Excellence in Drug Metabolism and Pharmacokinetics

Innovative CRO+Explorer
ICE Bioscience

DMPK Platform

In vitro ADME

Solubility
Lipophilicity
Buffer Stability
Permeability
Transporter
Plasma/Tissue Protein Binding
Blood to Plasma Ratio
Matrix Stability
Metabolism Stability
DDI
MetID

In vivo PK

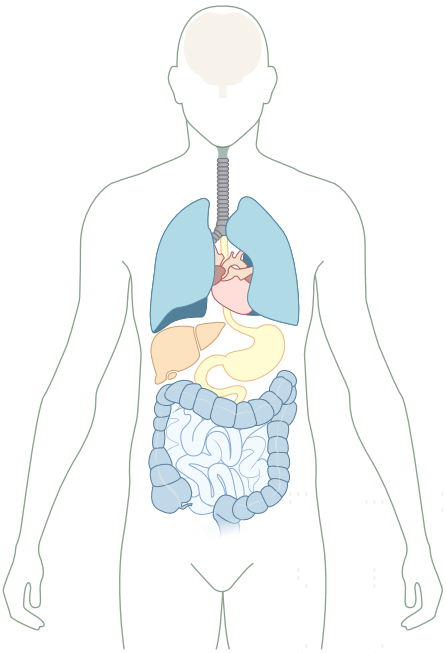
Screening PK
Tissue Distribution
Metabolism and Excretion
MetID
DDI risk evaluation
Surgery models
CNS solution
CNS PK、BBB
CSF （rodents), Brain
（Brain/Hippocampus/Corpus/
Cerebral cortex/Doesal raphe
nucleus/CSF/rodents）

Bioanalysis

Biological matrices(CSF/
Microsome/lysosome)
Method development
and validation
Capabilities
Small molecules
Therapeutic proteins
(mAb , BsAb , ADC etc.)
PROTAC
Peptides
Biomarkers
Covalent binding

Part I: ADME

Absorption (A): Absorption refers to the process by which a drug enters the bloodstream from its site of administration. For orally administered drugs, this involves passage through the gastrointestinal tract, followed by transport across the intestinal wall into the systemic circulation. Factors influencing absorption include the drug's solubility, permeability, and formulation, as well as physiological conditions like pH and gastrointestinal motility.



Distribution (D): Distribution describes how a drug is transported throughout the body once it enters the bloodstream. It involves the reversible transfer of the drug between the blood and tissues, influenced by factors such as blood flow, tissue permeability, and the drug's affinity for tissue and plasma proteins. The distribution phase determines the drug's concentration at various sites, affecting its therapeutic and toxic effects.

Metabolism (M): Metabolism is the chemical alteration of a drug by the body, primarily occurring in the liver. This process transforms the drug into metabolites, which can be either active or inactive. Metabolism usually occurs in two phases: Phase I reactions (e.g., oxidation, reduction) involving cytochrome P450 enzymes, and Phase II reactions (e.g., conjugation) that increase the drug's water solubility, facilitating its excretion. Metabolism is crucial for detoxifying drugs and preparing them for elimination.

Excretion (E): Excretion is the process by which drugs and their metabolites are eliminated from the body. This can occur via various routes, including the kidneys (urine), liver (bile), lungs (exhaled air), and skin (sweat). Renal excretion is the primary route for many drugs, involving filtration, secretion, and reabsorption processes in the kidneys. Efficient excretion prevents drug accumulation and potential toxicity.

In Vitro ADME Services Overview

Understanding ADME is crucial in the drug development process. ADME studies provide essential insights into how a drug behaves in the body, which directly impacts its therapeutic efficacy and safety. At ICE Bioscience, our comprehensive ADME services are designed to support drug discovery and development.

Service Category	Specific Service	Details
Physicochemical Property	Solubility	• **Kinetic Solubility:** Determines the rate at which a compound dissolves in a solvent. • **Thermodynamic Solubility:** Measures the equilibrium solubility of a compound in a solvent under specific conditions.
	Lipophilicity	• **LogD:** Measures the distribution of a compound between a lipophilic phase (usually octanol) and an aqueous phase, pH-dependent. • **LogP:** Measures the partition coefficient of a compound between octanol and water, independent of pH.
Permeability and Transporter	PAMPA (Parallel Artificial Membrane Permeability Assay)	Assesses passive permeability of compounds through a lipid membrane.
	Caco-2 and MDCK Assays	• **Caco-2:** Mimics the intestinal barrier to study absorption and transport mechanisms. • **MDCK-MDR1/BCRP:** Used for assessing permeability and P-glycoprotein-mediated transport.
	Transporters: SLCs and ABCs	• **SLCs (Solute Carrier Transporters):** Involved in the uptake of various molecules into cells. • **ABCs (ATP-Binding Cassette Transporters):** Mediate the efflux of drugs and metabolites out of cells.
Protein Binding	Plasma Protein Binding	Determines the extent of a compound's binding to plasma proteins, affecting its distribution and free drug concentration.
	Brain and LM Protein Binding	Evaluates binding to proteins in the brain and liver microsomes (LM), crucial for understanding drug penetration and metabolism.
	Red Blood Cell Partition Ratio	Measures the distribution of a compound between plasma and red blood cells, influencing drug efficacy and safety.
Metabolic Stability and DDI	Metabolic Stability	Assessed using liver microsomes, S9 fractions, hepatocytes, plasma, and whole blood to predict in vivo metabolism.
	Drug-Drug Interaction (DDI) Studies	• **Enzyme Stability:** Evaluates the stability of compounds in the presence of metabolic enzymes. • **GSH Trapping:** Detects reactive metabolites by trapping with glutathione. • **CYP and UGT Inhibition/TDI:** Studies the inhibitory effects of compounds on cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) enzymes, including time-dependent inhibition (TDI). • **CYP Induction:** Assesses the potential of compounds to induce CYP enzymes, affecting drug metabolism. • **CYP Phenotyping:** Identifies which CYP enzymes are involved in the metabolism of a compound.
	Specialized Enzyme Inhibition Studies	Inhibition of specific enzymes such as CYP11B1, CYP11B2, CYP4A11, CYP4F2, CYP17A1, CYP21A2, ERAP1, and URAT1, providing detailed insights into metabolic pathways and potential drug interactions.
MetID	Metabolite Identification	Identifies and characterizes metabolites in biological matrices.
Drug-Induced Liver Injury (DILI)	Cell Viability and Mitochondrial Toxicity	Measures the cytotoxic effects of compounds on liver cells to predict potential liver toxicity.
	Oxidative Stress Markers	• **SOD (Superoxide Dismutase) Activity:** Assesses the antioxidant defense system in cells. • **MDA (Malondialdehyde) Levels:** Evaluates lipid peroxidation as an indicator of oxidative stress.

