Drug Metabolism and Pharmacokinetics

Drug metabolism and pharmacokinetics (DMPK) sets up a bridge between chemistry, biology, pharmacology and toxicology in the whole process of drug research and development, from discovery to clinical trial.

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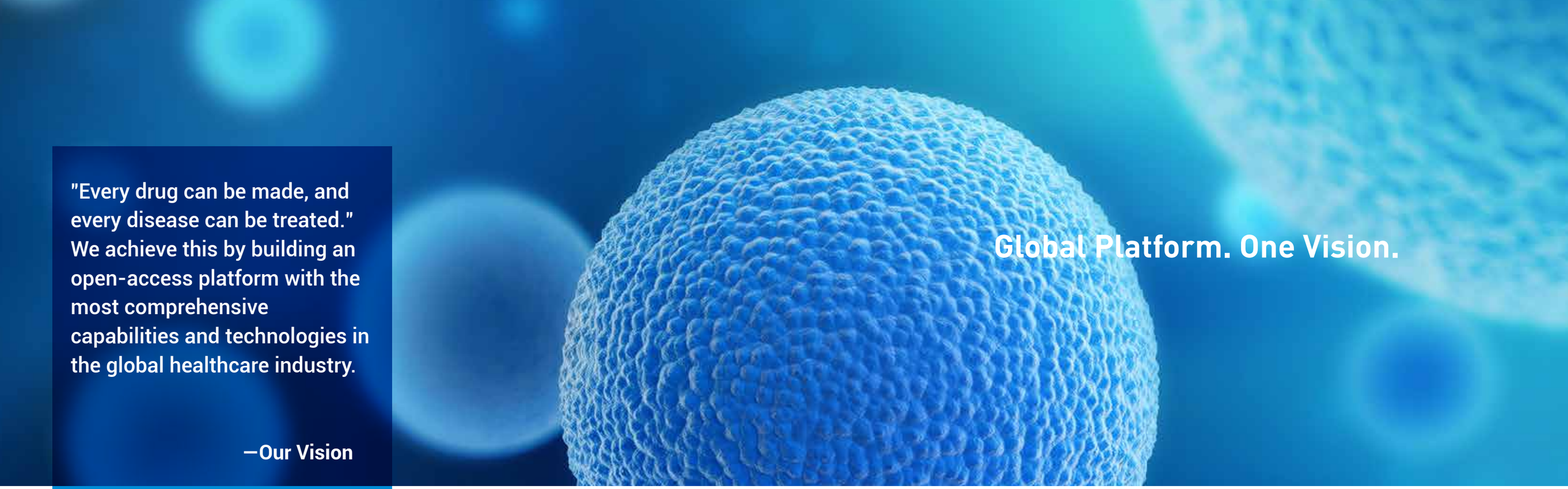
药明康德
WuXi AppTec

Contents



"Every drug can be made, and every disease can be treated." We achieve this by building an open-access platform with the most comprehensive capabilities and technologies in the global healthcare industry.

—Our Vision

Global Platform. One Vision.

About WuXi AppTec

As a global company with operations across Asia, Europe, and North America, WuXi AppTec provides a broad portfolio of R&D and manufacturing services that enable global pharmaceutical and healthcare industry to advance discoveries and deliver groundbreaking treatments to patients. Through its unique business models, WuXi AppTec' s integrated, end-to-end services include chemistry drug CRDMO (Contract Research, Development and Manufacturing Organization), biology discovery, preclinical testing and clinical research services, cell and gene therapies CTDMO (Contract Testing, Development and Manufacturing Organization), helping customers improve the productivity of advancing healthcare products through cost-effective and efficient solutions.

Integrated, End-to-end Enabling Platform

Small Molecule, Oligonucleotide and Peptide Drug Discovery Capability

Pharmaceutical Development and Manufacturing Capability

Drug R&D, and Medical Device Testing Capability

Clinical Research Capability

Cell and Gene Therapy R&D, Testing and Manufacturing Capability

WuXi AppTec's Laboratory Testing Division

We are passionately committed to our role in enhancing the quality of human lives. We are a unique, world-class, globally integrated testing platform that supports the entire continuum of the drug development journey. We enable scientists to transform their ideas into healthcare products that ultimately improve life from discovery through preclinical to clinical and beyond.

We are dedicated to providing the highest level of attention to you and your programs. Our subject matter experts (SMEs) are there with you every step of the way to collaborate and work through the unique challenges of drug discovery and development.

As your partner, you have access to a team that operates with complete transparency and industry-leading turnaround times. We provide the insights and high-quality data you need to advance your project to the next milestone while meeting international standards for regulatory compliance.

Integrated Testing Platform

The Laboratory Testing Division (LTD) has built three testing platforms—preclinical drug development, clinical drug development, and medical device testing.

Preclinical Drug Development Platform

WuXi AppTec IND (WIND) combines technical experience, program management, and regulatory expertise to facilitate the submission of clients' IND packages.

Clinical Drug Development Platform

The Laboratory Testing Division provides a complete lifecycle of pharmaceutical R&D to drive your drug from the early R&D stage to clinical trials.

Medical Device Testing Service Platform

The WuXi AppTec's Laboratory Testing Division provides end-to-end regulatory and technical expertise to support your medical device's entry into the market.

End-to-End Services

LTD provides pharmacokinetic, toxicological, bioanalytical, and medical device testing services. It works closely with other departments at WuXi AppTec to offer a one-stop service to clients.

DMPK Services

Comprehensive *in vitro* and *in vivo* ADME solutions from high-throughput screening in discovery through drug development phases.

Safety Assessment Services

Our skilled team of toxicologists provides comprehensive safety assessments across a wide range of species and through various routes of administration.

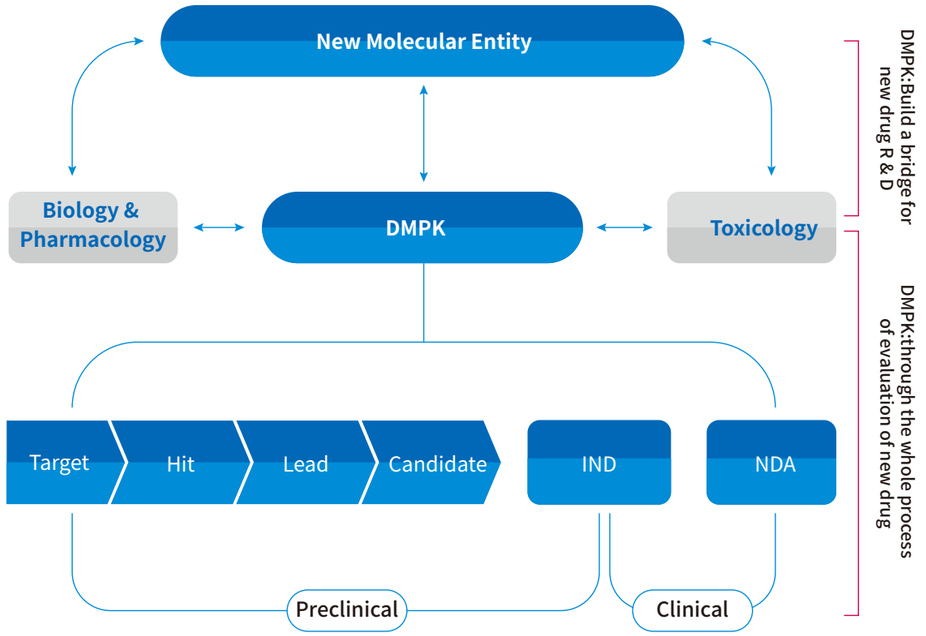
Bioanalytical Services

Supporting your drug discovery and seamlessly moving your program from preclinical to clinical development.

Drug Metabolism and Pharmacokinetics

The purpose of DMPK is to study the characteristic interactions of a drug and the body for its absorption, distribution, metabolism, and excretion. The pharmacokinetics study of innovative drugs and new dosage forms, combined with the mechanism of action and safety assessments of the drugs, helps drug R&D institutions discover more bioactive and safer molecular entities and elucidates the causes of drug efficacy and toxicity from the DMPK perspective.

The pharmacokinetics characteristics of New Molecular Entities (NMEs) should be studied and evaluated throughout the whole process of new drug research and development. In preclinical development, adequate pharmacokinetics studies can significantly improve the success rate of the translation of preclinical results to clinical manifestations. Pharmacokinetics assessments in clinical research stages play a crucial role in characterizing drug performance in humans and improving drug package inserts. During the research and development of new drugs, pharmacokinetics is equally essential as pharmacodynamics and toxicology. It is part of the journey of a compound from the chemist's shelf to becoming a therapeutic drug.



The DMPK Service Department of WuXi AppTec

The DMPK Service Department helps you quickly promote the R&D process from drug discovery to a new drug application.

The DMPK Service Department is one of the business units under the Laboratory Testing Division. It is primarily responsible for *in vivo* and *in vitro* pharmacokinetics studies. When you partner with WuXi AppTec for drug discovery and development services, you gain access to an integrated worldwide network of facilities that offer a full range of discovery screening, preclinical development, clinical drug metabolism, and pharmacokinetic (DMPK) platforms and services. These include *in vitro* absorption, distribution, metabolism, and excretion (ADME) studies and *in vivo* pharmacokinetic studies, metabolite identification studies, quantitative autoradiography studies, human radioactive substance balance, and metabolite safety evaluation studies. With locations in the United States (New Jersey) and China (Shanghai, Suzhou, and Nanjing), we can help you navigate every aspect of the drug development process—no matter what the stage.

DMPK Service Department has a diversified client community, covering more than 90% of the world's large pharmaceutical companies with more than 1,000 small and medium-sized biopharmaceutical companies, virtual companies, non-profit organizations, and academic institutions. As a global DMPK service provider, we are committed to delivering all clients with high-quality data, precise R&D strategies, and excellent services to meet the unique needs of every client and become the preferred partner for DMPK studies.

Drug Development Process	What WuXi DMPK can provide
Hit to lead	▪ High-throughput screening of DMPK experiments suitable for early R&D stages ▪ Experimental design and research strategy according to the characteristics of the project.
Lead optimization	▪ Pharmacokinetics study strategies and optimization recommendations based on the characteristics of test compounds
Preclinical candidate (PCC)	▪ Comprehensive interpretation of pharmacokinetics properties of test compounds ▪ Species selection to support GLP toxicological assessment on safety ▪ Support for PK/PD studies
Investigational New Drug (IND) application	▪ IND submission strategy and IND study packages (Support clients in submitting IND applications to regulatory agencies worldwide)
Clinical studies	▪ A basis for dosing frequency and dose setting ▪ Improved package inserts of drugs

Why Choose the WuXi AppTec DMPK Service Department

Customized Services

- Provides customized solutions at different stages of new drug research and development according to client projects.
- Offers a service model dedicated to improving project success.
- Excellent service system, rich experience, trustworthiness.

Integrated Services*

- WuXi AppTec's departments can work together to support a client's project. Our integrated services provide our clients with flexible development options that result in significant time and cost savings
- The Laboratory Testing Division offers a complete spectrum of IND-enabling services (WIND)

Digital Operations

- Precise and efficient digital operation management systems ensure hundreds of thousands of *in vitro* and *in vivo* experiments annually.
- Digitalization of the whole process from test article receiving to report submission protects client intellectual property.
- The project progress is transparent and visible. Clients can remotely review project progress in real-time.

Teams and Facilities

- Four global R&D centers have passed regulatory inspections from the AAALAC, FDA, NMPA, and EPA.
- We have nearly 1,000 R&D scientists, with over half of the team possessing a doctor's or master's degree.
- An orderly supply of animal resources ensures that clients' projects are timely executed.
- We serve more than 1,000 customers worldwide.

Innovation and Development

- To prospectively establish a research and testing service system for cutting-edge pharmaceutical R&D areas and new drug modalities.
- We successfully support IND applications in multiple innovative fields to ensure client success.
- Papers on DMPK studies submitted by our scientists are regularly published in notable international journals.

*For details, please refer to the introduction of the integrated enabling platform of WuXi AppTec and Laboratory Testing Division on pages 03-04

Customized Services

▶ Trustworthy Solution Provider

New drug discovery stage: developing R&D strategies and improving R&D efficiency

In the discovery stage, we can provide customized services for clients by designing step-by-step DMPK strategies according to the project characteristics, achieving a higher hit ratio during drug screening, which can shorten the R&D cycle of obtaining the regular pattern between structure-activity relationships (SAR) and DMPK properties. These include:

- Lead compound discovery: The design of an effective and rapid *in vitro* and *in vivo* high-throughput screening studies.
- Lead optimization and preclinical candidate (PCC): More comprehensive and in-depth pharmacokinetics studies will be conducted by interpreting previous data combined with preliminary pharmacodynamic and toxicological results to provide reliable experimental data and a solid research basis for PCC.

IND application stage: excellent service system, rich application experience

The DMPK Service Department provides precise application strategy and testing services in the IND application stage that adheres to indications and drug modalities. An excellent experimental system and data management system accompany successful applications of client projects. We offer:

- Support for submitting Investigational New Drug (IND) applications to regulatory authorities worldwide.
- More than ten years of experience by supporting over 500 IND submissions.
- Established pharmacokinetics research strategies for various disease areas and new drug modalities (peptides, antibodies, oligonucleotides, PROTACs, etc.).

▶ Proprietary service model dedicated to increasing project success

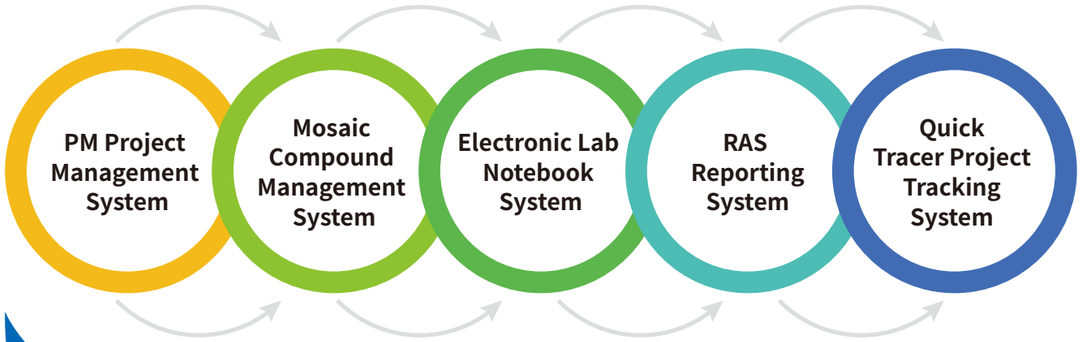
Our "Customer First" philosophy guides our approach and makes our customer and their programs our central focus. Each client has a dedicated Study Director who takes charge of the client's DMPK studies from screening to the IND stage. Study Directors with extensive experience in drug research and development and DMPK professional backgrounds offer a full range of project management services.

The Study Director works seamlessly with experienced scientists and researchers to provide clients with the comprehensive pharmacokinetic test service required to research and develop new drugs based on the research needs. The DMPK Service Department is divided into *in vitro*, *in vivo*, bioanalysis, and operation teams to serve clients better. The division achieves a higher degree of specialization and constantly improves our capability to ensure high-quality, efficient project delivery.

To address any unique challenges encountered in the project, DMPK Service Department will establish a group consisting of the Study Directors, senior DMPK experts, and functional heads to provide clients with rapid and professional solutions.

Digital Operations

Full-circle digital operation management:
precise and intelligent operation system, efficient and safe data delivery



Dynamic transparency of materials and systematization of intellectual property protection

By implementing comprehensive digital operation management from test article receipt to report submission, every project is delivered more efficiently and safely.

- Digital operations bring dual improvements in capacity and turnaround time to quickly locate resources and deliver reports.
- Intellectual property protection, quality, and compliance assurance are more optimized. Digital operation management provides real-time records while fully protecting client intellectual property.
- Close monitoring is achieved through the digital operating system, from project management, compound management, data management to automated report output. The clients can track the progress of their projects via this remote real-time monitoring system.

Why Choose the WuXi AppTec DMPK Service Department

Digital Operation System				
	Project Management	**Precise and intelligent process management** Systematic management from contract signing to project delivery makes the process more transparent and more intelligent.	**Automatic and efficient experiment management** Automatically creates millions of sample labels every year and scans codes to capture the dynamic trajectory of samples.	**Safe and compliant authority management** The system has a clear layered structure with authority limitations, so all users operate only within the scope of their authorization
	Compound Management	**100% Recording Accuracy of Compounds** The compounds are weighted on a high-precision balance with real-time transmission and recording of weighing data. Tens of thousands of weighing times occur per month, but scientists can accurately retrieve the inventory and usage of compounds.	**100% Accuracy of Aliquot and Solution Preparation** The Hamilton automatic workstation calculates the required amount of solvent according to the instructions and automatically prepares the solution with a target concentration and aliquot. Real-time data synchronization guarantees that the process is more reliable.	**10-second ability to locate storage position** After compound information is registered, the compound position can be located in batches in various ways with 100% accuracy within 10 seconds, ensuring efficient project delivery.
	Data Management	**Real-time recording of operating traces** An audit trail system can record all online operation traces in real-time, ensuring accurate and safe records.	**Standard recording template** The customized recording system was developed to precisely match DMPK experimental types and achieve online recording. The sample code can be acquired with one click instead of manual editing, accurately generated for 100,000 + cumulative projects.	**Secure data backup** VERITAS NetBackup software enables incremental backup of electronic data daily and full weekly backup, so data is secured and recoverable.
	Automated Reporting	**Avoidance of human writing errors** To avoid human errors, copying and pasting was eliminated from more than 100 steps which occurred in traditional editing.	**Man-machine collaboration to ensure quality** The human-computer synergy mode not only improves QC efficiency by 70%, but also allows scientists to shift their focus of work from data alignment to data plausibility, which doubles the accuracy of data and reports.	**More rapid report delivery** Report delivery can be achieved in less than 1.5 hours. Individual report writing times were reduced by 78% after project completion.

