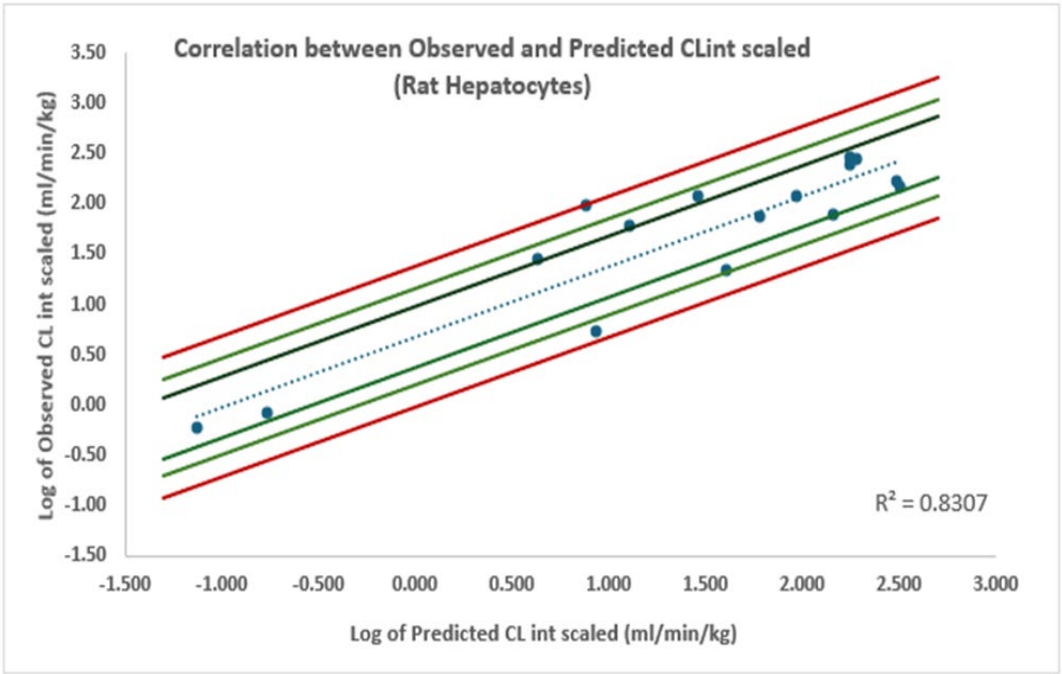
6.1. IVIVE (*In Vitro In Vivo* extrapolation)

While any *in vitro* study data comes with the underlying question of how accurately it reflects the *in vivo* situation, TCGLS has established *in vitro in vivo* correlation (IVIVC) with respect to:

a) Solubility in gastrointestinal fluid
b) Permeability
c) Metabolism using microsomes and hepatocytes

TCGLS has considered a set of compounds and performed metabolic stability, plasma protein binding, and blood-to-plasma ratio determination to extrapolate *in vitro* data to the predicted *in vivo* results. Further, these extrapolated values were compared with available *in vivo* data, and the correlation was good, suggesting a strong IVIVC.





6.2. Permeability Assay in the Presence of Cosolvent

The cosolvent-based permeability assay is a customised *in vitro* approach designed to address specific project needs, particularly for compounds with low aqueous solubility or poor recovery. By incorporating a defined percentage of cosolvents (within a tolerable limit for the cell monolayer) into the donor solution, this method enhances compound solubility and facilitates accurate assessment of passive permeability across biological membranes.

Additionally, it provides mechanistic insights into nonspecific binding, compound instability, or adsorption-related losses, thereby supporting a more comprehensive interpretation of permeability and recovery data.