Key Advantages

- **Flexibility:** Adaptable services tailored to each client's needs.
- **Customization:** Tailored ADME studies for small and large molecules.
- **Efficiency:** Fast, reliable results from our advanced facilities and experienced scientists.
- **Data Consistency:** High-quality, reproducible data using advanced technologies and standardized protocols.

In Vitro Permeability Study

The prediction of oral and blood-brain barrier (BBB) permeability is essential for the successful development of new drug candidates. The expression of transporters, receptors, and the presence of tight junctions significantly influence a molecule's ability to permeate these barriers. At ICE Bioscience, we offer a range of in vitro permeability models to evaluate these critical factors, ensuring comprehensive and accurate predictions for various types of molecules.

Model	Molecule Type	Application
PAMPA-BBB	Non-polar small molecules	Evaluates passive permeability through a simulated lipid bilayer.
MDCK	Small molecules	Assesses general epithelial permeability.
MDCK-MDR1	Small molecules	Studies the role of P-glycoprotein in drug efflux, relevant for BBB and intestinal permeability.
MDCK-BCRP	Small molecules	Examines interactions with breast cancer resistance protein.
Caco-2	Small molecules	Mimics the intestinal epithelium, widely used for predicting oral drug absorption.
bEnd.3 single-cell model	Biologics	Investigates BBB permeability using mouse brain endothelial cells.
hCMEC/D3 single-cell model	Biologics	Uses human brain microvascular endothelial cells to model BBB permeability.
bEnd.3 + U251 double-cell model	Biologics	Combines mouse brain endothelial cells with human glioma cells for a more comprehensive BBB model.
hCMEC/D3 + U251 double-cell model	Biologics	Integrates human brain endothelial and glioma cells to study BBB permeability.
hCMEC/D3, U251, and SH-SY5Y co-culture model	Biologics	A complex co-culture model including human brain endothelial cells, glioma cells, and neuroblastoma cells to provide a detailed analysis of BBB permeability.

PAMPA-BBB Assay Sample Data

Compound ID	Pex10^{-6} (cm/s)	-LOG Pe
Methotrexate	<0.0005	>9.34
	<0.0003	>9.49
Testosterone	36.65	4.44
	24.01	4.62
Naproxen	0.95	6.02
	0.79	6.10
Antipyrine	1.15	5.94
	1.29	5.89
Atenolol	<0.0002	>9.60
	<0.0004	>9.44
Verapamil	34.62	4.46
	38.34	4.42
Quinidine	34.13	4.47
	27.73	4.57

In vitro Transporter Study

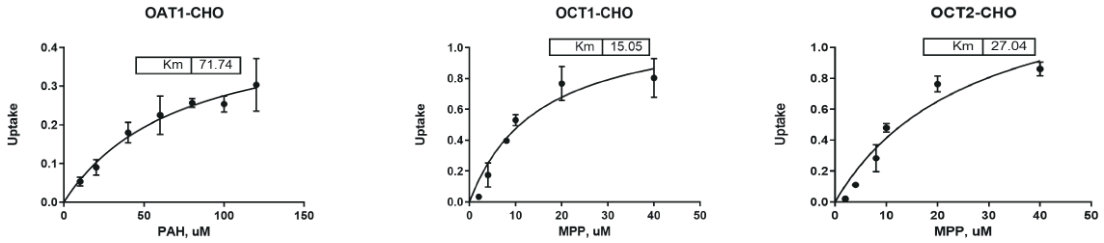
ATP-Binding Cassette (ABC) Family Transporter

The ATP-binding cassette (ABC) family of transporters plays a crucial role in the export of various substances across cellular membranes. These transporters are essential in mediating drug resistance and influencing drug pharmacokinetics and bioavailability.

- **P-glycoprotein (P-gp):** We evaluate drug interactions with P-gp to understand and overcome drug resistance, enhancing absorption and distribution, particularly in cancer therapeutics.
- **Breast Cancer Resistance Protein (BCRP):** Our services assess interactions with BCRP to improve drug bioavailability and disposition, helping to mitigate resistance mechanisms.
- **Multidrug Resistance Proteins (MRPs):** We study interactions with MRP1 and MRP2 to understand drug distribution and excretion, crucial for developing effective treatments for cancer and other diseases.

Solute Carrier (SLC) Superfamily Transporter

The Solute Carrier (SLC) superfamily comprises a diverse group of membrane-bound transport proteins that facilitate the movement of a wide variety of substrates, including ions, metabolites, and drugs, across cellular membranes. These transporters play critical roles in physiological processes and are significant in the pharmacokinetics and pharmacodynamics of therapeutic agents.



Transporter	Substrate	Inhibitor
OATP1B1	Estradiol-17β-Glucuronide	Rifampicin
OATP1B3	Estradiol-17β-Glucuronide	Rifampicin
OAT1	Para-aminohippurate	Probenecid
OAT3	Estrone-3-sulfate	Probenecid
OCT1	Metformin(1-methyl-4-phenylpyridinium (MPP+)	Verapamil
OCT2	Metformin(1-methyl-4-phenylpyridinium (MPP+)	Verapamil
MATE1	TEA(1-methyl-4-phenylpyridinium (MPP+)	Pyrimethamine
MATE2K	TEA(1-methyl-4-phenylpyridinium (MPP+)	Pyrimethamine

Drug-Induced Liver Injury

Drug-induced liver injury (DILI) remains a challenge in clinical practice and is still a diagnosis of exclusion. Although it has a low incidence amongst the general population, DILI accounts for most cases of acute liver failure with a fatality rate of up to 50%

- **General Categories of DILI:**

Direct: Drugs have direct and predictable toxicity on liver, biliary, sinusoidal endothelial and stellate cells.