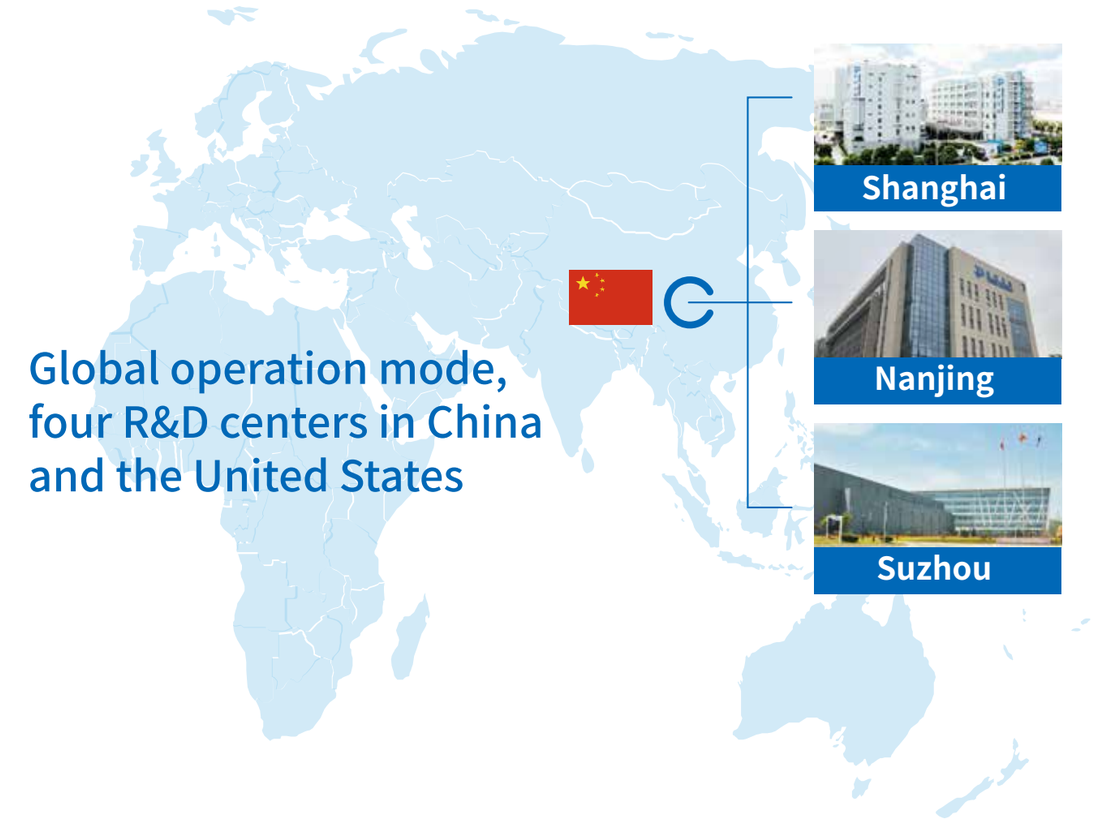
Remote control: project progress is transparent and visible, so that you can check the whole progress remotely and in real time

The QuickTracer online tracking system displays project progress in total transparency and real-time with one click on your PC or mobile phone.

QuickTracer Project Full Process Example

QuickTracer Project Progress Example

Team and Facility



Shanghai

Nanjing

Suzhou

Global operation mode, four R&D centers in China and the United States

R&D team:

Nearly 1,000 scientists, more than 50% of employees with PhDs / MS Degree

Adequate animal resources and efficient project management

Long-term supply agreements with global leading animal vendors ensure animal resources for the project's immediate execution.

Why Choose the WuXi AppTec DMPK Service Department

Cranbury, NJ

Successfully serve more than 1,000 global clients

Shanghai

In Vitro ADME Studies, Rodent Pharmacokinetics Studies, Drug Metabolite Identification Studies, Non-GLP Bioanalysis

Nanjing

In Vitro ADME Studies, *In Vivo* Pharmacokinetics Studies, Drug Metabolite Identification Studies, Pharmacokinetics Studies of Radiolabeled Test Articles, Non-GLP Bioanalysis

Suzhou

In Vivo Pharmacokinetics Studies, Non-GLP Bioanalysis

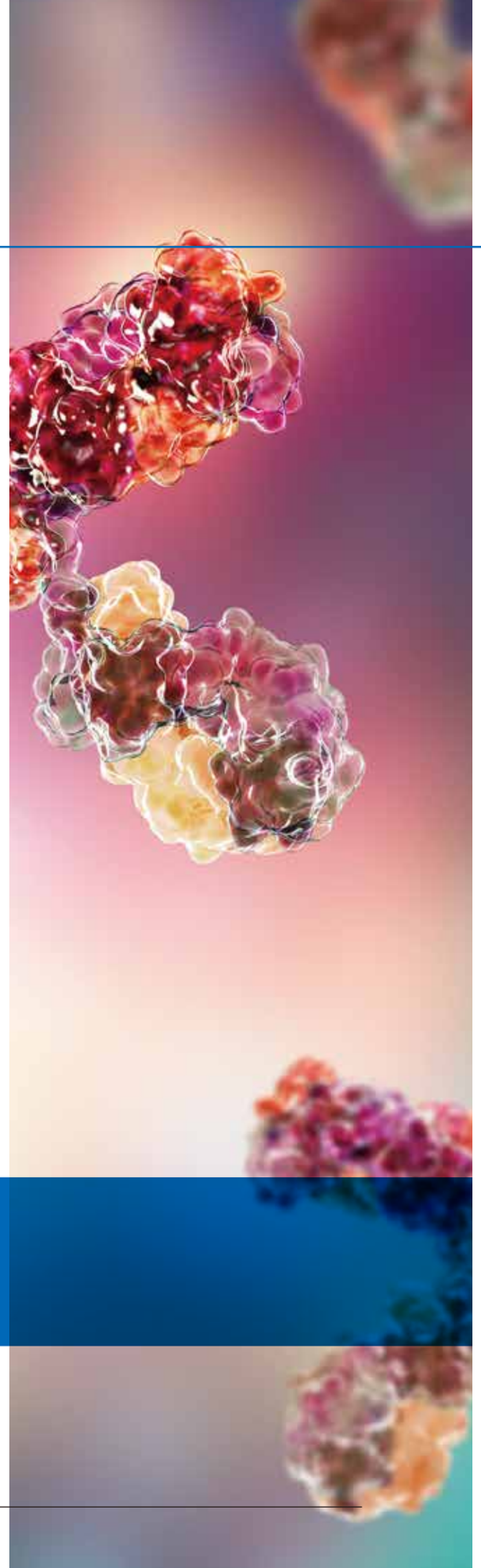
Cranbury, NJ

In Vitro ADME Studies, Rodent Pharmacokinetics Studies, Drug Metabolite Identification Studies, Pharmacokinetics Studies of Radiolabeled Test Articles, Non-GLP Bioanalysis

Innovation and Development

Our team of scientists continue to strive to improve our research capabilities and broaden our scope of drug-enabling platforms through innovative ideation and partnership. We have established pharmacokinetic research strategies and test service systems for each type of new drug modality. Our capabilities in new drug modalities help our clients and global partners achieve success.

With the deepening understanding of disease pathogenesis and drug mechanism of action, continuous innovation and breakthroughs occur in drug research and development (R&D). Novel therapeutic approaches and new drug modalities continue to emerge and occupy an increasingly important position. New drug modalities (such as therapeutic proteins, peptides, oligonucleotides, antibody-drug conjugates (ADC), proteolysis-targeting chimeras (PROTACs), etc.) are significantly different from conventional small molecule drugs in both structural characteristics and pharmacokinetic behaviors. Each new drug modality has unique research strategies, and higher requirements are required for the corresponding analytic ability. Appropriate pharmacokinetic studies and druggability evaluations are conducted for each new drug modality and are critical for researchers in the field.



Why Choose the WuXi AppTec DMPK Service Department

Our dedicated R&D team established a comprehensive set of pharmacokinetic research strategies and test systems from *in vitro* to *in vivo* for different new drug modalities. The R&D team includes scientific consultants and experts in various fields with more than 10 PhDs as the core members covering *in vitro* and *in vivo* ADME, metabolite identification, QWBA and isotope mass balance, and bioanalysis.

We collaborate with well-known pharmaceutical and biotech companies that have focused on new drug modalities for years. Extensive experience is attained by annually testing considerable amounts of new modality molecules.

Our scientists continuously share their DMPK knowledge and experience of new drug modalities in the field by publishing scientific articles in peer-reviewed journals, presenting research at international academic conferences, and completing multiple patent applications.

Part of our patents

01 *In Vitro* ADME Services

The absorption, distribution, metabolism, and excretion (ADME) properties of a drug represent the drug's disposition process by the body; ADME properties of a drug are determined by its structure. Measuring the ADME properties of a drug can provide an essential value for predicting its bioavailability and biological activity (i.e., whether a drug can reach its target and exhibit the corresponding therapeutic effect). In the 1990s, approximately 40-50% of drug candidates' failure rate was attributed to unacceptable ADME properties in drug development [1]. Therefore, in recent years, the R&D team has introduced ADME assessment at the early stage of drug discovery and development to exclude undesirable compounds early and quickly at a low cost and focused resources on promising compounds for subsequent development.

The failure caused by ADME properties of the drug decreased to 10% in the year 2000. This achievement has been key to the development of *in vitro* ADME research.In the early years, the primary approach to predicting human *in vivo* PK performance was from *in vivo* pharmacokinetics (PK) data in animals. Later, with the development of new synthetic strategies, the number of compounds for screening the ADME properties increased substantially. The study of their ADME properties also needed to be more extensive. *In vitro* ADME experiments emerged. Since the 1990s, the variety and availability of human-derived reagents have increased for *in vitro* drug metabolism studies, making *in vitro* ADME research more convenient [2]. In addition to providing high-throughput screening at a low cost, *in vitro* ADME studies can also eliminate many factors and investigate a single ADME property. Human PK performance is more accurately predicted by combining the data obtained from *in vitro* studies with *in vivo* animal experiments.

The desirable ADME properties vary considerably based on the disease target, the delivery route, frequency of administration, the pharmacokinetic-pharmacodynamic (PK-PD) relationship, and market competition strategies. These factors make it impossible to design a common strategy for all projects. Typically, in the Lead Finding (LF) stage, the solubility, lipophilicity, permeability, and microsomal stability of compounds are determined first. The obtained data are used to build the structure-property relationship of compounds, screen chemical structure categories, and confirm the priority of multiple structural backbones. At the Lead Optimization (LO) and Pre-Clinical Candidate (PCC) stages, comprehensive *in vitro* ADME studies are usually required. These include multi-species metabolic stability, plasma protein binding, transporters related to drug interaction, and inhibition of the drug-metabolizing enzymes combined with animal PK data to predict human PK performance. In the Investigational New Drug (IND) stage, a comprehensive evaluation of *in vitro* ADME properties is required and conducted per the requirements of drug registration authorities. It should be noted that the preliminary *in vitro* ADME study is usually not designed for regulatory submission, so the results are typically not submitted as application materials but can be submitted as supplementary materials [3].

Our *In Vitro* ADME Platform

In recent years, our *in vitro* ADME team has made progress in many ways. First, a variety of advanced automated liquid workstations and LC-MS/MS analysis technologies have been incorporated into *in vitro* ADME studies to improve further the assays' speed, quality, and throughput. Secondly, the *in vitro* assay portfolio of ADME studies has continued to expand, especially in the fields of drug-transporter interactions, drug metabolism studies of non-cytochrome P450 enzymes, *in vitro* ADME studies of peptide drugs, and protein binding studies of covalent inhibitors. The screen methods and experimental methodologies are relatively mature for traditional small molecule drugs, and our team has acquired vast experience and strategies in this area (Table 1). More new modalities with complex structures have emerged from the recent R&D pipelines, including proteolysis-targeting chimeras (PROTACs), oligonucleotides, and antibody-conjugated drugs (ADC). There are many challenges in the *in vitro* ADME study of these new modalities. We continuously expand the tools to support the ADME studies of these new modalities.

Table 1 *In vitro* ADME study objectives and main assay types at various stages of drug development

Development Stage	Purpose	Assay Types
Hit to lead	Screen chemical structure classes to provide 1 – 3 lead compounds with a reasonably diverse structural series	Solubility PKa Log P/D Plasma protein binding Hepatic microsomal stability Permeability, etc.
Lead optimization	Evaluate and improve the drug properties of lead compounds and help to obtain preclinical drug candidates with the best drug-like properties	Solubility Permeability Substrate or inhibitor screening of transporters Plasma protein binding Blood-to-plasma partition ratio Multiple species metabolic stability Inhibition of CYP enzymes and transporters Time-dependent inhibition of CYP enzymes CYP reaction phenotyping, etc.
Preclinical candidate (PCC)		
Investigational New Drug (IND) application	As required by new drug registration authorities, perform preclinical ADME evaluation. These parameters play an essential role in the species and dose selection of initial toxicity studies and complement the toxicokinetic data.	In addition to the above assays, CYP induction, identification of reactive metabolites, and other studies are needed in this stage.

Key features

Dedicated *in vitro* ADME Research and analytical teams

We have teams focused on *in vitro* ADME experiments and analysis, respectively, with first-class experimental, analytical capabilities and project throughput to ensure rapid and high-quality data delivery.

Professional assay system

The standard ADME assays are validated to ensure the accuracy and reliability of our experimental data:

- The standard assays are validated with commercial drugs. The results are highly consistent with the literature data.
- The assays are highly reproducible, with control compounds undergoing thousands of experiments. The existing assays are regularly validated using commercial drugs every year.
- When setting up a new assay, multi-dimensional investigations are performed to ensure the reliability of experimental data.

In Vitro ADME Services

Our Strengths

01

High-throughput and comprehensive service types

We can provide various services from screening to IND application, with more than 100 assays and one-stop service to the clients. Automation can ensure continuous improvement of throughput.

02

Fast data delivery

Based on solid technology, optimized operation, and improving throughput, we can save time and cost for the clients. For most screening assays, no more than five working days are needed from compound receipt to report submission.

03

High-quality data

The application of automation can reduce human error and improve data quality. A complete set of *in vitro* ADME evaluation services can be provided to meet the submission requirements from FDA, EMA, and NMPA for new drugs. At present, the IND applications of hundreds of drug candidates have been completed, and all of them have passed the on-site audit by the regulatory authority.

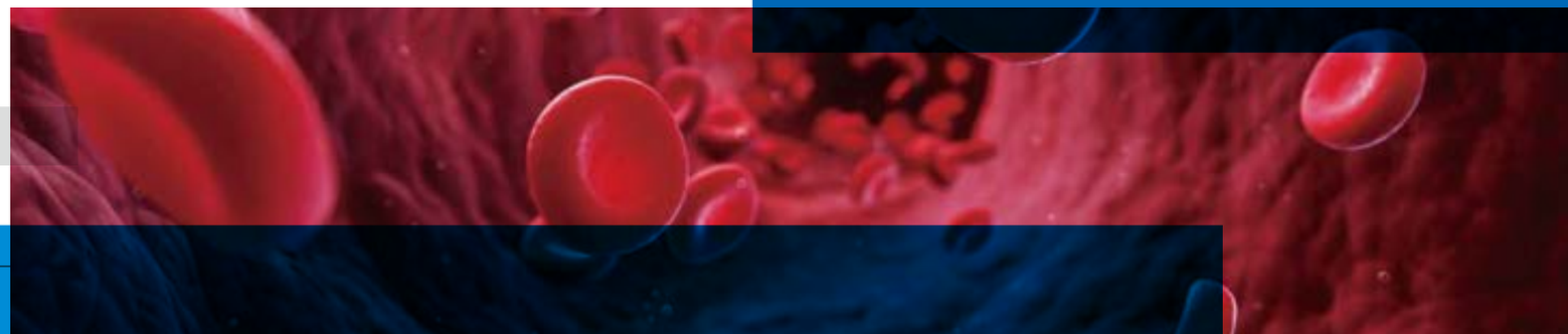
04

Experienced researchers

More than 70% of employees have a master's degree. The core team has more than 15 years of ADME research experience, providing the most valuable data in the shortest time to save resources and accelerate the development process.

Table 2 Main *in vitro* ADME assays

Attribution	Classification	Assay Type
Physicochemical Properties	/	Solubility (KS, TS), lipophilicity, pKa, solution stability
Absorption	Permeability	PAMPA, Caco-2, MDCKII, MDR1-MDCKII, MDR1-MDCKI, MDCK I
	Transporter	Efflux transporters: BCRP, P-gp, BSEP, MRP2, MATE1, and MATE2-K Uptake transporters: OATP1B1, OATP1B3, OATP2B1, OAT1, OAT3, OCT1, OCT2, PEPT1/2, and liver uptake experiments
Distribution	Red blood cell distribution	B/P Ratio
	Protein binding	Equilibrium dialysis, ultrafiltration, and ultracentrifugation
Metabolism	Matrix	Tissue, Plasma, Simulated gastric fluid/Simulated intestinal fluid, Buffer
	Cells, sub-cellular components, and others	Microsomes, S9, hepatocytes, lysosomes, mitochondria, recombinant enzymes, etc.
Metabolism-related drug-drug interactions	CYP and UGT	Reversible inhibition, Ki, time-dependent inhibition, kinact/KI, metabolic reaction phenotyping, CYP enzyme induction (enzyme activity and gene-level)



In Vitro ADME Services

Physicochemical Property Study

Study Purpose

The research has shown that successful drugs tend to have a "drug-like" property. At the *in vivo* level, the "drug-like" mainly refers to PK characteristics and safety, which can be viewed as the result of the balance between the SPR (structure-property relationship) and SAR (structure-activity relationship). The SPR study of drugs is an emerging drug discovery strategy, such as solubility, lipophilicity, dissociation constant (pKa), stability, and other property studies that significantly impact *in vitro* and *in vivo* pharmacology. For example, in the early stages, scientists can understand and optimize more factors affecting the experiment based on the physicochemical properties and help determine compound priority and promote the optimization of the molecular structure. This process also informs the research team of the factors affecting development loss to optimize the design to improve the compound's performance and success. As the table below illustrates, the physicochemical properties of a drug are significant in the whole ADME study.

Table 1 Types of High-throughput Screening Services

Physicochemical Properties	Test Item	Method
Solubility	Kinetic solubility	Shake flask method
	Thermodynamic solubility	Shake flask method
lipophilicity	Log D or Log P	Shake flask method, chromatography, potentiometric titration
pKa	pKa	Potentiometric titration
		UV spectrophotometry
Stability	Buffer stability (pH 1.2 to 11)	Shake flask method (manual and automated)
	Gastrointestinal stability (SIF, SGF, etc.)	

Method Introduction

▶Solubility

Solubility is a critical parameter used to guide compound selection and optimization during drug discovery. The advantage of early solubility screening is that compounds with poor absorption or bioavailability that resulted from poor solubility should be excluded promptly before more expensive screening experiments are performed. Therefore, the determination of solubility values should be performed as early as possible during drug development. Different types of solubility data are required for the characterization of compound properties in different R&D stages. Because of the large number of test articles, even with a small number of compounds in the early R&D stage, kinetic solubility can be one of the critical methods used for compound ranking. As compounds enter the later stage, different crystal forms are usually selected for the study, and thermodynamic solubility testing is a commonly used method in this stage. DMPK provides these two high-throughput screening methods for solubility measurement. Details on the experimental system are shown below:

Table 2 Kinetic Solubility Test Method

Theoretical Concentration	200 μM
Media	Aqueous buffer system, such as 50 mM phosphate buffer solution, pH 7.4; bio-relevant media, such as simulated gastrointestinal fluid
Percentage of DMSO	2% (routine), adjustable to 1%
Incubation Equilibration Time	24h
Equilibration Temperature	Room temperature or 37°C
Compound Required	100 μL of 10 mM DMSO stock solution
Analytical Method	HPLC-UV/LC/MS/MS
Turnaround Time	5 working days

Table 3 Thermodynamic Solubility Test Method

Sample Amount	2 mg solid (for solubility experiments) and 1 mg solid (for linearity curves preparation)
Media	Aqueous buffer system, such as 50 mM phosphate buffer solution, pH 7.4; bio-relevant media, such as simulated gastrointestinal fluid
Incubation Equilibration Time	24h
Equilibration Temperature	Room temperature or 37°C
Analytical Method	HPLC-UV/LC/MS/MS
Turnaround Time	5 working days

▶ Lipophilicity

Lipophilicity expressed as distribution coefficient (log D) in octanol/aqueous buffer, is an important physicochemical parameter influencing processes such as oral absorption, brain uptake, and various pharmacokinetic (PK) properties. For example, a drug exerts its therapeutic effects by interacting with specific receptors present inside the body. Physicochemical properties of a drug, such as lipophilicity and water solubility, play a crucial role in making the drug target-drug receptor interaction. DMPK provides services of high-throughput lipophilicity testing as shown below:

Table 4 Log D Test Method

Theoretical Concentration	67 μM
Oil Phase	N-octanol
Aqueous Phase	100 mM phosphate buffer, pH 7.4
Oil-water Volume Ratio	1:1 (v:v)
Percentage of DMSO	0.67%
Incubation Equilibration Time	1h
Equilibration Temperature	Room Temperature
Sample Volume Required	100 μL of DMSO stock solution at 10 mM sample concentration
Analytical Method	LC/MS/MS
Turnaround Time	5 working days

Table 5 Log P Test Method

Test Method	Description	Data Deliverable
Shake Flask	▪ Log D values got from 7 different pH values (the general buffer pH range from 1.8 to 12) ▪ Plot Log D vs. pH to derive Log P	▪ Log D vs pH (1.8 to 12) ▪ Log P
Chromatography	▪ Log P value is derived from the residence time of the compound on the column	▪ Log P
Potentiometric Titration	▪ Measurement of pKa ▪ Calculate Log P by the pKa curve	▪ Log P

▶pKa

The dissociation constant (pKa) of compounds affects the mode of drug existence in the body fluid. It is vital to get the pKa of compounds in the drug discovery and development stages. For example, it can help the scientist know how to perform the salt form screening. Due to the correlation of pKa, solubility, and lipophilicity, pH adjustment can be performed to improve the compound's solubility once the pKa value is obtained. Both potentiometric titration and spectrophotometry are routinely used in DMPK, and Sirius T3 instruments are applied to measure pKa.

Table 6 pKa Test Method

Test Method	Description
Potentiometric Titration	pH titration range from 3 to 11 ⩾ 5 mg compound solid powder required
Spectrophotometry	pH titration range from 2 to 12 ⩾ 100 μL of 10 mM mother solution required

▶ Buffer Stability

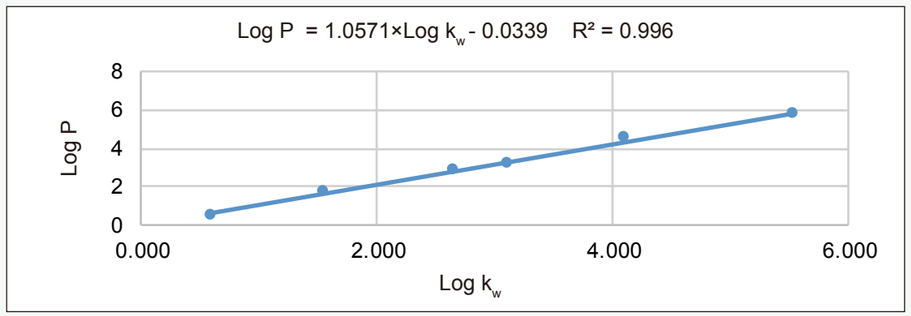
From a physiological point of view, the stability of the drug is affected by the *in vivo* environment, especially the pH of the gastrointestinal tract. Therefore, the stability of the drug is essential in different pH environments. Compounds should be stable enough to ensure they are not degraded in the gastrointestinal system before they enter the systemic circulation, allowing sufficient time to pass through the gastrointestinal tract into the blood circulation and bringing about ideal bioavailability. DMPK provides the buffer stability testing of a compound in a different medium, as follows:

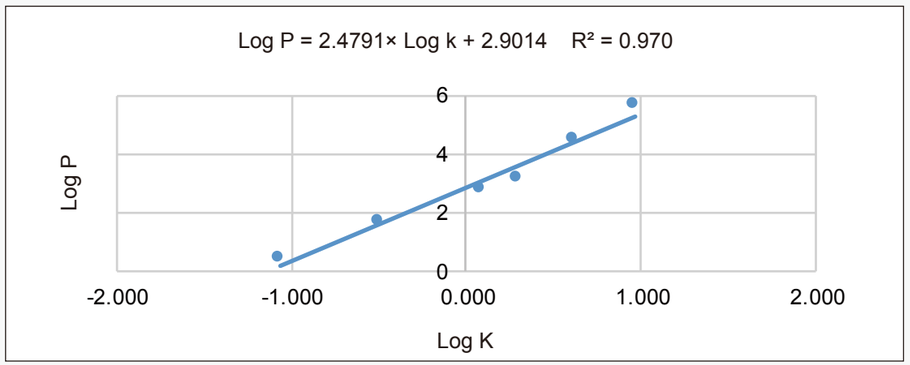
Table 7 Buffer Stability

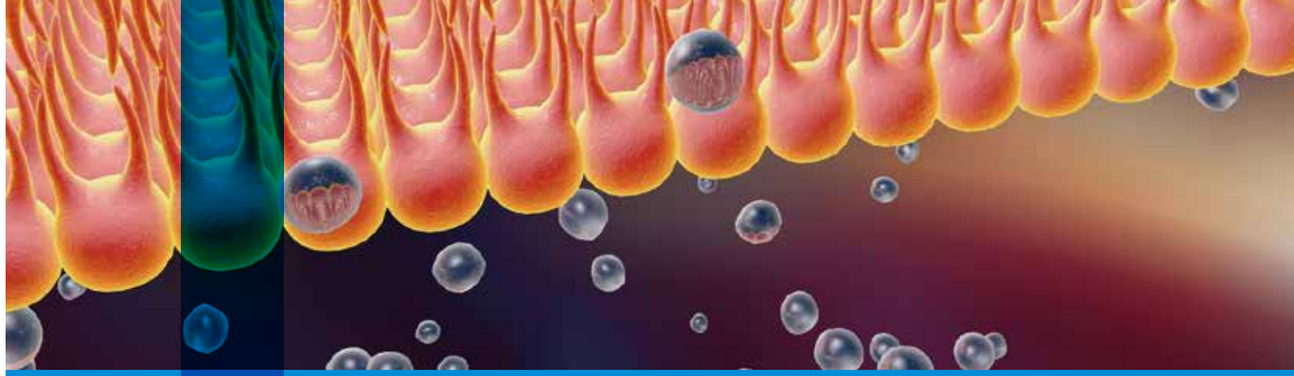
Theoretical Concentration (dose)	▪ 2 μM or other specified concentration
Media	▪ Aqueous buffer, such as 75 mM phosphate buffer solution pH 7.4; bio-relevant media such as simulated gastrointestinal fluids
Percentage of DMSO	▪ 1% (routine)
Incubation Equilibration Time	▪ 0h,1h,2h,6h,24h
Equilibration Temperature	▪ Room temperature or 37°C
Compound Required	▪ 100 μL of 10 mM DMSO stock solution
Analytical Method	▪ LC/MS/MS
Turnaround Time	▪ 5 working days

Example of Validation Data

Compounds that Log P-values within the range of 0-5 can be experimentally determined by the classic Shake-Flask method, which is fast and accurate for high lipophilicity compounds (Log P >5). Two chromatography test methods were developed for detection aiming at efficient and accurate data acquisition. The verification data are shown below:







In Vitro ADME Services

Permeability and Transporter Study

Study Purpose

Most discovery projects focus on developing orally administered drugs that are primarily absorbed across the intestinal mucosa. Therefore, it is imperative to select an appropriate in vitro permeability study model in drug development that provides reliable data and accurately predicts human absorption. In addition to artificial membrane models such as PAMPA and simulated model systems, the data supplied by cell-based permeability models are more instructive for optimizing lead compounds [4]. The permeability data obtained from these assays has many factors, including aqueous solubility, cell partitioning, influx/efflux transport, and tight junction modulability, making it helpful in estimating oral bioavailability and total biological distribution. Factors also include estimating permeation into the central nervous system (CNS) or blood-brain barrier (BBB).

Transporters are a group of carrier proteins that influence drugs' pharmacokinetics, pharmacodynamics, and toxicological properties. Drug-related transporters in humans mainly contain two types of transporter super-families. These are ATP-binding cassette (ABC) transporters and solute carrier (SLC) transporters. Transporters can affect the absorption, distribution, elimination, and other *in vivo* processes of drugs, affecting the efficacy and safety of drugs and playing an essential role in drug-drug interactions (DDIs) [5]. Usually, *in vitro* testing is the first step in assessing transporter-mediated DDIs.

Platform Introduction

The WuXi AppTec *in vitro* permeability and transporter platform provides four models to evaluate the permeability of drugs. Combined with the needs of different stages in drug development, different solutions are customized for sponsors to meet the requirements of high-throughput screening, mechanistic research, and application. PAMPA is a non-cell-based model suitable for high-throughput screening [6] [7], mainly used to assess passive transcellular transport of compounds in the early stage of drug development. In addition, three cell-based models are provided for permeability assessment: wild-type MDCK II cells, MDR1-MDCK II cells (MDCK II cells transfected with the human MDR1 gene encoding for the efflux transporter, P-glycoprotein (P-gp)), and Caco-2 cells. Compared with the PAMPA model, cell-based models comprehensively monitor the facilitated diffusion and the active transport processes during absorption, thus providing more reliable data to support the permeability assessment *in vitro*. Details of the models are shown in Table 1:

Table 1 *In Vitro* Permeability Models

Model		Features	Assay Type
Non-cell-based Model	PAMPA	▪ To predict passive transcellular transport in the early stage of drug development ▪ Suitable for high-throughput screening	▪ Egg-PAMPA ▪ BBB-PAMPA
Cell-based Models	Wild-type MDCK II Cells	▪ To assess both passive and active transport processes ▪ Madin Darby canine kidney epithelial cells ▪ 4 to 7 days culture	▪ Unidirectional (A-B), bidirectional (A-B and B-A) permeability assays
	MDR1-MDCK II Cells	▪ To assess both passive and active transport processes ▪ MDCK II cells transfected with the human MDR1 gene encoding for the efflux transporter, P-gp, which can specifically express efflux transporter P-gp ▪ 4 to 7 days culture	▪ Unidirectional and bidirectional permeability assays ▪ Concentration-dependent permeability assay
	Caco-2 cells	▪ To assess both passive and active transport processes ▪ An *in vitro* model widely used to evaluate small intestinal absorption ▪ Originated from a human colon carcinoma and differentiated to cells that resemble mature small intestinal enterocytes and express carrier proteins similar to the small intestine under the specific culture conditions ▪ Expressing multiple transporters, such as efflux transporters P-gp, Breast Cancer Resistance Protein (BCRP), etc. ▪ 21 to 28 days culture	▪ Unidirectional and bidirectional permeability assays ▪ Concentration-dependent permeability assay

WuXi AppTec's *in vitro* permeability and transporter platform provides various *in vitro* models for assessing transporter-mediated DDIs. We can evaluate substrates or inhibitors of specific transporters using three types of cell-based models. These include human embryonic kidney cells (HEK 293 cells) stably transfected with transporter genes such as human OATP1B1, OATP1B3, OATP2B1, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2-K, PEPT1, and PEPT2 for DDI studies with SLC transporters; MDR1-MDCK I and MDR1-MDCK II cells specifically expressing P-gp, and Caco-2 cells expressing efflux transporters such as P-gp and BCRP for DDI studies with efflux transporters P-gp and BCRP [8]. MDR1-MDCK I cells can better predict P-gp substrates at the blood-brain barrier *in vivo* and are mainly used in central nervous system-related drug development. In addition, we provide the vesicles models for efflux transporters BSEP and MRP2 and establish a model for the comprehensive assessment of hepatic uptake using primary hepatocytes. Details of the models are in Table 2.

Table 2 *In vitro* Transporter Models

Model		Features	Assay Type
Cell-based Models	HEK-293 Cells-transfected with SLC Transporters	▪ HEK-293 cells transfected with the human SLC transporter gene, which specifically express the SLC transporter ▪ To assess transporter-mediated DDIs for OATP1B1, OATP1B3, OATP2B1, OAT1, OAT3, OCT1, OCT2, MATE1, MATE2-K, PEPT1, and PEPT2	▪ SLC transporter substrate assessment assays ▪ SLC transporter inhibition assays
	MDR1-MDCK II Cells	▪ MDCK II cells transfected with the human MDR1 gene encoding for the efflux transporter, P-gp ▪ To assess P-gp-mediated DDIs	▪ P-gp substrate assessment assay ▪ P-gp inhibition assay
	MDR1-MDCK I Cells	▪ MDCK I cells transfected with the human MDR1 gene encoding for the efflux transporter, P-gp ▪ To assess P-gp-mediated DDIs ▪ To predict P-gp substrates at BBB *in vivo*	▪ P-gp substrate assessment assay ▪ P-gp inhibition assay
	Caco-2 Cells	▪ A human colon cancer cell that expresses multiple transporters, such as efflux transporters P-gp and BCRP ▪ To assess P-gp and BCRP mediated DDIs	▪ P-gp and BCRP substrate assessment assays ▪ P-gp and BCRP inhibition assays
	Primary Hepatocytes	▪ Suspension primary hepatocytes ▪ To assess hepatic uptake ▪ Species: human, monkey, dog, rat, and mouse	▪ Hepatic uptake assay
Non-cell-based Models	Vesicles	▪ Vesicles expressing efflux transporters BSEP and MRP2 ▪ To assess BSEP and MRP2 mediated DDIs	▪ BSEP and MRP2 substrate assessment assays ▪ BSEP and MRP2 inhibition assays

Example of Validation Data

WuXi AppTec's *in vitro* permeability and transporter platform pays excellent attention to the reproducibility of the system and the consistency with *in vivo* data or literature data. For example, the validation of a Caco-2 permeability test was carried out with 29 commercial drugs. This validation experiment had been repeated three times in triplicate each time. The results were reproducible, and the P_{app} (A-B) values of these 29 model drugs correlated very well with the human absorption data [9] (R^2 of 0.93) (Figure 1). In addition, the verification of system reproducibility was performed once every year, and the data correlation was greater than 0.9 between years. For example, the correlation R^2 of P_{app} (A-B) values for 18 model drugs tested from 2016 to 2020 was 0.98 (Figure 2).

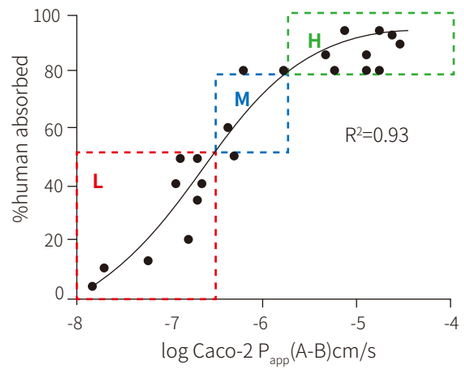


Figure 1. Correlation between P_{app} (A-B) values of 29 model drugs in Caco-2 cells and their oral absorption in humans. These 29 model drugs are acetaminophen, alprenolol, atenolol, carbamazepine, cimetidine, cisapride, clozapine, daunorubicin, dexamethasone, diclofenac, etoposide, imipramine, ketoprofen, granisetron, loperamide, methotrexate, metoprolol, minoxidil, omeprazole, pindolol, prazosin, propranolol, quinitidine, ranitidine, saquinavir, sulfasalazine, topotecan, and verapamil, respectively. L, M, and H represent low, medium, and high permeability, respectively.

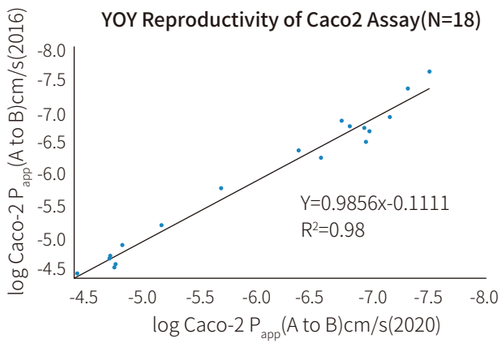


Figure 2. Year-to-year correlation of P_{app} (A-B) values of 18 model drugs in Caco-2 cells. These 18 model drugs were carbamazepine, cimetidine, daunitidine, dexamethasone, diclofenac, etoposide, ketoprofen, loperamide, methotrexate, metoprolol, minoxidil, omeprazole, pravastatin, propranolol, quinidine, ranitidine, sulfasalazine, and topotecan, respectively.