Indirect: This category also includes liver injury associated with some immunotherapies, as well as the reactivation of hepatitis B viral infection triggered by exposure to certain immunomodulatory or immune suppressive agents.

Idiosyncratic: It is believed to be precipitated by the interplay of several critical factors including the toxicological properties of the drug in conjunction with selective host-related factors and environmental conditions.

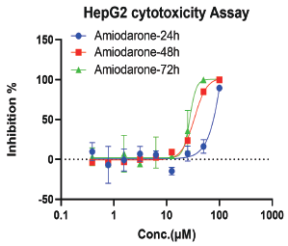
- **DILI Assay:**

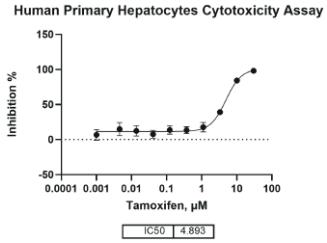
Amiodarone and tamoxifen are the DILI+ drugs included in the drug-Induced Liver Injury Severity and Toxicity (DILIst) Dataset. Amiodarone can cause cute hepatic necrosis, hepatic steatosis, secondary sclerosing cholangitis and acute fatty liver. Tamoxifen cause hepatic steatosis and peliosis hepatis.

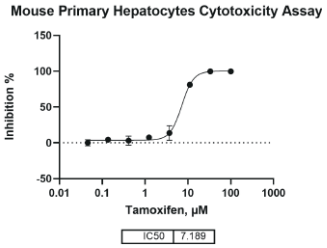
After incubation for 24h, 48h, and 72h, cell viability tests were conducted on cell cultures. Our data shows amiodarone can induce damage to HepG2 cells, and this experiment can be used as a routine DILI assay for drug screening evaluation.

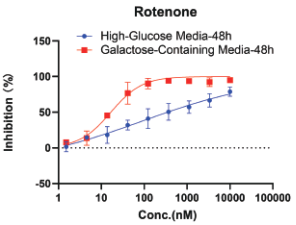
After 24h of incubation, tamoxifen causes significant damage to the viability of human and mouse primary hepatocyte.

When HepG2 cells are cultured in galactose medium, they exhibit increased sensitivity to mitochondrial toxins compared to when cultured in glucose medium. The ratio of the IC50 values obtained from cells cultured in glucose medium versus those in galactose medium serves as an indicator of mitochondrial toxicity.The known mitochondrial toxins rotenone shows mitochondrial toxicity in the galactose/glucose assay.









Drug-Drug Interaction

The ICH M12 guideline, finalized in May 2024, standardizes methodologies for assessing drug-drug interactions (DDIs) across regulatory bodies, facilitating global drug development. It covers enzyme- and transporter-mediated interactions, includes detailed recommendations for evaluating metabolite interactions, and encourages model-informed drug development approaches, ultimately improving consistency and efficiency in predicting and managing DDIs during drug development

We offer comprehensive and precise Drug-Drug Interaction services designed to evaluate the potential interactions of your drug candidates with cytochrome P450 (CYP) enzymes and UDP-glucuronosyltransferases (UGTs). Our services include:

- **CYP Inhibition/UGT Inhibition:**

Percentage Inhibition (Single Point): We assess the inhibitory effect of compounds at a single concentration to rank compounds based on their inhibition potential.

IC50 Determination: We measure the half-maximal inhibitory concentration (IC50) to categorize compounds as follows:

Potent inhibition: IC50 < 1 μM

Moderate inhibition: 1 μM < IC50 < 10 μM

No or weak inhibition: IC50 > 10 μM

Ki Measurement: We determine the inhibitory constant (Ki) to define the inhibitory affinity of the compounds.

- **CYP Induction:**

  > HepG2-Reporter Assay:

  PXR (Pregnane X Receptor): CYP3A and CYP2C.

  AhR (Aryl Hydrocarbon Receptor): CYP1A2.

  CAR (Constitutive Androstane Receptor): CYP2B6.

  > Primary Hepatocytes:

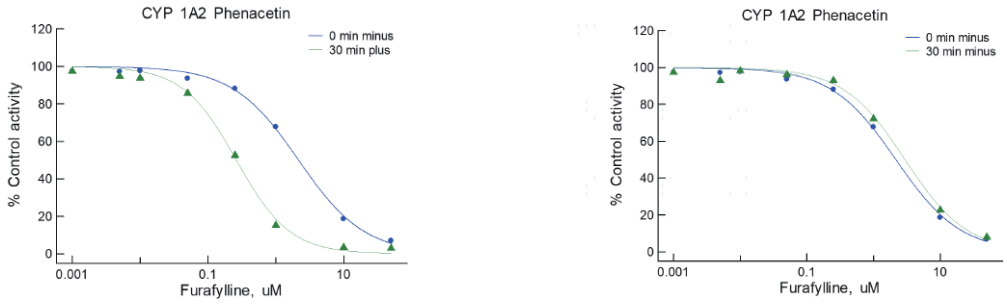
  mRNA Quantification: Using qPCR to measure mRNA levels.

  Enzyme Activity: Utilizing LC-MS/MS to assess enzyme activity.

CYP Isoform	UGT Isoform
CYP 1A2	UGT1A1
CYP 2A6	UGT1A3
CYP 2B6	UGT1A4
CYP 2C8	UGT1A6
CYP 2C9	UGT1A9
CYP2C19	UGT2B7
CYP 2D6	
CYP 2E1	
CYP3A4(Midazolam)	
CYP3A4(Testosterone)	
CYP2J2	

Time-Dependent Inhibition (TDI) in Human Liver Microsomes (HLM)

Our TDI services in HLM are designed to evaluate the inhibitory potential of compounds over time on cytochrome P450 (CYP) isoforms.