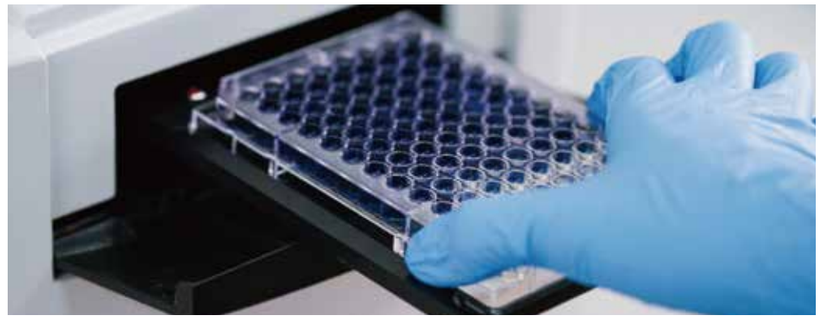
In Vitro ADME Services

Drug Distribution and Protein Binding Study

Study Purpose

After entering the blood, the drug will be distributed along with the blood flow into various tissues and organs of the body. Generally, the drug is first distributed into the tissues with a high blood flow rate, such as the liver and kidney. It then enters the tissues with a low blood flow rate, such as muscle and fat. The tissue distribution of drugs depends on physiological factors such as tissue blood flow rate and physiological barriers and drug properties such as lipophilicity, plasma protein binding rate, and tissue affinity.

Plasma protein binding is an important parameter to characterize the drug distribution process. In the blood, most small molecule drugs are reversibly bound to plasma proteins. In the absence of active transport processes, only free drugs can penetrate the biological membrane into the corresponding tissues, exert pharmacological effects or undergo metabolism and excretion. When the distribution process reaches equilibrium, the bound drugs in the plasma and tissues are in dynamic equilibrium with free drugs. Plasma protein binding may cause multiple effects, such as slowing down metabolism, reducing clearance, prolonging elimination half-life, and limiting drug distribution into target tissues. Plasma protein binding is of great significance in building the PK/PD model, predicting drug-drug interactions, evaluating drug toxicity, predicting human PK parameters and dose [10] [11].

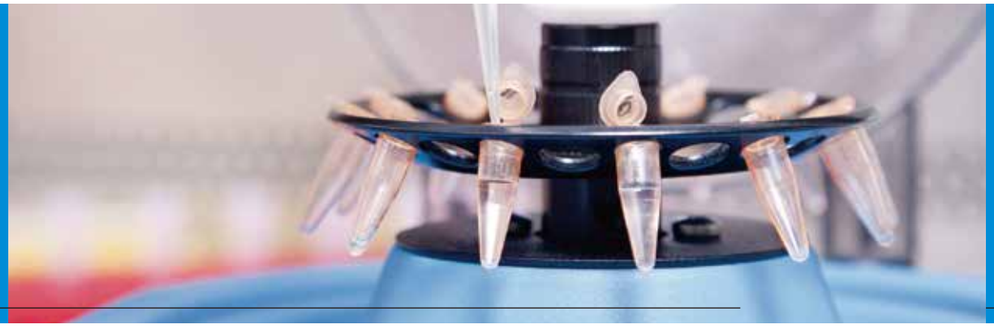


Platform Introduction

Our drug distribution and protein binding study platform utilizes three models to investigate plasma protein binding: equilibrium dialysis, ultracentrifugation, and ultrafiltration. The data obtained by these methods are in agreement with those in the literature. In addition to plasma protein binding, this platform can also carry out the study of drug binding to human serum albumin and α1-acid glycoprotein. Drug binding studies can be conducted to other biological matrices, such as tissue homogenate, hepatocytes, liver microsomes, whole blood, and drug distribution studies such as drug partition ratio in blood and plasma.

Model	Features	Assay Type
Equilibrium dialysis (both devices[a]: HTD and RED)	▪ High throughput, simple ▪ Reliable results and not susceptible to non-specific binding ▪ Long dialysis time, prone to fluid volume migration, Gibbs – Donnan effect, and protein leakage	▪ Plasma protein binding and tissue homogenate binding ▪ Albumin/α1-acid glycoprotein binding ▪ Microsomal/hepatocyte binding ▪ Cell culture medium and other matrix protein binding
Ultracentrifugation	▪ Suitable for compounds with severe non-specific adsorption to semipermeable membranes or devices ▪ Absence of fluid shift, plasma dilution, and membrane leakage ▪ Long centrifugation time/Low throughput ▪ Possible false high binding ▪ Possible redistribution of the drug into plasma proteins during centrifugation	▪ Plasma protein and tissue homogenate binding ▪ Albumin/α1-acid glycoprotein binding ▪ Microsomal/hepatocyte binding ▪ Binding to other matrix proteins
Ultrafiltration	▪ The rapid separation process and suitable for drugs that are unstable in plasma ▪ Membrane leakage ▪ Susceptible to non-specific adsorption	▪ Plasma protein binding ▪ Albumin/α1-acid glycoprotein binding ▪ Binding to other matrix proteins

[a] HTDialysis 96-well high throughput equilibrium dialysis device (HTD device) and Thermo Scientific rapid equilibrium dialysis device (RED device)



In Vitro ADME Services
Drug Distribution and Protein Binding Study

Example of Validation Data

We selected a series of drugs with high, medium, and low protein binding rates, respectively, and performed a plasma protein binding assay using in-house methods. The results obtained correlate well with the literature results.

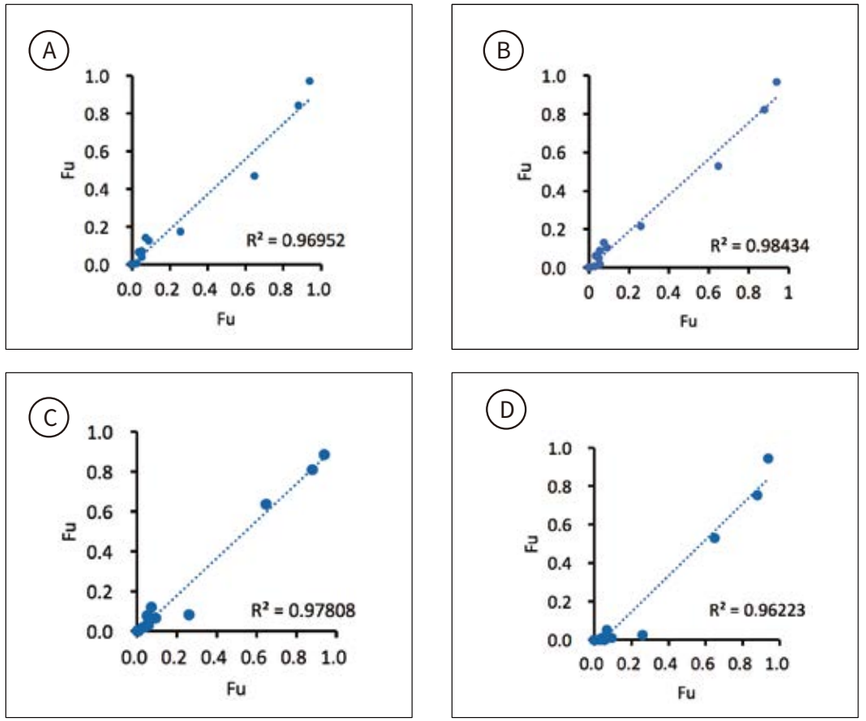


Figure 1 Correlation of free fraction of commercial drugs in human plasma between literature data (abscissa)[12] and in-house data (ordinate). Each data point represents the mean of three data. A. equilibrium dialysis (HTD) method; B. equilibrium dialysis (RED) method; C. ultracentrifugation; D. ultrafiltration; The following drugs were analyzed: Amiodarone hydrochloride, Atenolol, Chlorpromazine Hydrochloride, Clozapine, Diclofenac sodium salt, Imipramine, Metoprolol, Montelukast, Propafenone hydrochloride, Quinidine, Saquinavir, Topotecan hydrochloride, Verapamil hydrochloride, Ceftriaxone, Ibuprofen, and Cyclosporine.

In Vitro ADME Services
Metabolic Stability Study

Study Purpose

Drug metabolism (or drug biotransformation) is the process of structural transformation of drugs under the catalysis of drug-metabolizing enzymes after absorption and distribution in the body. It is the major elimination route from the body for most drugs. The liver is the most important organ for drug metabolism. The other clearance routes include renal excretion and biliary excretion. About 75% of marketed drugs were reported to be eliminated from the body primarily through the metabolic route (13). The metabolism of drugs in the body is a very complex process. Research in the metabolic process of drugs is of great significance for improving the *in vivo* pharmacokinetic properties of drugs, such as reducing the systemic clearance of drugs, increasing the oral bioavailability, addressing the potential metabolism-related issues, and predicting the human dose.

In general, drug-metabolizing enzymes convert lipophilic drug molecules into more hydrophilic metabolites, which the kidneys and bile can readily excrete. The drug metabolic reactions can be classified into two types: phase I and phase II. Phase I metabolism, such as oxidation, involves adding functional groups or exposing the functional groups from a molecule. Phase II metabolism involves conjugation reactions, such as glucuronidation and sulfonation. Drugs can be metabolized by Phase I enzymes, Phase II enzymes, or Phase I, followed by a Phase II enzyme.

In the early stage of drug discovery, the study of *in vitro* metabolic stability is an important method to analyze drug metabolism. The conversion rate of compounds in the biological matrix can be measured by incubating compounds with a specific biological matrix (e.g., liver microsomes, hepatocytes, etc.) *in vitro*. *In vitro* kinetic parameters such as metabolic rate and intrinsic clearance can be obtained, and *in vivo* clearance can be further predicted by relevant models.

Platform Introduction

The *in vitro* experimental models for a metabolic stability study can be classified as cells, subcellular fractions (S9, cytosol, and microsomes), and recombinant enzymes. WuXi AppTec's Metabolic Stability Laboratory can provide stability experiments of microsomes, S9, hepatocytes, and plasma from multiple species and tissues. Details are listed in the following table.

Model		Features	Assay Type
Phase I metabolism	Liver microsomes	▪ Contains phase I and phase II enzymes of endoplasmic reticulum from a hepatocyte, such as cytochrome P450 enzymes, flavine-containing monooxygenases, esterases, amidases, and epoxide hydrolases, UDP-glucuronyltransferases, etc. ▪ Primary experimental model	Liver microsomal stability (+/- NADPH)
			Flavin monooxygenase stability (+/- NADPH)
	Liver cytosol	▪ It contains the soluble phase I enzymes such as esterases, amidases, aldehyde oxidase, epoxide hydrolases, and soluble phase II enzymes	Cytosolic stability
Phasic II metabolism	Liver microsomes + UDPGA	▪ Study of phase II metabolic reactions by specific cofactors	Liver microsomal stability (+ NADPH, + UDPGA)
	Liver S9	▪ It contains both liver microsomal and cytosolic fractions with a more complete metabolic profile, but a low level of microsomal enzymes	Liver S9 stability (+/- cofactors)
	Hepatocytes	▪ Contains intact cell membranes and near physiological concentrations of phase I and phase II metabolic enzymes and cofactors	Hepatocyte stability
Extrahepatic Metabolism	Intestinal microsomes	▪ Contains drug-metabolizing enzymes in the endoplasmic reticulum membrane of enterocytes	Intestinal microsomal stability (+/- NADPH)
	Intestine S9	▪ Contains intestinal microsomal metabolizing enzymes and soluble enzymes	Intestinal S9 stability (+/- cofactors)
	Lung microsomes	▪ Contains drug-metabolizing enzymes in the pulmonary endoplasmic reticulum membrane	Lung microsomal stability (+/- NADPH)
	Kidney microsomes	▪ It contains drug-metabolizing enzymes in the endoplasmic reticulum membrane of the kidney	Renal microsomal Stability (+/- NADPH)
	Plasma stability	▪ It contains metabolic enzymes in plasma	Frozen or fresh plasma
	Whole blood stability	▪ It contains metabolic enzymes in plasma and blood cells	Fresh whole blood
	Other matrix	▪ Other *in vitro* metabolic stability models as needed	Other tissue homogenates, subcellular fractions
Recombinant enzyme	Recombinant enzyme stability	▪ FMO1, FMO3, FMO5, GSTA1, GSTM1, MAOA, MAOB, and CYP3A4, etc.	Half-life CLint

Example of Validation Data

The following figure shows the *in vitro* and *in vivo* correlation of liver intrinsic clearance of 13 drugs (acetaminophen, midazolam, buspirone, chlorpromazine, diltiazem, promazine, metronolol, propranolol, quinidine, verapamil, diclofenac, tolbutamide, and warfarin). The abscissa is the liver intrinsic clearance measured by an *in vitro* liver microsomal stability assay. The ordinate is the liver intrinsic clearance calculated from the *in vivo* clearance reported in the literature [14]. The red, blue, and green lines represent the 2-, 3- and 5-fold error lines, respectively. Sixty-nine percent of compounds were within the range of 2-fold error.

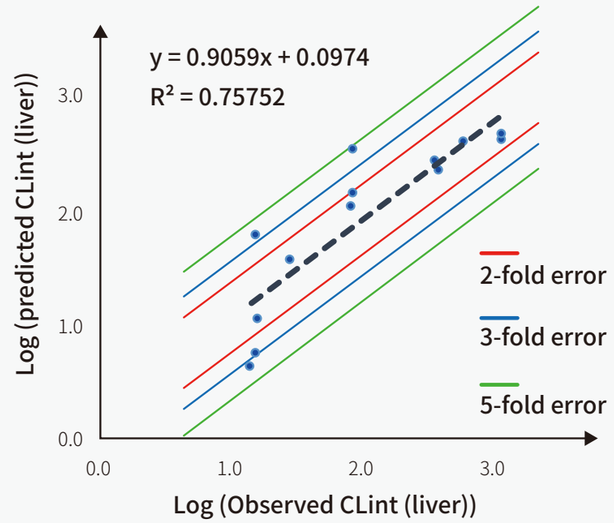


Figure 1. *In Vitro – In Vivo* Correlation of Hepatic Intrinsic Clearance for 13 Drugs (Liver microsomal stability assay)

In Vitro ADME Services

Metabolism-related Drug Interaction Study

Study Purpose

Metabolism is the leading clearance mechanism responsible for about 75% of the marketed drugs [15]. Among the drugs that have undergone metabolism, about 48% and 24% are mediated by cytochrome P450 (CYP) enzymes and non-CYP enzymes, respectively (Figure 1a). The contribution of CYPs to the metabolism of those marketed drugs ranges from 1% to 40%, with CYP3A4 being the most (Figure 1b). The contribution of non-CYP enzymes to the metabolism of those marketed drugs ranges from 2% to 45%, with uridine diphosphate glucuronosyltransferase (UGT) contributing the most (Figure 1c) [16]. Although drugs can be metabolized in several organs, drug metabolism primarily occurs in the liver and intestine. Hepatic metabolism occurs primarily through the CYP family of enzymes but also through non-CYP enzymes (e.g., UGTs). Although CYP enzymes are somewhat similar at all species, the isoform types and expression levels of CYPs vary significantly among different species and even among diverse human populations. The isoform types and expression ratios of CYPs also differ in different tissues, with the highest expression in the liver and intestine. That makes the prediction of human metabolic profiles across populations difficult. It is even more complicated when trying to predict human profiles based on animal data. The most valuable and predictive methods begin with human CYPs, either recombinantly expressed or derived from liver microsomes.

Drug interactions (DDI) are primarily examined *in vitro* by probing well-defined enzymatic reaction bench markers to elucidate the potential mechanism of DDIs and obtain kinetic parameters for further studies. Metabolic enzymes mediated DDIs include determining the main routes of drug elimination and assessing the contribution of relevant metabolic enzymes to drug disposition (enzyme metabolic reaction phenotyping experiments). It also includes investigating the drug's effect on metabolic enzymes (enzyme inhibition or induction experiments). DDI involves the influence of one drug, the "perpetrator," with the metabolic or pharmacokinetic behavior of a co-administered drug, the "victim." If the perpetrator inhibits a CYP, this can decrease the metabolic clearance of a victim that CYP primarily metabolizes. Likewise, if the perpetrator activates the CYP, it will expedite the victim's clearance. Similarly, if the victim is a prodrug converted to the parent or active drug by a CYP, then inhibitive or activating perpetrators will decrease or increase the serum concentrations of the active drug, respectively. Inhibition of cytochrome P450 enzymes causes toxic side effects that may be improved by changes in treatment regimens. Still, drug interactions may lead to severe adverse effects, which lead to some drugs being terminated at an early stage of development and/or even withdrawn from the market.

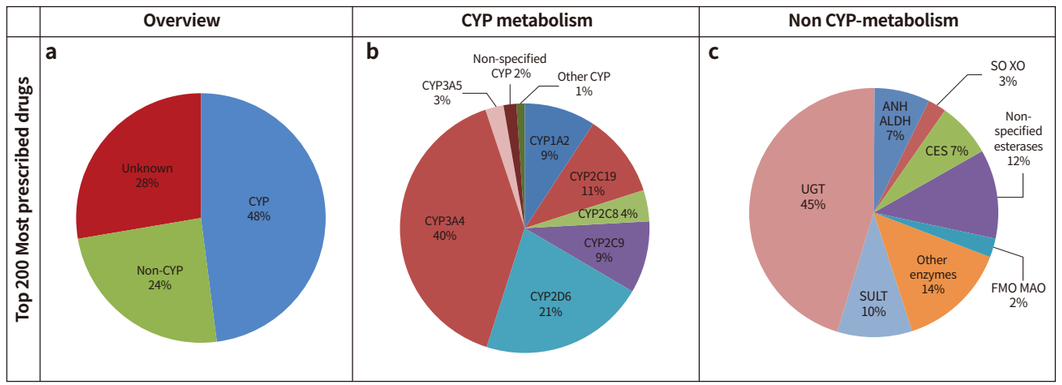


Figure 1. Metabolic pathways of drugs, data derived from Anitha Saravanakumar et al

Note: Figure 1a: enzymes involved in drug metabolism, Figure 1b: cytochrome P450 enzymes (CYPs) involved in drug metabolism, Figure 1c: non-cytochrome P450 enzymes (CYPs) involved in drug metabolism.