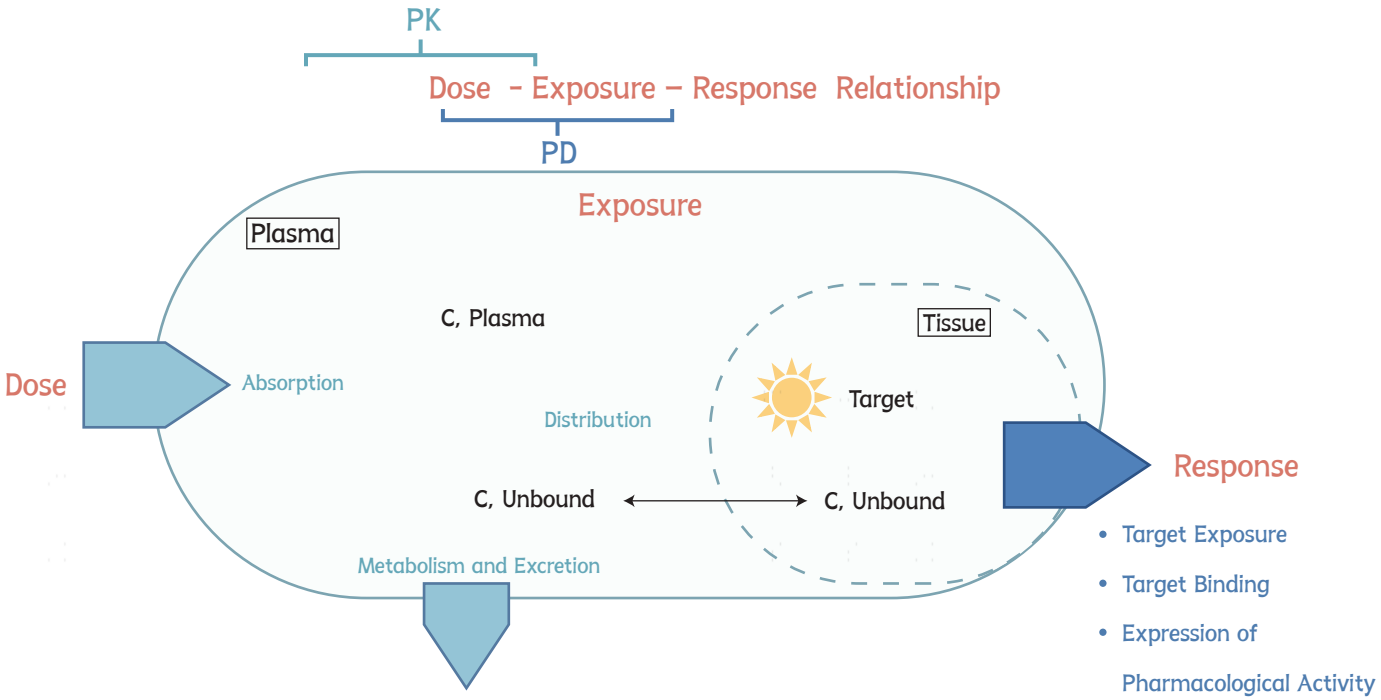


Furafylline show TDI for CYP1A2 by the 10-fold IC50 shift

CYP Isoform	Compound ID	Substrate	0 min minus/μM	30 min plus/μM	30 min minus/μM
CYP 1A2	Furafylline	Phenacetin	2.16	0.26	2.96
CYP 2A6	Xanthotoxin	Coumarin	0.91	0.03	0.71
CYP 2B6	ThioTEPA	Bupropion	8.09	1.94	11.02
CYP 2C8	Gemfibrozil	Paclitaxel	IC 50 > 50	3.05	IC 50 > 50
CYP 2C9	Ticrynafen	Diclofenac	0.54	0.10	0.76
CYP2C19	Flouxetine	(S)-mephenytoin	11.80	1.63	10.62
CYP 2D6	Paroxetine	Dextromethorphan	0.37	0.05	0.55
CYP 2E1	Diethyldithiocarbamate	Chlorzoxazone	IC 50 > 50	14.13	44.24
CYP 3A4	Mifepristone	Midazolam	4.13	0.30	3.63
CYP 3A4	Mifepristone	Testosterone	2.61	0.38	6.00

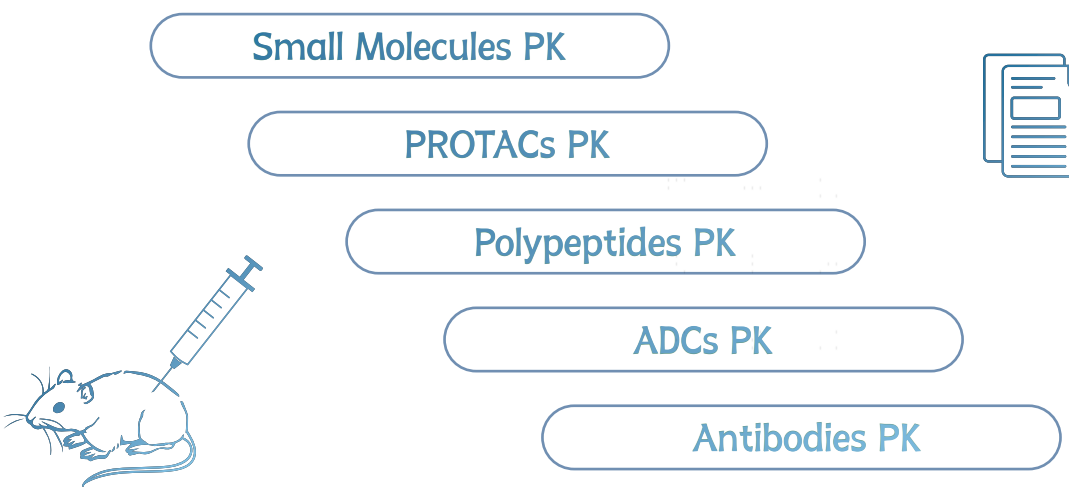
Part II: In vivo PK

In vivo pharmacokinetics (PK) plays a critical role in drug discovery by providing comprehensive data on the ADME of drug candidates in living organisms. This information is crucial for understanding the drug's behavior in the body, optimizing dosage forms, and predicting human pharmacokinetics. In vivo PK studies help identify potential issues such as poor bioavailability, rapid clearance, or toxic metabolites early in the development process, enabling the selection of drug candidates with favorable pharmacokinetic profiles and reducing the risk of late-stage.



Comprehensive *In Vivo* Pharmacokinetic Services

We offer specialized PK services tailored to a diverse range of therapeutic modalities. Our expertise spans from small molecules to complex biologics, ensuring precise and reliable PK data to support your drug development process. Here are the key PK services we provide:



- **Formulation Screening**
- **Plasma PK Design:**
  - Full PK
  - Rapid PK
  - Snapshot PK
  - Cassette PK
  - CNS PK
- **Tissue Distribution:**
  - Brain Tissue Distribution
  - Lung Tissue Distribution
  - Other Target Tissue Distribution
- **Metabolism and Excretion**
  - Metabolite Analysis and Metabolites Identification
  - Excretion: Urine/Feces/Bile
- **General Toxicology Services**
  - Acute Toxicity Test
  - Subacute Toxicity Test
  - Long-term Toxicity Test

SPF Animal House and IVC Cages

Species Supported: Mouse, rat, dog, monkey

Facilities: Specific Pathogen-Free (SPF) animal housing with Individual Ventilated Cages (IVC) to ensure the highest standards of care and research accuracy.

Administration Methods

Administration Methods	Description
Intravenous Injection	Direct delivery of drugs into the bloodstream for immediate systemic distribution.
Oral Administration	Non-invasive method for studying the absorption and first-pass metabolism of orally administered drugs.
Intraperitoneal Injection	Delivery of drugs into the peritoneal cavity, commonly used in rodent studies for systemic exposure.
Subcutaneous Injection	Administration of drugs under the skin for slow and sustained drug release.
Intramuscular Injection	Injection of drugs into muscle tissue for rapid absorption into the bloodstream.
Intravenous Drip	Continuous intravenous infusion of drugs, allowing for controlled and steady-state drug delivery.

Local Drug Delivery Methods	Description
Pulmonary Inhalation	Direct delivery of drugs to the lungs to study pulmonary absorption and local effects.
Intrathecal Drug Delivery	Administration of drugs into the spinal canal to bypass the blood-brain barrier, targeting the central nervous system.
Cutaneous Administration	Topical application of drugs on the skin.
Lateral Ventricle Administration	Delivery of drugs directly into the brain's lateral ventricles.

Intubation Techniques

Intubation Technique	Description
Jugular Vein Intubation	Allows for direct administration of compounds and continuous blood sampling, providing accurate and consistent PK data.
Hepatic Portal Vein Intubation	Used to study the first-pass metabolism of drugs by delivering compounds directly to the liver, enabling detailed analysis of hepatic drug metabolism.
Bile Duct Intubation	Facilitates the collection of bile to study biliary excretion and metabolism of drugs, providing insights into drug clearance and enterohepatic circulation.
Femoral Vein Intubation	Provides an alternative route for drug administration and blood sampling, often used in long-term studies and for compounds with specific vascular targets.
Endobronchial Intubation	Allows for the administration of drugs directly into the lungs, used to study pulmonary absorption and distribution of inhaled medications.

Sample Collection

Tissue
Heart
Liver
Spleen
Lung
Kidney
Brain
Intestines
Skin
Fat
Muscle
Spinal Cord

Blood Collection Method
Retro-orbital Vein
Jugular Vein
Hepatic Portal Vein
Abdominal Aorta Vein
Saphenous Vein
Heart

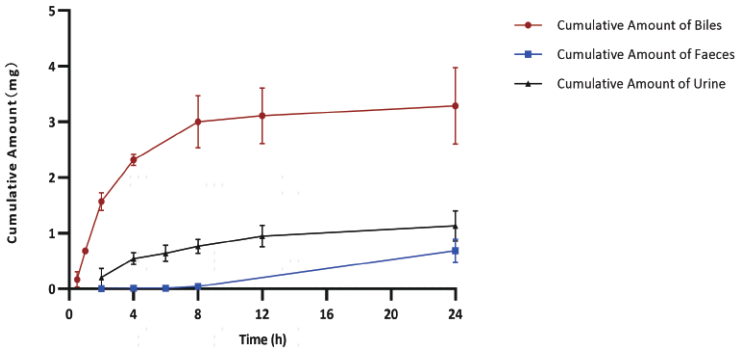
Subsample Category	Specific Subsamples
Brain	Cerebral cortex
	Hippocampus
	Corpus striatum
	Dorsal raphe nucleus
	Cerebrospinal Fluid
	Cerebellum
	Mesencephalon
	Brainstem
	Callosum
	Conarium

Subsample Category	Specific Subsamples
Intestines	Duodenum
	Cecum
	Ileum
	Colon
	Jejunum
	Mesenteric lymph nodes

Featured Services: In Vivo Biliary Duct Cannulation (BDC)

Biliary Duct Cannulation (BDC) services are primarily used to study the metabolism and excretion of drugs in vivo. By performing BDC surgery, we can directly collect bile samples, which provide detailed information on drug metabolism. The specific aspects of BDC services include:

- Biliary Duct Cannulation Surgery
- Metabolic Cage
- Bilirubin Determination
- Intravenous Infusion
- Collection of Bile, Urine, and Feces
- Metabolite Identification
- Cumulative Excretion Rate



Cumulative excretion of bile, feces, and urine in SD rats following intravenous administration of Rifampicin at 30 mg/kg. The graph illustrates the cumulative amounts of bile (red), feces (blue), and urine (black) over a 24-hour period, highlighting the primary routes of elimination for the drug.

Overcoming Challenges in PROTACs PK with Strategic Solutions

PROTACs (Proteolysis Targeting Chimeras) represent a groundbreaking class of therapeutic agents with the potential for high efficacy. However, their unique characteristics present several pharmacokinetic challenges that need to be addressed to optimize their effectiveness in vivo:

- Poor Absorption
- Low Exposure
- Low Bioavailability
- Low Dose Requirements
- Long Half-Life
- Good Effectiveness

Animal Feeding Strategies

We use advanced animal feeding protocols to optimize the administration of PROTACs, ensuring consistent and accurate delivery during preclinical studies.

Use of HP-β-CD as an Excipient

Hydroxypropyl-β-cyclodextrin (HP-β-CD) is used to increase the solubility and stability of PROTACs. This excipient enhances the bioavailability of poorly soluble compounds, improving their systemic exposure.