ADH: alcohol dehydrogenase, ALDH: aldehyde dehydrogenase, CES: carboxylesterase, FMO: flavin monooxygenase, MAO: monoamine oxidase, UGT: uridine diphosphate glucuronosyltransferases, XO: xanthine oxidase, AO: aldehyde oxidase

Platform Introduction

WuXi AppTec's DDI platform provides tier-based DDI assays which meet the requirements of different stages in drug discovery and development, namely lead finding (LF), lead optimization (LO), preclinical candidate (PCC), and investigational new drug (IND).

Model	Study Purpose	Assay Type
Cytochrome P450 enzyme reversible inhibition test	Evaluate the inhibitory effect of compounds on the activity of human liver microsomal cytochrome P450 isozymes (e.g., CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A) using probe substrates for each isozyme	5 in 1 DDI
		DDIM
	Evaluate the inhibition constants (Ki) of compounds on human liver microsomal cytochrome P450 isozymes (e.g., CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A) using probe substrates for each isozyme	Ki
Reversible inhibition test of recombinant human uridine diphosphate glucuronosyltransferases	Evaluate the inhibitory effect of compounds on recombinant human uridine diphosphate glucuronosyltransferase (UGT enzymes) activities such as UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15	UGTI
Cytochrome P450 enzyme Time-dependent inhibition test	Evaluate the time-dependent inhibitory effects of compounds on human liver microsomal cytochrome P450 isozymes (e.g., CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A)	TDI
	Evaluate the kinetic parameters (k_{inact}/K_I) for the irreversible inhibition of cytochrome P450 isozymes (e.g., CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A) of compounds	k_{inact}/KI
Cytochrome P450 Enzyme Metabolic Reaction Phenotyping Experiment	Identify cytochrome P450 isozymes involved in the ***in vitro*** metabolism of compounds in human liver microsomes and determine the relative contributions of various isozymes in metabolism (e.g., CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A)	PNT
Metabolic Reaction Phenotype of Recombinant Human Uridine Diphosphate Glucuronosyltransferases	Identify recombinant human uridine diphosphate glucuronosyltransferases involved in compound metabolism (e.g., UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15)	PNTU
Cytochrome P450 enzyme induction test	Evaluation of the induction potential of a compound on enzyme activities and gene expression levels of cytochrome P450 isozymes (e.g., CYP1A2, CYP2B6, CYP3A4, and CYP2C8, CYP2C9, and CYP2C19) by *in vitro* hepatocyte induction experiments	HI

Example of Validation Data

Both CYP3A4 and CYP2C enzymes are induced via activation of the pregnane X receptor (PXR). FDA and NMPA guidance recommend if the investigational drug causes CYP3A4 and the results suggest that a clinical study is warranted, the sponsor should evaluate the potential of the investigational drug to induce CYP2C. WuXi AppTec established a unique CYP2C (CYP2C8, CYP2C9, and CYP2C19) induction test platform through long-term exploration and validation (Figure 2 and Figure 3). The maximum group of induction of known inducers in our test system was significantly greater than those reported in the literature. It provided a more sensitive method for evaluating CYP2C induction and predicting the risk of drug interactions while providing a reference basis for clinical drug interaction study protocols.

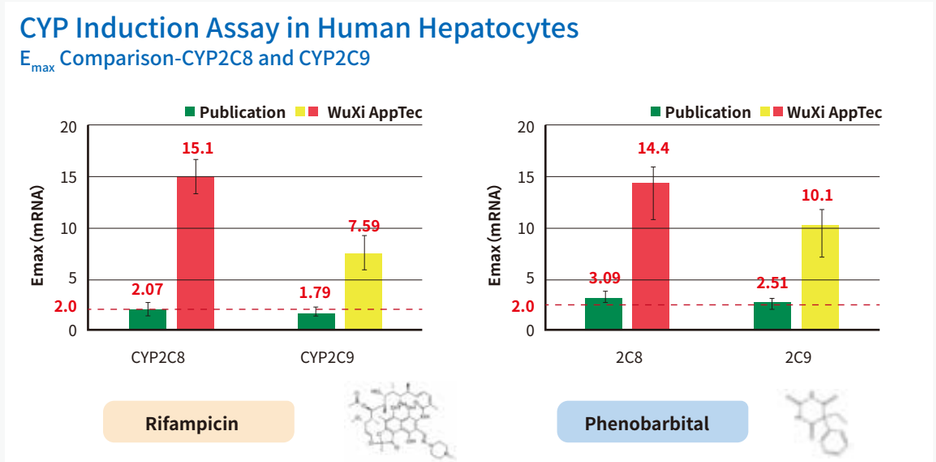


Figure 2. Emax Values of Rifampicin and Phenobarbital in Human Hepatocytes: CYP2C8 and CYP2C9

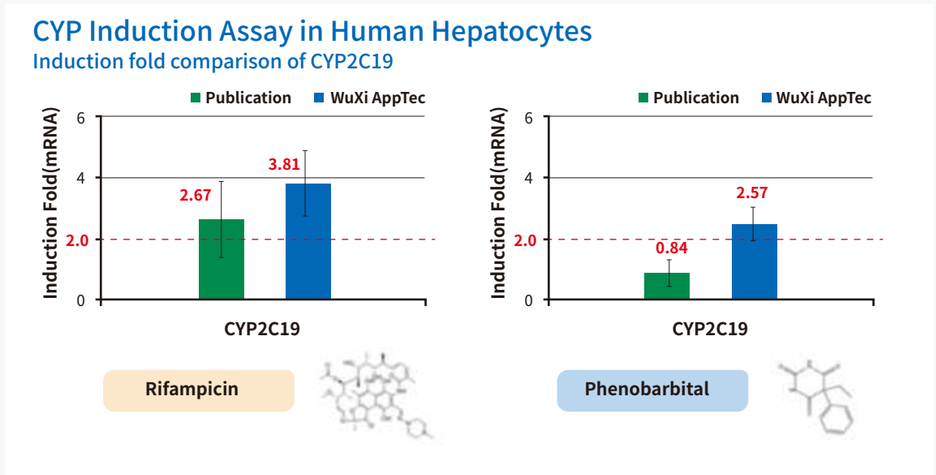


Figure 3. Induction fold of CYP2C19 in hepatocytes by rifampicin and phenobarbital

REFERENCES

1. Frank R, Hargreaves R. 2003. Clinical biomarkers in drug discovery and development. Nature Reviews Drug Discovery. 2(7):566-80

2. Di L, Feng B, Goosen TC, Lai Y, Steyn SJ, Varma MV, Obach RS. 2013. A perspective on the prediction of drug pharmacokinetics and disposition in drug research and development. Drug Metabolism and Disposition. 41(12):1975-93

3. Nomeir, Amin A. 2010. ADME Strategies in Lead Optimization (book chapter) 25-88 in Early Drug Development: Strategies and Routes to First in Human Trials (book) Editor(s): Mitchell N. Cayen DOI:10.1002/9780470613191

4. Press, B. & Di, G. D. Permeability for intestinal absorption: Caco-2 assay and related issues. Curr. Drug Metab. 9, 893-900 (2008)

5. Giacomini, K.M. et al. Membrane transporters in drug development. Nature reviews drug discovery 9, 215-236 (2010)

6. Lipid-PAMPA with the MultiScreen Filer Plates, Millipore #PC040EN00, June 2004

7. Kansy, M., Senner, F. & Gubernator, K. Physicochemical high throughput screening: parallel artificial membrane permeability assay in the description of passive absorption processes. J. Med. Chem. 41, 1007 1010 (1998)

8. Food and Drug Administration Center for Drug Evaluation and Research (2020). Clinical Drug Interaction Studies —Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry (FDA Maryland)

9. Suzanne, S. et al. Towards Prediction of *in vivo* intestinal absorption using a 96-well Caco-2 Assay. J. Pharm. Sci. 99, 32463265 (2010)

10. Smith, D.A. et al. 2010. The effect of plasma protein binding on *in vivo* efficacy: misconceptions in drug discovery. Nature Reviews Drug Discovery 9, 929 939

11. Di L, Breen C, Chambers R, Eckley ST, Fricke R, Ghosh A, Harradine P, Kalvass JC, Ho S, Lee CA, Marathe P, Perkins EJ, Qian M, Tse S, Yan Z, Zamek-Gliszczynski MJ. 2017. Industry Perspective on Contemporary Protein-Binding Methodologies: Considerations for Regulatory Drug-Drug Interaction and Related Guidelines on Highly Bound Drugs, Journal of Pharmaceutical Sciences, 106(12), 3442-3452

12. Obach RS, Lombardo F, Waters NJ. 2008. Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 drug compounds. Drug Metabolism and Disposition, 36(7):1385-1405

13. Di L. 2014. The role of drug-metabolizing enzymes in clearance. Expert Opinion on Drug Metabolism and Toxicology. 10(3):379-93

14. Poulin P, Haddad S. 2013. Toward a new paradigm for the efficient *in vitro-in vivo* extrapolation of metabolic clearance in humans from hepatocyte data. Journal of Pharmaceutical Sciences. 102(9):3239-51

15. Di L. 2014. The role of drug-metabolizing enzymes in clearance. Expert Opinion on Drug Metabolism and Toxicology. 10(3):379-93

16. Anitha Saravanakumar et al. 2019. Physicochemical Properties, Biotransformation, and Transport Pathways of Established and Newly Approved Medications: A Systematic Review of the Top 200 Most Prescribed Drugs vs. the FDA-Approved Drugs Between 2005 and 2016

02 *In Vivo* Pharmacokinetics

Pharmacokinetics (PK) describes how the body affects the absorption, distribution, metabolism, and excretion (ADME) processes of specific xenobiotics/chemicals or their metabolites after administration. Various dose routes can be performed, including intravascular administration (intravenous bolus or infusion) and extravascular administration (oral, subcutaneous, and intraperitoneal injection). It is of great reference significance to do administration method studies in the pre-clinical stage when selecting a clinical administration route. *In vivo*, pharmacokinetic studies play an essential role in new drug discovery and development stages.

The early stage of new drug research and development (R&D) mainly focuses on the target validation, lead compounds discovery, and optimization affirmed by routine *in vivo* pharmacokinetics and specific tissue distribution experiments. Its purpose is to understand the drug Structure-activity relationship (SAR) and guide the dose selection for late pharmacodynamics studies. Therefore, project quality and turnaround time (TAT) are significant.

In the late stage of new drug development, a study mainly focuses on preclinical candidate compounds selection and IND (Investigational New Drug) filing. The requirements for relevant animal experiments and biological sample analysis are stricter as possible compounds move onto the development stage. More detailed and well-targeted *in vivo* pharmacokinetic experiments are required for this phase of research. For example, dose-escalation studies are necessary to explore the linear relationship between dose and dose-response, investigate the effects of different formulations or different salt forms on *in vivo* exposure, *in vivo* pharmacokinetics, and cross-over experiments.

Our *InVivo* Pharmacokinetics Platform

The WuXi AppTec DMPK Service Department is committed to providing a comprehensive R&D platform for *in vivo* PK experiments, assisting new drug R&D projects with the most effective *in vivo* research methods, and providing accurate and efficient solutions to cope with all the challenges that occur in the new drug development.

The protocol is designed specifically to fulfill clients' needs and their experiment purposes, including formulation screening, dose and sampling time point setting, administration route, and sample matrix selecting. In formulation screening studies, the compound stability and solubility in its solvent are measured based on its physicochemical properties before *in vivo* study starts.

Dosing strategies can be discrete dosing or cassette dosing (N-in-1 cassette). Cassette dosing is an attractive procedure for high-throughput screening large numbers of candidate compounds (up to 4 candidate compounds and one reference compound) in drug discovery through assessing their pharmacokinetic properties. Administration routes include but are not limited to intravenous, oral administration, intraperitoneal injection, intramuscular injection, subcutaneous injection, intraventricular administration, intranasal administration, and intratracheal administration. Sampling duration can be set at 24 hours or above according to compound characteristics and project requirements.

The routine matrices are collected from *in vivo* pharmacokinetic experiments, including plasma, serum, brain, cerebrospinal fluid, bile, urine, feces, liver, gastrointestinal, and other specific tissues and fluids. If it is necessary to investigate the drug concentration of a specific tissue, the tissue or tissue fluid can be collected from blood sampling at different time points to obtain the ratio of tissue drug concentration to plasma concentration (for example, collecting brain and blood to assess the brain/plasma drug concentration ratio).

We are always committed to providing a comprehensive in vivo pharmacokinetic service platform for our clients. We focus on client demands to establish the corresponding experimental system and pharmacokinetic screening strategies, including data interpretation and data application. We are eager to satisfy all the *in vivo* pharmacokinetics assessing requirements proposed by our clients.

In Vivo Pharmacokinetics

Table 1 Development Strategy

Drug Development Process	Services Provided	Assay Type
Hit to lead	▪ Identification of PK issues ▪ *In vivo* screening and compounds ranking ▪ Proof of Concept (POC) based on compound exposure	▪ *In vivo* PK, for example: - Studies on large and small animals that administered through intravenous or oral administration route - Brain penetration studies (e.g., central nervous drugs)
Lead optimization	▪ The screen on a specific “soft-spot.” ▪ PK/PD relationship ▪ Fully assess ADME properties in animals ▪ Provide support for identifying safety assessment species ▪ Provide a basis for predicting safety dose for further clinical studies	▪ *In vivo* PK optimization and validation studies, including cassette or discrete dosing on suitable species to screen PK properties and *in vivo* PK studies in higher species (e.g., dogs and monkeys) - Routine PK studies in rodents and non-rodents, including PK studies on different dosing methods, dosage, dosing frequency, dosage forms, salt forms, and other studies. - Tissue distribution and drug metabolism studies: collecting and analyzing tissues and other biological samples such as cerebrospinal fluid (CSF), bile, urine, and so on. - Maximum tolerated dose (MTD) studies: single dose or repeat dose ▪ IPK/PD studies - Dose-effect relationship in the pharmacology model
Preclinical candidate (PCC)		
Investigational New Drug (IND) application	▪ Present declared data (detailed preclinical test compound characterization) to regulatory authorities ▪ Demonstrate that compounds are promising and can be conducted for further safety and efficacy evaluation studies	▪ Single-dose and multiple-dose PK studies in rodent and non-rodent ▪ Repeated-dose studies with appropriate doses in rodents and non-rodents ▪ Tissue distribution study ▪ Bile, feces, urine excretion study ▪ I*n vivo* metabolite identification ▪ Isotope tracer studies (if required)
Clinical studies	▪ Seek and verify suitable dosage and dose routes for human ▪ Determine DMPK properties under the target dosage regimen (dosage and dose route have been decided) ▪ Reasonable analysis on the safety and efficacy studies presented from the test compound	▪ Estimation based on maximum recommended starting dose (MRSD) - Estimation based on NOAEL (No Observed Adverse Effect Level) - Estimation based on biological exposure - Estimation based on minimum anticipated biological effective level (MABEL)

Key Features

▶High standards of animal welfare

- Good animal care and use program with oversight of animal welfare. The animal facility is compliant with China, US, and EU first-class standards.
- All animal facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).
- Animal facilities in Shanghai and Suzhou have acquired PHS Animal Welfare Assurance. Animal facility in New Jersey have acquired OLAW assurance.

DEPARTMENT OF HEALTH & HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

April 29, 2019

Re: Assurance Approval for
A3869-01

Steve Qing Yang, Ph.D.
Executive VP and Chief Operating Officer
WuXi AppTec (Shanghai) Co. Ltd.
1-204, 288 Fute Zhong Road
Shanghai, China

Dear Dr. Yang

I am pleased to inform you that the Office of Laboratory Animal Welfare (OLAW) reviewed and approved your institution's Animal Welfare Assurance. This Assurance is duly document in defining your relationship with the PHS. It sets forth the responsibilities and procedures of your institution regarding the care and use of laboratory animals.

Your Assurance, identification number **F16-00231 (A3869-01)**, became effective on **April 24, 2016** and will expire on **April 30, 2024**. Please include the Assurance number on all correspondence to OLAW. A copy of the signed Assurance document is enclosed. The signature page provides verification of approval by OLAW and specifies the period during which your institution's Assurance is effective.

Thank you for your cooperation in submitting an Assurance in compliance with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy), revised in August 2015.

If I may be of any further assistance, please do not hesitate to contact me.

Sincerely,

Glenn W. Morgan
Director, Division of Assurances
Office of Laboratory Animal Welfare

Enclosure: As Stated

cc:
Jia Min, D.V.M.
Deming Xu, Ph.D.

March 15, 2018

Deming Xu, Ph.D.
Chairman, WuXi IACUC (Shanghai)
and Executive Director
WuXi AppTec Co., Ltd.
288 Fute Zhong Road
Waigaoqiao Free Trade Zone
Shanghai, 200131
P.R. China

Dear Dr. Xu

The AAALAC International Council on Accreditation has reviewed the correspondence dated January 5, 2018 pertaining to Council's letter of October 27, 2017. Council is pleased to inform you that with the Attending Veterinarian's establishment of anesthetic and analgesic guidelines for the use of non-pharmaceutical-grade agents and the Institutional Animal Care and Use Committee's review of the justification for the use of non-pharmaceutical-grade anesthetics, the animal care and use program at WuXi AppTec Co., Ltd., Shanghai, People's Republic of China, conforms with AAALAC International standards as set forth by the *Guide for the Care and Use of Laboratory Animals*, NRC 2011. Council additionally acknowledges and appreciates your addressing the recommended suggestions for improvement. Therefore, the criteria for maintaining **FULL ACCREDITATION** have been satisfied.

Council looks forward to following your program activities and wishes you continued success. As a reminder, AAALAC International requires an Annual Report from accredited units detailing changes made during the year. In the interim, AAALAC International expects to be apprised in a timely manner of significant programmatic changes or concerns should they occur. Please note that, at your request, AAALAC International will provide your institution with a separate letter simply verifying that your animal care and use program is accredited. Should you also wish to distribute an electronic copy of this letter to program staff, a Portable Document Format (pdf) version will be sent upon request.