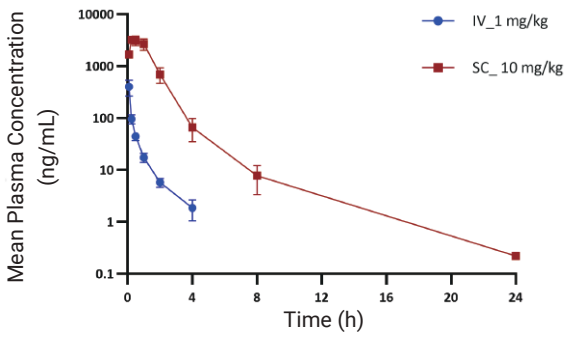
Optimizing Drug Delivery Formulations

We develop and optimize drug delivery formulations to enhance the absorption and bioavailability of PROTACs. Tailored formulation strategies help in achieving effective therapeutic levels while maintaining safety.

Monitoring and Identification of Metabolites In Vivo

Our advanced metabolite monitoring and identification techniques allow us to thoroughly understand the metabolism and excretion profiles of PROTACs. This helps in optimizing dosing regimens and minimizing potential off-target effects.

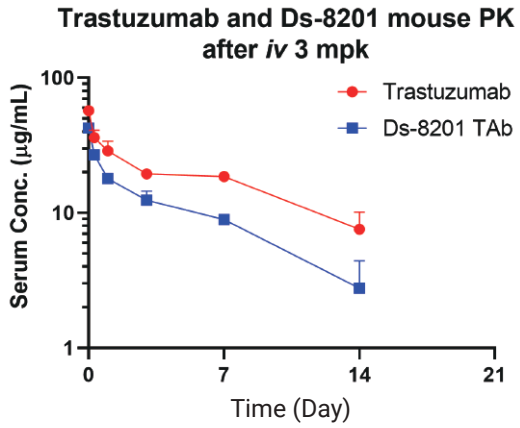
Mean Plasma Conc. of ARV-771 male mouse IV 1 mg/kg_SC 10 mg/kg PK

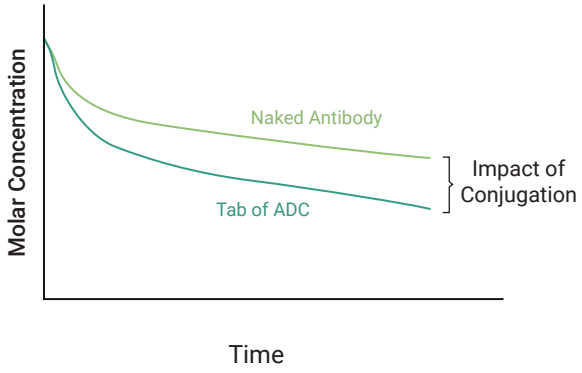


Pharmacokinetic profile of ARV-771 in male subjects following intravenous (IV, 1 mg/kg) and subcutaneous (SC, 10 mg/kg) administration. The graph illustrates the concentration-time curves, highlighting the differences in absorption and distribution between the two routes of administration.

Analysis of Conjugation Impact on ADC Pharmacokinetics

The conjugation of a payload to an antibody significantly impacts the pharmacokinetics of ADCs, leading to faster clearance and altered distribution compared to naked antibodies. This analysis underscores the importance of considering these changes in the design, dosing, and therapeutic strategies for ADCs to ensure their effectiveness and safety in clinical applications.





Comparative Analysis of ELISA and LC-MS Assays for ADC Pharmacokinetics

Both ELISA and LC-MS assays offer valuable insights into the pharmacokinetic properties of ADCs. Both methods provide consistent and reliable PK measurements. The LC-MS assay stands out as a powerful tool for the pharmacokinetic analysis of ADCs, offering superior sensitivity, specificity, and quantitative accuracy. Our expertise in LC-MS technology enables us to provide detailed and reliable PK profiles, supporting the optimization and successful development of ADC therapeutics.

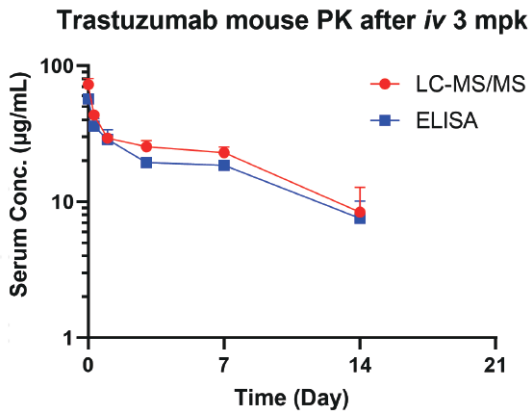
Advantages of LC-MS Assay for ADC Pharmacokinetics

High Sensitivity: Detects very low concentrations of analytes for accurate measurement.

Specificity and Multiplexing: Simultaneously quantifies multiple ADC components, providing a comprehensive profile.

Quantitative Accuracy: Offers precise and reproducible quantification of drug concentrations.

Broader Dynamic Range: Suitable for detailed kinetic studies across various dosing regimens.



PK	Unit	ELISA assay	LC-MS assay
$t_{1/2}$	day	7.54±3.17	7.53±3.09
C_0	μg/ml	57.57±0.62	73.39±9.75
AUC_{last}	day*μg/ml	252.5±11.61	270.95±86.98
CL	ml/day/kg	8.97±1.71	7.48±2.17

Chapter III: Non-GLP Bioanalysis

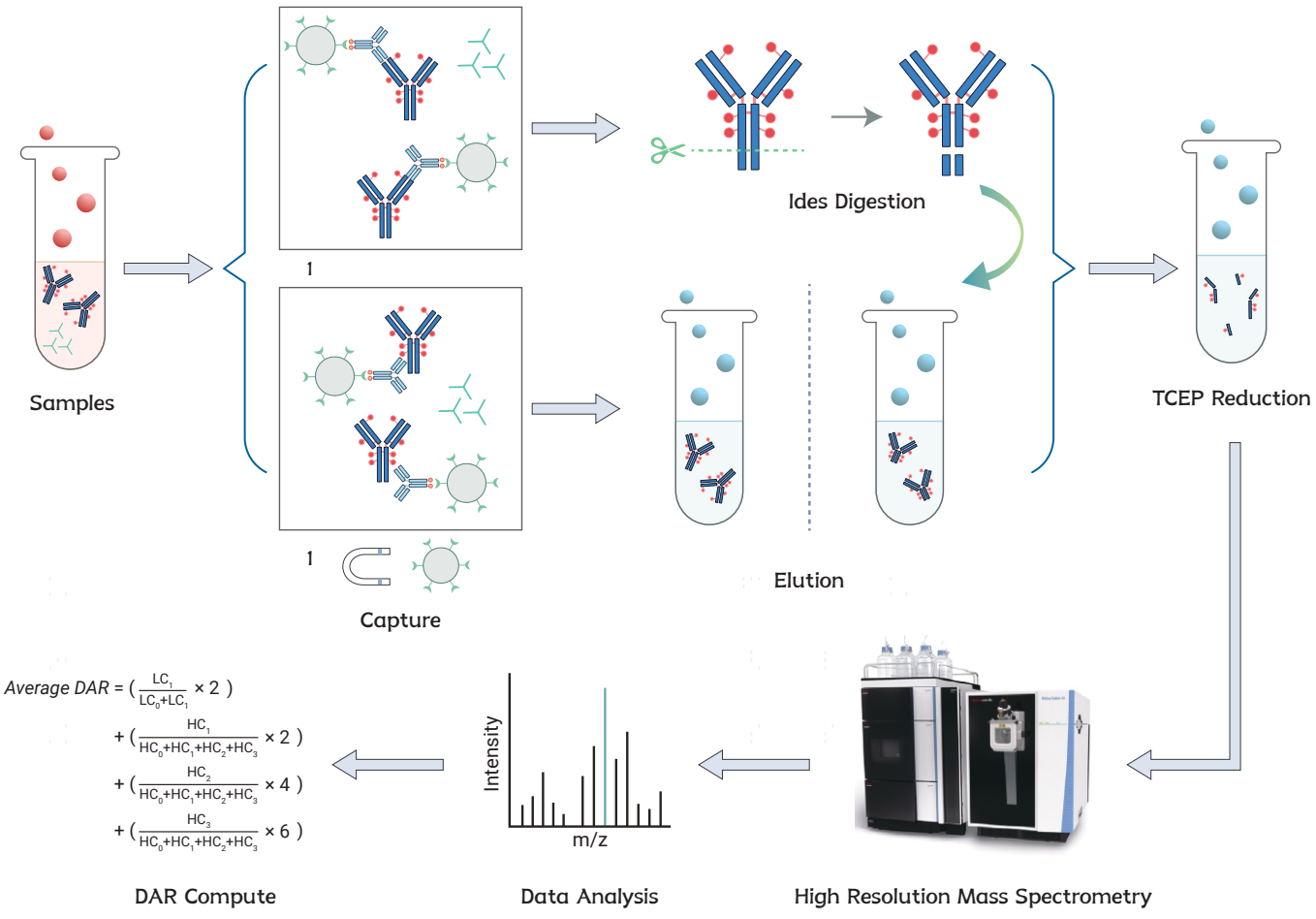
We provide comprehensive bioanalytical services that encompass the precise quantification and stability assessment of small molecules, as well as the more complex analysis of large molecules such as Antibody-Drug Conjugates (ADCs) and polypeptides. Utilizing advanced technologies like LC-MS/MS and Ligand Binding Assays (LBA), we deliver accurate and reliable data for both small and large molecule therapeutics, addressing the unique challenges associated with each.

Key Bioanalysis Services:

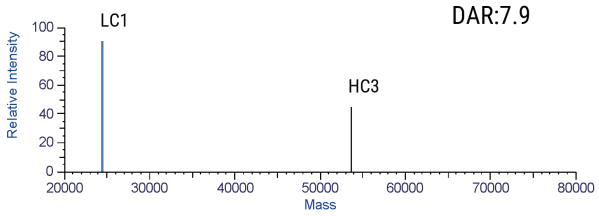
- **Method Development and Validation:** We develop and validate robust bioanalytical methods to ensure accurate and reliable measurements.
- **Biomarker Analysis:** We identify and quantify biomarkers to support pharmacodynamic (PD) studies and evaluate therapeutic efficacy.
- **Metabolite Identification:** Using advanced techniques, we identify and characterize drug metabolites, providing insights into metabolic pathways.
- **Sample Analysis:** Employing state-of-the-art LC-MS/MS and other analytical technologies, we perform high-throughput sample analysis for a variety of biological matrices.

Plasma Stability of ADC

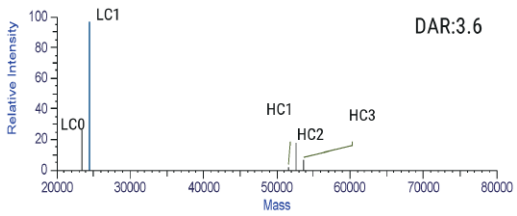
The plasma stability of Antibody-Drug Conjugates (ADCs) is significantly influenced by the Drug-Antibody Ratio (DAR) and the free payload release rate. The number of drug molecules attached to each antibody, known as the DAR, affects the overall stability and pharmacokinetics of the ADC, playing a crucial role in its therapeutic performance. Additionally, the rate at which the drug (payload) is released from the ADC in plasma impacts its effectiveness and potential toxicity, making controlled release essential for targeted action.



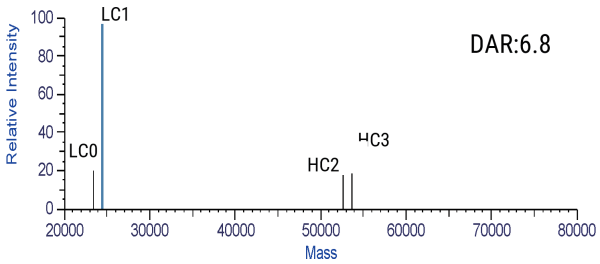
Flow Chart for Analyzing the DAR Value of ADC Drugs in Biological Samples



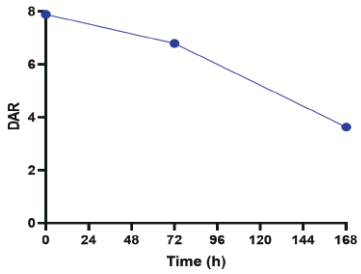
Stability of DS-8201 in plasma samples at 0 day