Sincerely,

Andrew W. Gindy, D.V.M., M.S.
President, Council on Accreditation

AWG:cmz
001209

cc: Steve Yang, Ph.D., Chief Operating Officer and International Official

▶Experienced and professional teams

WuXi AppTec is equipped with finely differentiated professional teams, including preclinical formulation, *in vivo* experiments, surgery, veterinarian, innovation, and operation, to fully ensure the high quality and efficient implementation of experiments through close cooperation. More than one-third of our animal laboratory researchers acquire AALAS (American Association for Laboratory Animal Science) certifications for animal technicians. These certifications provide sufficient assurance of animal welfare and team's work quality and efficiency. The qualification of this team can meet and even exceed clients' expectations and is in line with international requirements.

- Preclinical formulation: This team has experienced preclinical formulation screening research experts and is equipped with advanced instruments which enable selecting suitable preclinical formulation within 24 hours.
- *In vivo* experiments: This team has accumulated technologies and experience of more than 15 years. They continue updating existing technicals and developing industry-leading new technologies, such as mouse saphenous vein, rat jugular vein puncture, and other continuous blood sampling methods.
- Surgery: This team is experienced in many difficult and complex technicals such as aseptic surgical operation and sample collection. They had overcome many technical difficulties, such as lymphatic cannulation in rats and biliary cannulation in mice.
- Veterinarian: This team is experienced and professional in veterinary and animal care to fully ensure animal welfare.
- Innovation: The team continues to develop new capabilities and has made achievements in inhalation administration, intraventricular administration, in vitro release testing, and *in vitro* skin permeation study.

Our Strengths

Short lead-time

Efficient operation leads to rapid result delivery. Early screening study: 5-7 days; IND study: 2 weeks.

01

Sufficient animal resources

A well-organized animal supply ensures timely project implementation. Long-term supply agreements with world-class suppliers guarantee this.

02

Reliable experimental results

Provide a complete set of pharmacokinetics evaluation services meeting the FDA, OECD, and NMPA New Drug Application (IND) requirements, with high data reproducibility.

03

Comprehensive experiment types

Able to conduct studies from discovery to development stages, elect animal species from rodent to non-rodent, perform a variety of administration routes, and complete multiple sample collections to meet the diverse needs proposed by the clients.

04

Advanced instruments and equipment

The wide application of an industry-leading automated cage washer, automated blood sampling instrument, automated liquid workstation, and IT intelligent tools help ensure the efficient execution of customer projects and obtain stable and reliable experimental results.

05

Professional solution

Provide experienced data trouble-shooting analysis and professional solutions.

06

In Vivo Pharmacokinetics

Rodent PK Study

Study Purpose

An ideal drug needs to have an appropriate dosage and dosing regimen to obtain the application advantages of clinical studies and commercial success. An optimal oral drug requires suitable solubility, mainly assessed by passive diffusion absorption, high bioavailability, multiple metabolic elimination pathways, and adequate safety windows. Candidate compounds are obtained by providing appropriate drug metabolism and pharmacokinetics (DMPK) screening methods and evaluation strategies in the drug design, screening, and optimization stages that establishes a quantitative structure-property relationship (QSPR) which optimizes the compound properties in terms of absorption, distribution, metabolism, and excretion (ADME), and provides the basis for subsequent clinical trials by establishing an *in vivo-in vitro* correlation model. In the early phase of drug discovery, high-throughput in vitro screening has the advantages of being fast and low cost. With drug research development, it is necessary to carry out animal experiments to evaluate the absorption, distribution, metabolism, and excretion characteristics of candidate compounds. Many pharmaceutical companies and research institutions increasingly favor rodent PK studies for their easy-to-build *in vivo-in vitro* relationship, lower demand for compounds, shorter TAT at a lower cost, diverse animal models, and mature *in vivo* technologies. There are many types of rodent PK projects that can meet new drug discovery and development needs in each stage of preclinical new drug development and provide reference and guidance for extensive animal PK experiments, pharmacodynamics experiments, and toxicology experiments.

Facilities Introduction

The rodent barrier facilities of WuXi AppTec DMPK are located in Shanghai, Suzhou, Nanjing and New Jersey, respectively. All facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). In addition, animal facilities in Shanghai and Suzhou have PHS Animal Welfare Assurance, and animal facility in New Jersey has OLAW assurance. The DMPK barrier facility can house more than 10,000 rodents, including rats, mice, guinea pigs, and hamsters.

Animal facilities have rodent surgical suites that distinguish surgical, preoperative, intraoperative, and postoperative areas. They also have a professional design to ensure efficient and high-quality surgery and perioperative care.

The facility environment is controlled with advanced electronic systems and monitored in real-time to ensure the environmental conditions meet global standards. A two-corridor design in this animal facility can minimize cross-contamination. The peripheral auxiliary area has a cage washing room and a special feed bedding storage room. The cage washing room is equipped with an international top brand automatic cage washer to ensure the highest level of cage sanitation.

The facility has an independent Institutional Animal Care and Use Committee (IACUC), veterinary care unit and engineering team to ensure the health and welfare of animals.

Assay Type

Table 1 From Early Screening to IND Submission

Development Phase	Assay Type
Hit to lead	▪ Fast PK: Rat and mouse PK (oral or intravenous administration) ▪ Targeted PK experiment: For example, study on the central nervous system and brain/plasma ratio determination ▪ Routine PK experiments: Study-specific disease model based on PK results and exposure-based Proof of Concept (POC) model testing
Lead optimization	▪ PK study in rats and mice (administered through oral, intravenous, subcutaneous, intramuscular, and intraperitoneal routes. Species selection can be consistent with toxicology and efficacy studies) Single or multiple doses ▪ Exposure studies with different formulation ▪ Collection of different biological samples, such as cerebrospinal fluid, urine, and feces ▪ Tissue distribution validation studies, specific tissues can be collected such as brain, heart, liver, and lung
Preclinical candidate (PCC)	▪ Single-dose or multiple-dose experiment following specific administration route ▪ PK experiments with different salt forms or crystal forms ▪ PK experiments with different dosage forms, including bridging studies with clinical formulations ▪ Tissue distribution study: Multiple tissues can be collected, such as the heart, liver, spleen, lung, kidney, small intestine, large intestine, muscle, and fat ▪ Excretion study: Biological samples collection such as bile, feces, and urine ▪ Maximum tolerated dose experiment (MTD) and dose escalation experiment
Investigational New Drug (IND) application	▪ PK studies of single-dose and single intravenous administration (both rodents and non-rodents, at least three females and three males) ▪ Oral administration PK studies with high, medium, and low doses (rodents and non-rodents, at least three females and three males for each dose group) ▪ PK studies of medium dose and multiple oral administration (rodents and non-rodents, at least three females and three males) ▪ Tissue distribution studies after a single oral administration (at least three females and three male rodents at each time point, typically setting five time points, and no less than 13 tissues can be taken; tissue distribution isotope experiment - if needed) ▪ Biliary excretion studies with medium dose and single oral administration (rodent or non-rodent, at least three females and three males) ▪ Urine and fecal excretion studies with medium dose and oral administration (rodent or non-rodent, at least three female and three male, isotope excretion and mass balance study - as appropriate) ▪ Identify major metabolites in plasma and excretion (rodent or non-rodent, isotope identification of metabolites in plasma and excreta - if needed)

In Vivo Pharmacokinetics
Rodent PK Study

Animal Species

Table 2 Some Animal Species and Strains/Stocks

Species	Strain/Stocks
Mouse	ICR (CD-1)
	C57BL/6
	BALB/c
	FVB
	DBA/1
	DBA/2
	SJL
	129
	BALB/c Nude
	NU/NU Nude
	CD-1 Nude
	CB-17 SCID
	SCID Beige
	NOD SCID
	OB Mouse
	DB Mouse
	P-gp/BCRP KO Mouse
Rat	Sprague Dawley
	Wistar Han
	Wistar
	Brown Norway
	F344 (CDF)
	Lewis
Hamster	LVG Golden Syrian Hamster
Guinea Pig	Harley Guinea Pig

Route of Administration

Drug administration is one of the crucial parts of the *in vivo* study. It is essential to select the appropriate drug administration route in the early and late stages of drug development. Our team has developed more than 30 routes of administration based on customer needs and market foresight and provides high-quality rodent *in vivo* PK services to thousands of customers worldwide. This team has also established many unique technical capabilities, such as intravenous infusion for more than seven consecutive days. The team has launched many high-quality administration technologies for the respiratory system, ophthalmology, and nervous system and provided many trouble-shooting solutions for customer projects.

The team's biggest dosing technical competencies are listed in the table below:

Table 3 Team's Major Dosing Technical Capabilities

Route of Administration	Rat	Mouse	Hamster	Guinea Pig
Intravenous bolus injection	√	√	√	√
Intravenous Infusion	√	√	√	√
7-day continuous intravenous infusion	√	√	√	√
Oral gavage	√	√	√	√
Capsule administration	√	√	√	√
Intramuscular injection	√	√	√	√
Subcutaneous injection	√	√	√	√
Intradermal injection	√	√	√	√
Intraperitoneal injection	√	√	√	√
Intestinal administration	√	√	√	√
Intranasal administration	√	√	√	√
Sublingual administration	√	√	√	√
Transdermal administration	√	√	√	√
Intrathecal injection	√	√	√	√
Intracerebroventricular administration	√	√	N	N
Eye drop administration	√	√	√	√
Intravitreal injection	√	√	√	√
Intra-articular injection	√	√	√	√
Rectal administration	√	√	√	√
Vaginal administration	√	√	√	√
Intravesical administration	√	√	√	√
Intratracheal administration	√	√	√	√
Inhalation administration	√	√	√	√
Mini-pump implant	√	√	√	√

In Vivo Pharmacokinetics
Rodent PK Study

▶ Case Sharing of Classic Administration Route:

In Vitro Permeation Test (IVPT):

Overview: The transdermal drug delivery system (TDDS) has always been an attractive field as it can provide a therapeutic window larger than whole-body delivery. Topical skin formulations are widely used for topical administration, exert local effects, and cause systemic effects through skin absorption. Methods of evaluating topical drug transdermal delivery are divided into *in vivo* and *in vitro* [1]. Skin absorption is mainly a passive diffusion process. Literature has proven that the data obtained from *in vitro* studies complying with the appropriate protocol are of great reference values. Compared with traditional *in vivo* experiments, *in vitro* methods can measure the drug concentration retained in the skin and the amount that diffused through the skin into the receptor chamber. This method allows the exploration of skin permeability at the early screening stage. No live animals are used in this study, and multiple replicates can be applied at the same or different compounds. This feature is especially suitable for comparing the skin penetration of the same compounds in various formulations and screening the transdermal properties of different compounds [2].

Table 4. *In Vitro* Model for Investigating Drug Penetration

Model		Features	Available data presented in the report
Simulated Skin Model	Strat-M®[3]	▪ Strat-M ® film is designed to mimic the structure and lipid composition of human skin ▪ Relatively low cost ▪ Test and optimize drug formulations with good reproducibility for early drug/formulation development	▪ Accumulative permeation profile ▪ Permeation flux profile ▪ Permeation coefficient (K_p) (determined when permeation flux reached steady state)
Skin Model [4]	Rodents	▪ Small size and relatively low cost ▪ Penetration properties of drugs are overestimated	▪ Accumulative permeation profile ▪ Permeation flux profile ▪ Drug retention in different skin layers (stratum corneum, epidermis, and dermis can be separated, except rodents) ▪ Permeation coefficient (K_p) (determined when permeation flux reached steady state)
	Bama Miniature Pig	▪ Skin histology resembles human skin ▪ Higher cost	
	Porcine Ear	▪ Skin histology resembles human skin ▪ Obtained from food industry waste. Animal age and species are unselectable ▪ Commonly used to evaluate the active ingredients in cosmetics	

In Vivo Pharmacokinetics
Rodent PK Study

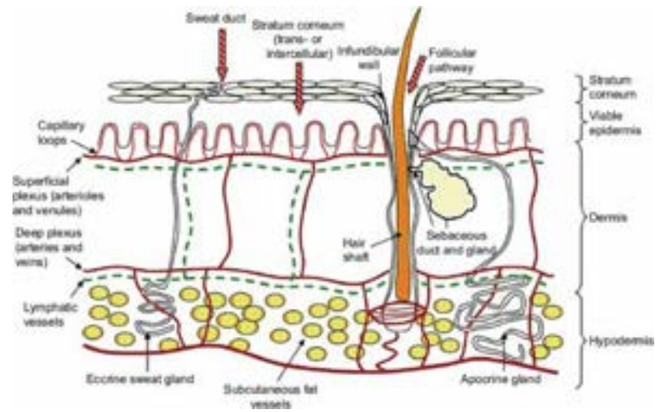


Figure 1. Skin Structure

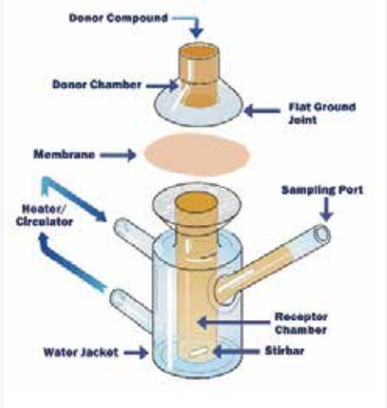


Figure 2. Franz Cell

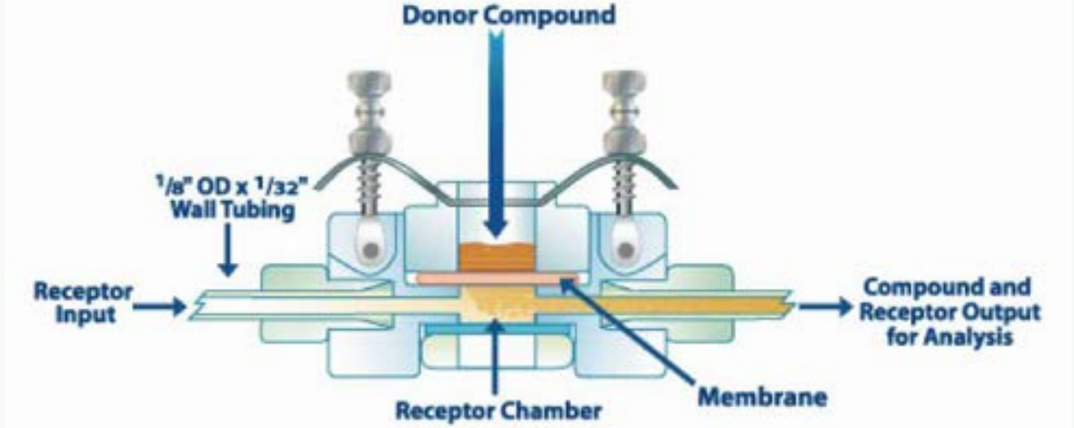


Figure 3. Flow-through In-line Cell

Research Data

In vitro, transdermal experiments tested the drug permeability of different batches of drugs using Bama minipigs' skin. The data showed no significant difference in the *in vitro* permeation characteristics between the test and the reference.

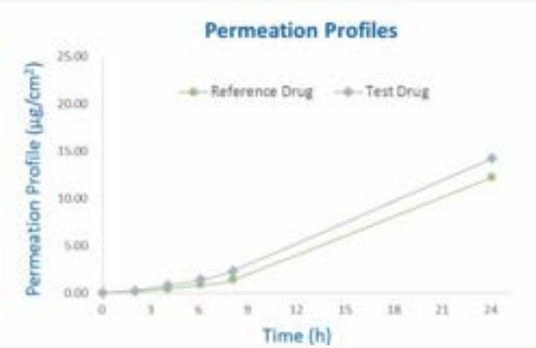


Figure 4. 24h Permeation Profile using Bama Miniature Pig Skin

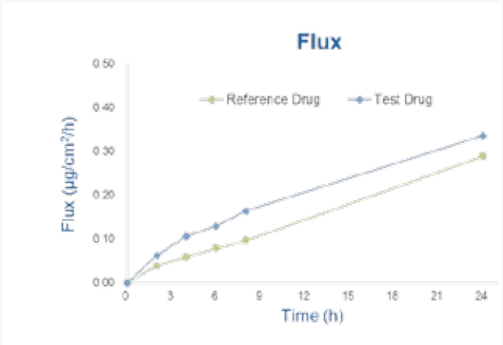


Figure 5. 24h Permeation Flux Profile using Bama Miniature Pig Skin

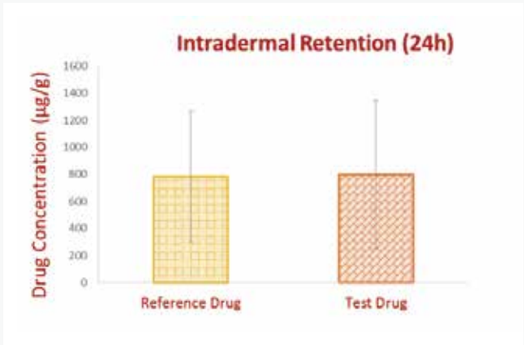


Figure 6. 24h Skin Retention of a drug in Bama Miniature Pigs' Skin

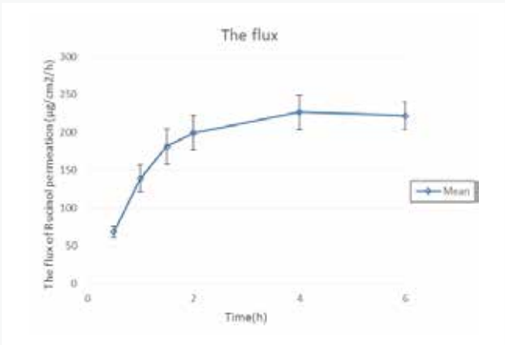


Figure 7. Permeation Flux Reached Steady State using Strat-M ® Membranes

Surgical Models

Table 7 Partial Surgical Models

Surgery Model		Rat	Mouse	Hamster	Guinea Pig
Vascular Cannulation	Carotid	√			√
	Jugular vein	√	√	√	√
	Femoral artery	√			
	Femoral vein	√			
	Hepatic portal vein	√			
Non-vascular Cannulation	Bile duct	√	√		
	Duodenum	√	√		
	Mesenteric lymph	√			
	Cisterna magna cannulation	√			
Non-survival Surgery	*In situ* small intestine perfusion	√			
	Isolated liver perfusion	√	√		
	In situ brain perfusion	√	√		
	Nasal lavage	√	√	√	√
	Bronchoalveolar lavage	√	√	√	√

▶Case Sharing of Classic Surgical Model:

In situ single-pass intestinal perfusion (SPIP) model

Overview: To date, the most used model for predicting the absorption process in humans is the Caco-2 cell monolayer system. While drug permeability correlates well with drug absorption in humans and the Caco-2 studies, there are many limitations when studying carrier-mediated transport or monitoring intestinal metabolites [4]. *In situ* single-pass intestinal perfusion (SPIP) is the closest experimental method that mimics oral administration and directly measures compound absorption rate. The intestinal structure is similar in both humans and rodents, and there is a strong correlation between effective permeability (P_{eff}) and fraction absorbed (F_a) between these two species (R^2 = 0.8 to 0.95). It is easy to control the intestinal environment in SPIP studies, such as drug concentration in the perfusate, perfusate pH, perfusion flow rate, and target intestine perfusion part of the study. More importantly, the SPIP enables simultaneous measurement of the effective permeability (P_{eff}) and flux of drugs appearing in mesenteric blood (P_b) [5, 6]. The team can use SPIP models in rodents to assess the intestinal permeability of test compounds and monitor their metabolites.