Stability of DS-8201 in plasma samples at 7 day



Stability of DS-8201 in plasma samples at 3 day



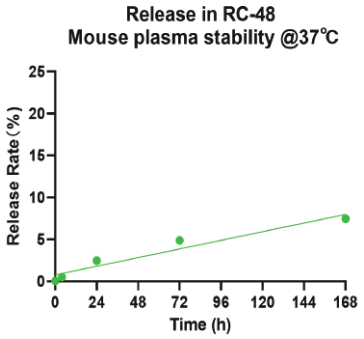
DAR in DS-8201 plasma stability @37℃

High-resolution mass spectrometry analysis of the stability of DS-8201 in plasma samples at different times

Determination of Released Payload and Total Antibody

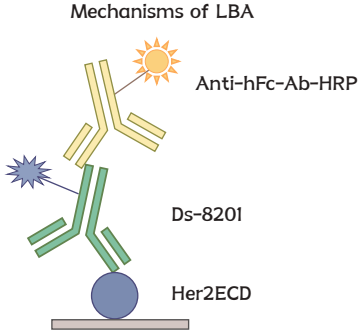
We measure the concentration of the payload in various biological matrices (e.g., blood, tissues) to understand its release profile. This data is critical for evaluating the therapeutic potential of the ADC and ensuring that the payload is released at the desired site of action.

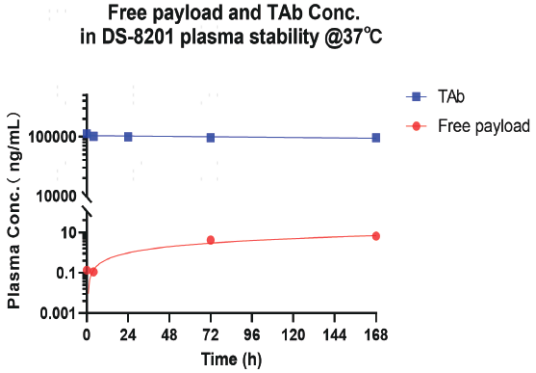
Techniques such as Liquid Chromatography-Mass Spectrometry (LC-MS) or Ligand Binding Assays (LBA) are employed to accurately quantify the total antibody levels. Assessing total antibody levels helps in understanding the distribution, metabolism, and clearance of the ADC, which is essential for dose optimization and reducing potential side effects.



Free Payload of RC48

Time(h)	Concentration (ng/mL)
0	0.463
4	4.026
24	19.19
72	38.113
168	58.201





Biomarker Analysis

Biomarkers play a pivotal role in the diagnosis, monitoring, and treatment of various diseases. In bioanalytical research, the accurate detection and quantification of biomarkers are essential for understanding disease mechanisms and developing effective therapeutics. At ICE Bioscience, we specialize in the analysis of both small and large molecule biomarkers, including endogenous peptides such as neurotransmitters and bradykinin, which are crucial indicators of physiological and pathological states.

Understanding Biomarkers in Bioanalysis

Biomarkers are measurable indicators of biological processes, conditions, or diseases. They can be proteins, nucleic acids, lipids, or metabolites. In the context of bioanalysis, biomarkers help in:

- **Disease Diagnosis:** Identifying specific biomarkers associated with diseases to facilitate early diagnosis.
- **Therapeutic Monitoring:** Measuring biomarker levels to monitor the efficacy and safety of treatments.
- **Pathophysiological Understanding:** Studying biomarker changes to gain insights into disease mechanisms.

CASE STUDY: LC-MS/MS Detection of Bradykinin in Plasma and Cerebrospinal Fluid

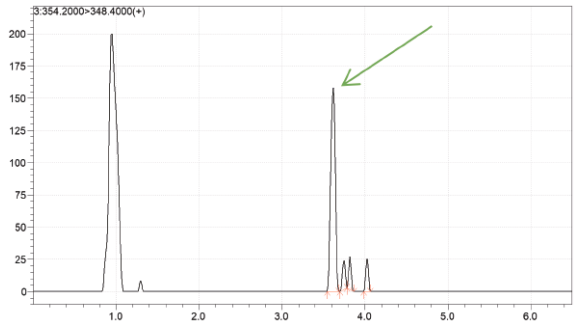
Bradykinin (BK) is a vasoactive peptide composed of nine amino acids (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), generated from kininogen by kallikrein. It plays a crucial role in the kallikrein-kinin system and serves as an important serum biomarker for diagnosing Alzheimer's Disease (AD). Due to its short half-life and difficulty in detecting it in the central nervous system, bradykinin is primarily measured in peripheral blood to reflect central changes associated with AD pathology.

Challenges and Solutions:

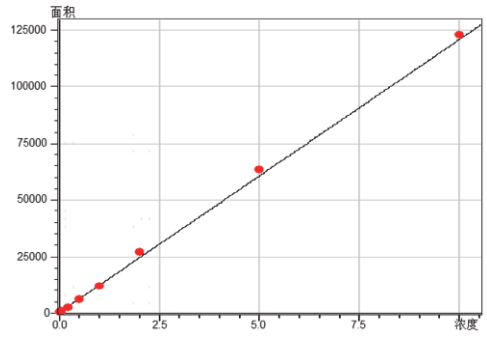
- **Sample Adsorption:** Used special low-adsorption materials and reagents to prevent sample loss.
- **Sample Stability:** Collected samples at low temperatures and added enzyme inhibitors to stabilize bradykinin in plasma.
- **Interference and Recovery:** Employed in-house developed solid-phase extraction (SPE) to enrich and purify plasma samples, achieving a lower limit of quantification (LLOQ) of 10 pg/mL.

Advantages:

- **Cost-Effective:** No need for commercial kits.
- **High Throughput:** Automated processing of 96-well plate samples.
- **Low Sample Volume:** Requires only 50 microliters of sample.
- **High Specificity:** Precise quantification without cross-reactivity.



Sample chromatogram showing the Lower Limit of Quantification (LLOQ) for bradykinin at 10.0 pg/mL with a retention time (RT) of approximately 3.6 minutes.



Calibration curve for bradykinin quantification with the equation Y = 12031.0X + 477.142 and a correlation coefficient (R^2) of 0.9967.

ICE Innovative CRO+Explorer
ICE Bioscience

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.

To learn more, please visit: en.ice-biosci.com