Validation Studies

Table 8 Results of Validation Studies

Group	Test Compound	P_{eff} from Literature ($\times 10^{-6}$ cm/s)[7,8]	P_{eff} from Verification Studies ($\times 10^{-6}$ cm/s)	Average P_b ($\times 10^{-6}$ cm/s)
High Permeability	Carbamazepine	62-160	85.60±20.54	34.18±14.47
	Propranolol	41-69	59.69±7.31	34.47±6.48
	Metoprolol	14.3-50.2	23.71±0.92	1.51±0.03
Medium Permeability	Ranitidine	14.7-30	16.84±3.79	13.77±6.64
	Dexamethasone	16-24	17.79±6.39	3.82±0.82
	Atenolol	1.8-16	3.46±1.20	3.15±1.36
Low Permeability	Topotecan	1.7-55.8	1.70±2.91	11.49±1.46
	Nadolol	2.7-5.3	3.52±8.89	3.22±2.72

In Vivo Pharmacokinetics

Non-rodent (Large Animal) PK Study

Study Purpose

In the early stage of new drug discovery and development, target validation, lead discovery, and lead optimization are the main focus points. The experimental content primarily includes *in vivo* PK studies and tissue distribution studies. In the later stage, drug development selects a candidate molecule for clinical development and the investigational new drug (IND) application. At the preclinical stage, especially for Investigational New Drug (IND) Application, the study is required to be conducted in two species, rodent and non-rodent-usually dog, monkey, or minipig[9].

WuXi AppTec DMPK Service department has international standard facilities that can hold thousands of large-high-quality laboratory animals. Our experienced and dedicated team with professional staff is committed to providing better experimental techniques and surgical skills applied during large animal studies. Furthermore, a close corporation with researchers from Bioanalysis & Formulation team help us simplify the regulatory guidelines and quickly obtain a wide variety of PK assays when a compound is administered to a non-rodent. We will also provide reliable data for evalutating the dose level for a compound in pharmacodynamics, toxicology and preclinical pharmacology.

Facilities Introduction

The large animal research facilities located in Suzhou and Nanjing have Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International) accreditation. The facility in Suzhou also has an OLAW-approved Animal Welfare Assurance (AWA) that is required by the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals.

The facilities have an advanced electronic management system that meets international standards. To reduce cross-contamination, we have different personnel and logistics flow directions and independent rooms for Veterinary Care, Necropsy, Cagewash, and Animal Housing. The Cage Washer Room is equipped with automatic cage washers to achieve the highest degree of sanitation.

WuXi AppTec has an Institutional Animal Care and Use Committee (IACUC) to ensure that the animal research program complies with applicable laws, regulations, policies, and guidelines. The Engineering Department, Veterinarian Team, and Husbandry Team ensure our facility complies with AWA and AWR requirements and provides good animal welfare, management, and maintenance of facilities, Animal records are carefully archived to ensure efficient data retrieval for each animal.

We also have a professional and experienced Formulation Team with advanced pharmaceutical preparation equipment to improve our pharmaceutical preparation capabilities and quality control. Equipment includes but is not limited to a Polarizing Microscope, Mastersizer 3000 laser diffraction particle size analyzer, NanoDrop One Spectrophotometer, and Retsch Planetary ball mill PM100, Waters HPLC.


Clean Corridor


Cage WasherRoom


Feed Storage Room


Fruit Storage Room


Surgery Room


Necropsy Room

Assay Type

Table 1 Assay Type: From early drug screening test to IND application

Development Phase	Study Type
Hit to lead	▪ Quick screening: e.g., PK study, oral dosing or intravenous administration in dogs or monkeys
Lead optimization	▪ PK study in dogs or monkeys (oral/intravenous/subcutaneous/intramuscular/intraperitoneal administration; the species of animals is consistent with that used in Toxicology studies and Pharmacodynamics Studies) ▪ Single-dose or multiple-dose studies ▪ AUC (area under the curve) studies of different dosage forms ▪ Studies are required to collect different biological sample matrices, such as cerebrospinal fluid, urine, feces, etc. ▪ Tissue distribution studies are required to collect specific target tissues, such as the brain, heart, liver, and lung
Preclinical candidate (PCC)	▪ Single-dose or multiple-dose in a particular administration route ▪ PK studies for the selection of different salt forms or crystal forms ▪ PK studies with various dosage forms, e.g., Formulation Bridge Study applied for Phase I ▪ Tissue distribution studies are required to collect other tissues, such as heart, liver, spleen, lung, kidney, small intestine, large intestine, muscle, and fat ▪ Excretion studies are required to collect bile, feces, and urine ▪ Maximum Tolerated Dose Study (MTD) and Dose Range Finding Study (DRF)
Investigational New Drug (IND) application	▪ PK Study: single-dose via intravenous administration (rodents and non-rodents, at least three females and three males) ▪ PK Study: single oral administration of high, middle, and low dose levels (rodents and non-rodents, at least three females and three males for each dose level) ▪ PK Study: multiple oral administration of middle dose level (rodents and non-rodents, at least three females and three males) ▪ Biliary Excretion Study: single oral administration of middle dose level (rodents and non-rodents, at least three females and three males for each dose level) ▪ Urinary and Fecal Excretion Study: single oral administration of middle dose level (rodents and non-rodents, at least three females and three males) ▪ Identification of major metabolites in plasma and excreta (at least five rodent or non-rodent animals)

Animal Species

Table 2 Large animal species and strain

Species	Strain
Monkey	Cynomolgus macaques
	Rhesus macaques
	Marmoset
Dog	Beagle
Pig	Bama mini-pig
Rabbit	New Zealand White
	Chinchilla
	Dutch-Belted
	Japanese White

Route of Administration

Drug administration is a vital component of *in vivo* PK studies. Proper administration route selection is of great significance during early drug screening and late drug development. A variety of administration routes have been developed according to client demand and forward-looking marketplace strategies to provide high-quality *In vivo* PK Study service for thousands of clients worldwide. Specialized and high-quality skills and techniques include 72-hour continuous intravenous infusion in animals without anesthesia, ocular administration, transdermal administration, and central nervous system (lateral cerebral, cerebellomedullary cistern, intrathecal administration) administration. We constantly strive to provide better service and continuous improvement.

Table 3 Team's Key Dosing Technical Capabilities

Route of Administration	Classification	Dog	Monkey	Pig	Rabbit
Intravenous	Bolus	√	√	√	√
	Infusion	√	√	√	√
Oral administration	Gavage	√	√	√	√
	Tablet	√	√	√	√
	Capsule	√	√	√	√
Transdermal administration	Ointment	√	√	√	√
	Patch	√	√	√	√
	Gel	√	√	√	√
	Films	√	√	√	√
	Spray	√	√	√	√
Intradermal injection	/	√	√	√	√
Subcutaneous	/	√	√	√	√
Intramuscular	/	√	√	√	√
Intraperitoneal	/	√	√	√	√
Nasal Sprays	/	√	√	N	N
Sublingual	/	√	√	√	√
Intra-articular injection	/	√	√	N	√
Rectal	/	√	√	√	√
Vaginal	/	√	√	√	√
Intrathecal injection	/	√	√	N	N
Intraosseous administration	/	N	√	N	N
Intravesical	/	√	√	N	N
Surgical method	Intestinal administration	√	√	N	N
	Portal Vein Administration	√	√	N	N
	Intracerebroventricular administration	√	√	N	N
	> 24-hour infusion	√	√	√	N
Ocular administration	Eye drops	√	√	√	√
	Intracameral injection	√	√	√	√
	Intravitreal injection	√	√	√	√
	Subconjunctival injection	√	√	√	√
	Retrobulbar injection	√	√	√	√

Table 4 Maximum administrable volumes for different routes [10, 11, 12]

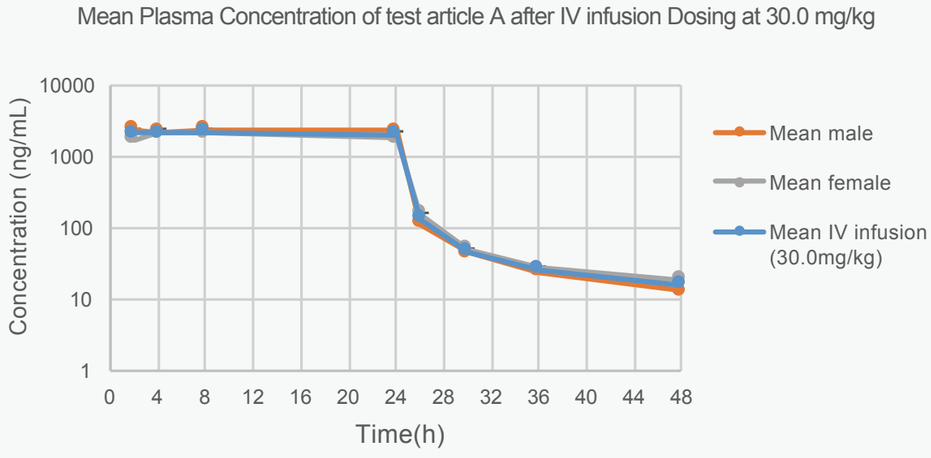
(*ml/kg* except where underlined- which is *ml/site*)

SPECIES	Oral		Subcutaneous Injection (SC)		Intramuscular (IM)		Intraperitoneal (IP)		Intradermal (ID)	Intravenous (IV) (bolus)	
	IDEAL	LIMIT	IDEAL	LIMIT	IDEAL	LIMIT	IDEAL	LIMIT		IDEAL	LIMIT
Rabbit	10	15	1	7.5	0.25	0.5	5	20	*0.1*	5	10
Dog	5	15	1	5	0.25	0.5	10	20	*0.1*	2.5	5
Swine	10	15	1	2	0.25	0.5	5	20	*0.1*	2.5	5
Non-Human Primate	5	15	2	5	0.25	0.5	5	10	*0.1*	2.5	5

▶Case sharing

24 hour IV infusion

Historical data: 24hrs (30 mg/kg) IV infusion via femoral vein cannulation in cynomolgus monkeys (three males and three females). Results are as follows:



Surgical Models

Table 5 Partial Surgical Models

Classification		Species			
		Monkey	Dog	Pig	Rabbit
Vascular cannula	Femoral vein cannulated	√	√	N	N
	Hepatic portal vein cannula	√	√	N	N
	A peripherally inserted central catheter (PICC)	√	√	√	N
Non-vascular cannula	Bile duct cannula	√	√	N	N
	Intestinal cannula	√	√	N	N
	Mesenteric lymph duct cannula	√	N	N	N
	Cervical lymphatics cannula	N	√	N	N
	Thoracic duct cannula	N	√	N	N
	Cisterna magna cannula	√	√	N	N
Special operations	Whole-body tissue perfusion	√	√	√	√
	Nasal mucosal lavage	√	√	N	N
	Bronchoalveolar lavage	√	√	N	N
	Intraarticular puncture	√	√	√	√
	Bone marrow aspiration	√	√	N	N
	CSF puncture	√	√	N	√
Biopsy	Liver Biopsy	√	√	N	N
	Skin Biopsy	√	√	√	√
	Muscle Biopsy	√	√	N	N

▶Case sharing of CMC surgical model

Cisterna Magna Cannula (CMC)

[Overview]

For drugs acting directly on the central nervous system, accurate detection of drug concentration in the brain contributes to evaluate the ability of drugs towards the target site. Moreover, the direct measurement of drug concentration of Cerebrospinal Fluid (CSF) has more accuracy than using the concentration of free drug in plasma to predict the drug concentration in the brain [13] [14]. The drug concentration of CSF can be used as an effective indicator for evaluating drug exposure for those CNS (Central Nervous System) drugs and provide a basis for these drug candidates to move to the next stage.

We have established Cisterna Magna cannulation (CMC) models in monkeys and canines according to the literature [15] since 2009, which allows us to collect CSF samples consecutively without anesthesia to obtain relevant PK curves and evaluate the drug's ability to cross the BBB (blood-brain barrier). The CMC model animal lasts up to 2 years, and we have standby CMC model animals for use anytime to shorten the leading time. Nearly 200 projects, including IND applications, were completed by 2020.

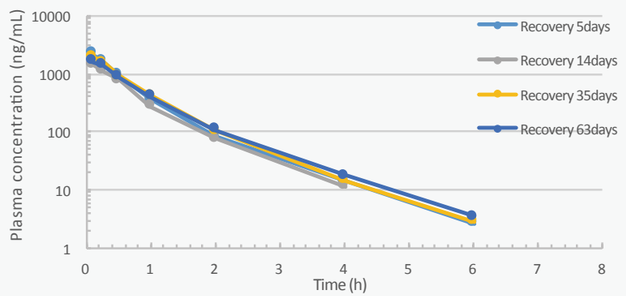
[Validation Experiment]

Objective: To compare the stability of the CMC model in dogs with different surgery recovery times.

METHODS: Lidocaine hydrochloride injection drug (25 mg/dog) was administered intravenously in 3 male beagle dogs five days, 14 days, 35 days, and 63 days after surgery. The plasma and CSF samples were collected at the same time point, and the results of drug concentration were as follows:

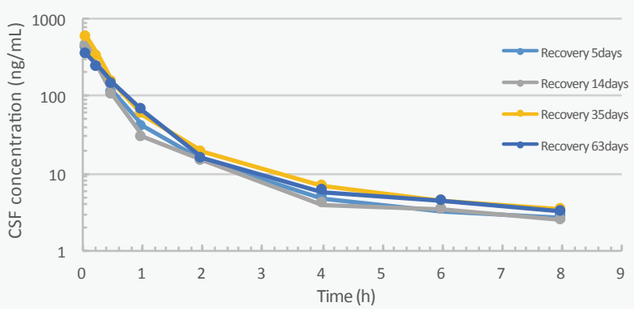
Comparision of exposure after different recovery duration mean plasma concentration of Lidocaine Hydrochloride

Figure 9: Plasma Concentration-Time Profiles



Comparision of exposure after different recovery duration mean CSF concentration of Lidocaine Hydrochloride

Figure 10: Drug Concentration-Time Profiles in CSF



Conclusion: The data showed a slight deviation in drug concentration between the plasma and CSF matrix on postoperative days 5, 14, 35, and 63, indicating that our animal surgical model is reliable with consistent data.

Table 6 List of biological sample collection of large animals

Sample Collection	Monkey	Dog	Pig	Rabbit
Whole blood	√	√	√	√
Plasma	√	√	√	√
Serum	√	√	√	√
White blood cell	√	√	√	√
Erythrocytes	√	√	√	√
PBMC	√	√	√	√
Stratum corneum	√	√	√	√
Epidermis	√	√	√	√
Dermis	√	√	√	√
Subcutaneous tissue	√	√	√	√
Ocular tissues *	√	√	√	√
Brain tissue **	√	√	√	N
Bone marrow	√	√	√	√
Organs (Table 7)	√	√	√	N
Intestinal fluid	√	√	√	N
Gastric fluid	√	√	√	N
Bronchoalveolar lavage fluid	√	√	√***	√***
Joint fluid	N	√	√	N
Rectal mucosa	√	√	√	√
Oral mucosa	√	√	√	√
Nasal mucosa	√	√	√	√
Cerebrospinal fluid	√	√	√***	√***
Cerebrospinal fluid (multiple consecutive)	√	√	N	N
Lymph (surgical)	√	√	N	N
Bile (surgical)	√	√	N	N
Urine (metabolic cage)	√	√	√	√
Urine (puncture)	√	√	√	√
Urine (Cannula)	√	√	N	N
Feces (metabolic cage)	√	√	√	√

* The samples of ocular tissues include but are not limited to conjunctiva, cornea, iris, lens, ciliary body, retina, choroid, sclera, optic nerve, aqueous humor, vitreous body, and tear.
**Samples of various brain tissues include but are not limited to the caudate nucleus, cerebellum, cerebral cortex, white matter of the cerebrum, cingulate gyrus, cingulate sulcus, corpus callosum, external capsule, internal capsule, globus pallidus, hippocampus, hypothalamus, pituitary gland, midbrain, medulla oblongata, optic nerve, optic chiasm, pons, shell, and spinal cord.
*** Terminal Collection

Table 7 Organ Matrix and Tissue Matrix

Tissue/Organ	
Adrenal Gland (bilateral)	Prostate
Aorta	Sialaden (submandibular, bilateral)
Bone (femur, distal)	Seminal Vesicle (bilateral)
Bone and Bone Marrow (sternum)	Skeletal Muscle (biceps femoris)
Brain	Skin (including tattoo number, if any)
Epididymis (bilateral)	Duodenum
Esophagus (thoracic)	Jejunum
Eyeball and Optic Nerve (bilateral)	Ileum
Heart	Spinal cord (cervical, thoracic, lumbar)
Kidney (bilateral)	Spleen
Cecum	Stomach (fundus and pylorus)
Colon	Testis (bilateral)
Rectum	Thymus
Liver and Gall Bladder	Thyroid (bilateral) Gland and Parathyroid Gland (bilateral or unilateral)
Lungs and Main Bronchi	Trachea (thoracic)
Submandibular Lymphatic Nodes	Bladder
Mesenteric Lymph Nodes	Uterus (including Cervix)
Sciatic Nerve	Vagina
Pancreas	Oviduct (bilateral)
Hypophysis	Ovary (bilateral)

In Vivo Pharmacokinetics
Preclinical Formulation Screening

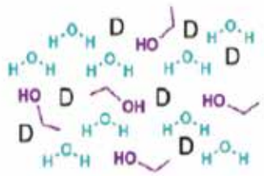
Study Purpose

In the drug discovery process, formulation plays a crucial role in assessing the biological characteristics of molecules. Increasing the exposure of the test compound in studies *in vivo* is one of the main goals of early animal experiments [16, 17]. Choosing a suitable preclinical formulation not only optimizes *in vivo* PK exposure but also promotes the optimization, selection, and development evaluation of compounds to provide data support for the design and development of clinical formulations [18]. For the test compounds in the early screening stage, the unsatisfactory physical and chemical properties, the insufficient compound amount, and the tight time are commonly encountered. In response to these challenges, the DMPK of WuXi AppTec established a team for preclinical formulation screening for PK studies, pharmacodynamic and toxicological studies. The team has more than ten years of experience and can perform rapid formulation screening and preparation services for large and small animal experiments. They also provide more than nearly 1,000 arms of formulations for preclinical animal experiments every week. They can use limited (milligram) compounds to offer a suitable formulation for preclinical animal experiments within 24 hours.

Study Methods

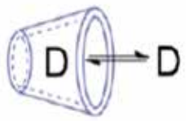
The formulation team can select vehicles that meet the requirements of the PK study within one working day. In formulation screening, solubilization technology (such as pH adjustment and cosolvent) is a commonly used technical means. For some poorly soluble compounds, a cosolvent plus surfactant or complexing agent plus pH adjustment are typically used in combination. In addition, the DMPK formulation team can customize the formulation screening strategy according to differentiated project needs.

▶ Strategy [19]



Co-solvent

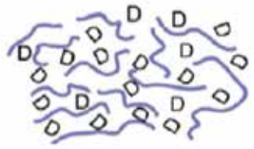
Surfactant



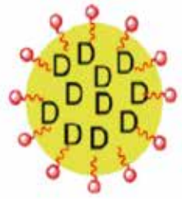
Cyclodextrins

Particle size reduction

Salt



Amorphous solid dispersion



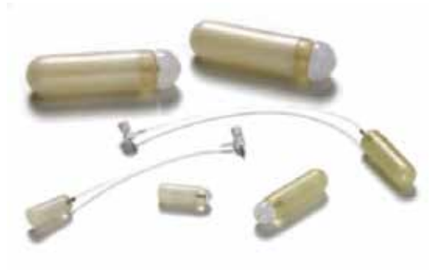
Lipid-Based Formulation

▶ Instrument

Osmometer

Siri T3 for The pKa Measurement

SPEX® SAMPLEPREP Mill



Small Animal Implantable Osmotic Pump

Malvern-3000——Particle Size Tester

Polarized Light Microscope

Retsch Wet Ball Mill

Covaris® E200x ULtra Ultrasonic Disperser

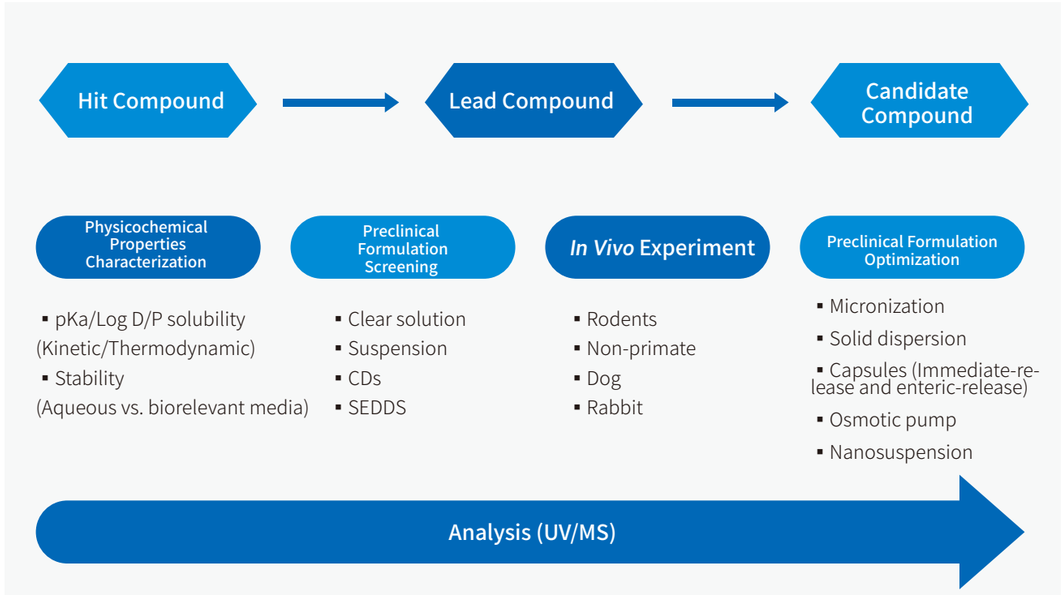
Small Animal Capsule

Lyophilizer

Retsch Ball Mill

Experimental Flow

The formulation team provides formulation screening and formulation services for pharmacokinetic studies from the screening stage to the IND application stage. It provides formulation screening services for pharmacodynamic tests and preclinical toxicological experiments. The team can select suitable excipients to dissolve the compound to tackle the solubility issue according to the physical and chemical properties of each specific drug substance (such as dissociation constant pKa, lipophilicity, aqueous solubility). The most appropriate formulation for a drug substance is selected by *in vitro* solubility assessment, homogeneity, and particle size analysis. Further screening is performed from eligible candidates to ensure the formulation with the best *in vivo* bioavailability or experimental objectives is achieved.



REFERENCES

1. Guideline, O. E. C. D. 428-Guideline f or the Testing of Chemicals-Skin Absorption: *in vitro* Method." Organization for Economic Cooperation and Development, Paris (2004)

2. Oh, Luke, et al. *In Vitro* Skin Permeation Methodology for Over-The-Counter Topical Dermatologic Products." Therapeutic Innovation & regulatory science 54.3 (2020): 693-700

3. Haq, Anika, et al. Strat-M ® synthetic membrane: Permeability comparison to human cadaver skin.' International journal of pharmaceutics547.1-2 (2018): 432-437

4. Kim, J.S., Mitchell, S., Kijek, P., Tsume, Y., Hilfinger, J. and Amidon, G.L., 2006. The suitability of an in-situ perfusion model for permeability determinations: utility for BCS class I biowaiver requests. Molecular Pharmaceutics, 3 (6), pp.686-694

5. Chiou, W.L. and Barve, A., 1998. Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. Pharmaceutical Research, 15 (11), p.1792

6. Cao, X., Gibbs, S.T., Fang, L., Miller, H.A., Landowski, C.P., Shin, H.C., Lennernas, H., Zhong, Y., Amidon, G.L., Lawrence, X.Y. and Sun, D., 2006. Why is it challenging to predict intestinal drug absorption and oral bioavailability in humans using the rat model. Pharmaceutical Research, 23 (8), pp.1675-1686

7. Salphati, L., Childers, K., Pan, L., Tsutsui, K. and Takahashi, L., 2001. Evaluation of a single-pass intestinal perfusion method in rats for the prediction of absorption in man. Journal of pharmacy and pharmacology, 53 (7), pp.1007-1013

8. Dahlgren, D., Roos, C., Sjögren, E. and Lennernäs, H., 2015. Direct *in vivo* human intestinal permeability (Peff) determination with different clinical perfusion and intubation methods. Journal of pharmaceutical sciences, 104 (9), pp.2702-2726

9. Technical Guidelines for Non-clinical Kinetic Studies of Chemical Drugs

10. Diehl. K et al. " A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volume. " Journal of Applied Toxicology. 21: 15-23. 2001

11. Turner P.V., Brabb T. Pekow C., and Vasbinder M.A ". Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider. " JAALAS. 50 (5): 600-613. 2011

12. Rollin B.E., Kesel M.L., The experimental animal in biomedical research, Vol. 2. Care, husbandry, and well-being, an overview by species

13. Maurer TS, DeBartolo DB, Tess DA, Scott DO (2005) Relationship between exposure and non-specific thirty-binding of three central nervous system drugs in mice. Drug Metab Dispos 33:175-181

14. Liu X, et al. (2009) Unbound drug concentration in brain homogenate and cerebral spinal fluid at steady state as a surrogate for unbound concentration in brain interstitial fluid. Drug Metab Dispos 37:787-793

15. Gilberto DB, Zeoli AH, Szczerba PJ, Gehret JR, Holahan MA, Sitko GR, Johnson CA, Cook JJ, Motzel SL. Contemp Top Lab Anim Sci. 2003 Jul; 42 (4): 53-9

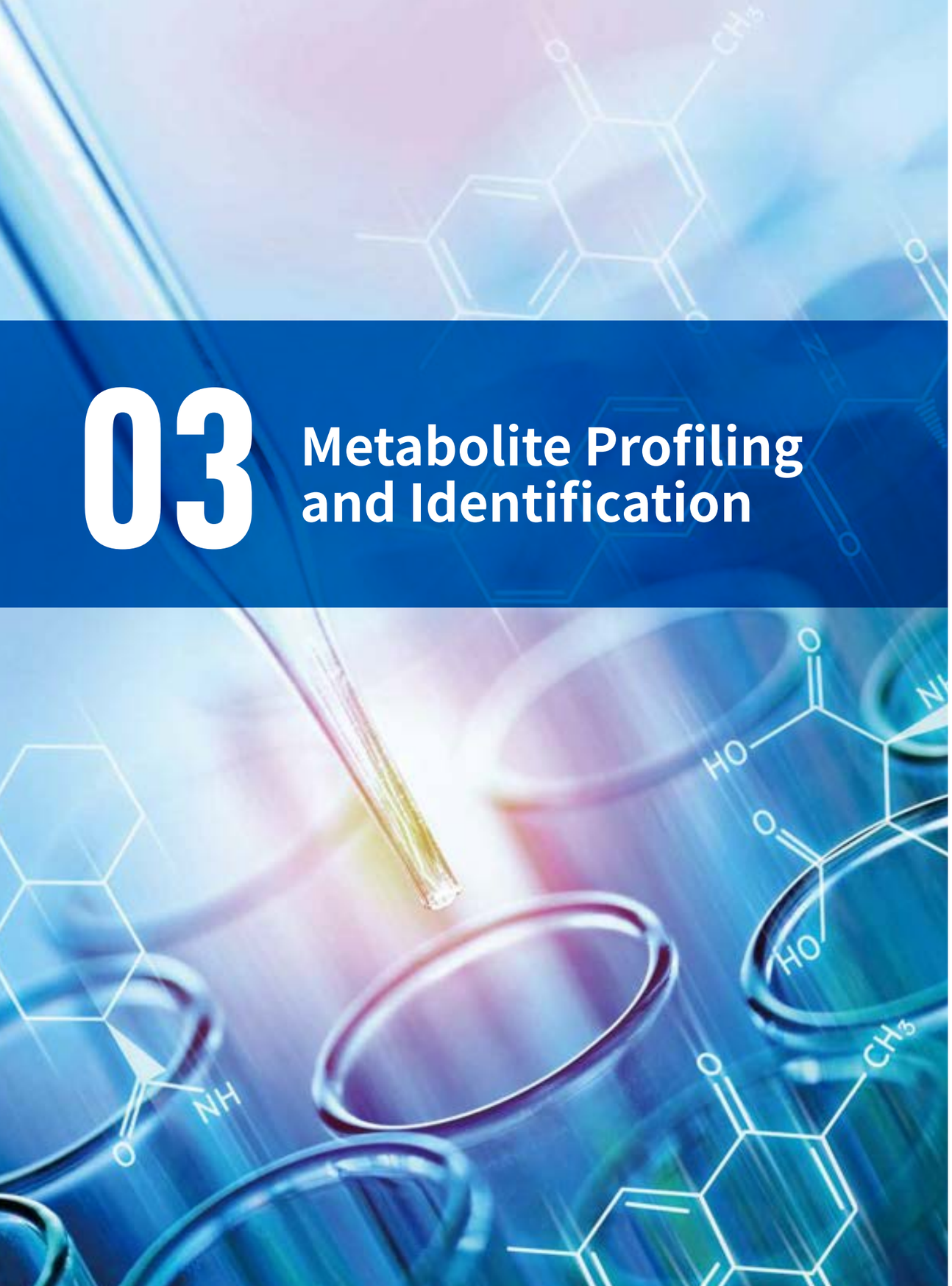
16. Seshadri Neer Vannan. Preclinical formulations for discovery and toxicology: physicochemical challenges. Expert Opin. Drug Metab. Toxicol. (2006) 2 (5): 715-731

17. Michael J Waring. Lipophilicity in drug discovery. Expert Opin. Drug Discov. (2010) 5 (3)

18. Dai W -G, Pollock-Dove C, Dong L C, Li S. Advanced screening assays to rapidly identify solubility-enhancing formulations: high throughput, miniaturization, and automation. Adv Drug Deliv Rev. 2008; 60 (6): 657 72

19. Hywel D. Williams, Natalie L. Strategies to Address Low Drug Solubility in Discovery and Development. Pharmacol Rev 65:315 499, January 2013

03 Metabolite Profiling and Identification



Drug metabolism studies run through the entire process of drug development. The information about metabolic pathways and metabolite structures of test compounds is vital for the design and optimization of lead compounds, selecting clinical drug candidates and supporting clinical studies, drug-drug interaction evaluation, and metabolites in safety testing in drug development. Metabolite profiling and identification (MetID) studies are helpful to understand clearance pathways to find potentially active or reactive metabolites and observe disproportionate or unique metabolites. Consequently, it leads to a better understanding of the effects of biotransformation on drug efficacy and safety.

In the process of screening lead compounds, metabolite identification can screen the formation of reactive metabolites and other potentially problematic metabolites. Based on results from *in vitro* metabolite identification, metabolic soft spots can be quickly identified for optimizing the structure of lead compounds and thus obtain better efficacy.

In the preclinical study stage, *in vitro* MetID studies of multiple species can be used to obtain cross-species metabolic differences. Comparing the *in vivo* and *in vivo* metabolism data of animals can determine if there is a good *in vitro-in vivo* correlation of metabolism, which can further ensure the reliability of using *in vitro* metabolism to predict *in vivo* metabolism in humans.

In the phase I clinical trial results from metabolite profiling in human plasma, urine, and feces can reveal qualitative and semi-quantitative exposure of drug-related components to humans and drug clearance and metabolic pathways in humans. It can be determined if the selection of toxicological animal species is appropriate after comparing the exposure of metabolites to human and tox species when repeated doses are assessed.

Radio-profiling can effectively study the metabolism, distribution, and clearance of a drug in an animal or human. Profiling and identification of Radiolabeled metabolites in plasma can determine qualitatively and semi-quantitatively drug-related components in the circulating system. The results can establish the basis for deciding the dose-exposure relationship of drugs and may help interpretation if toxicity observed is associated with the parent drug or its major metabolites in plasma. Profiles of Radiolabeled metabolites in bile, urine, and feces can provide information on the drug clearance and metabolic pathways *in vivo*, which can further help the experimental design of metabolizing enzyme phenotyping and transporter substrate analysis.

Our MetID Platform

We provide comprehensive MetID services, including MetID in biological matrices to support drug discovery and safety studies, metabolite isolation, and identification by NMR. The services cover lead compound optimization in the drug discovery stage to the clinical trial stage, including radiolabeled ADME study in animals and humans.

Our team of scientists can design bespoke experiments to overcome unique and complex challenges in metabolism study. We work hand in hand with pharmaceutical industries, research centers, and departments in WuXi to solve problems quickly and efficiently. We ensure timely delivery of reports that meet relevant regulatory requirements (FDA, EMA, NMPA, etc.). Our flexible, customized MetID solutions are designed to meet the requests of our customers.

Drug Development Process	Drug metabolite profiling and identification study can provide assistance	Assay Type
Hit to lead	▪ Guide the selection and optimization of lead compounds through metabolic soft spot analysis and reactive metabolite screening	▪ Metabolic soft spot analysis ▪ Reactive metabolite screening
Lead optimization	▪ Guide the structure optimization of lead compound by metabolic soft spot analysis as well as reactive metabolite analysis ▪ Evaluate major *in vitro/in vivo* metabolites to guide optimization of compound structure	▪ Metabolic soft spot analysis ▪ Reactive metabolite screening ▪ *In vitro* Met ID ▪ *In vivo* MetID in animals
Preclinical candidate (PCC)	▪ Evaluate correlation of metabolism in *in vitro/in vivo* to guide drug candidate selected ▪ Evaluate the *in vitro* metabolism cross-species. Select species for the subsequent toxicology studies.	▪ *In vitro* MetID ▪ Cross-species comparison of metabolites in *in vitro* MetID ▪ *In vivo* MetID in animals
Investigational New Drug (IND) application	▪ Provide complete documents of *in vitro/in vivo* metabolite profiling and identification study ▪ Support preclinical investigational new drug application (FDA/NMPA/EMA)	▪ Comparison cross-species of metabolites in *in vitro* MetID ▪ *In vivo* MetID in animals ▪ Radiolabeled MetID in animal
New drug application (NDA)	▪ Customized solution for complex problems encountered in the late stages of drug research ▪ Analyze the metabolite distribution of drugs in human circulation and obtain drug metabolism/elimination pathways ▪ Assessment of major metabolic and/or elimination pathways ▪ Evaluation of the safety of metabolites, assessing whether the toxicological species is appropriate and covers the metabolite exposure in humans	▪ Radiolabeled MetID in human ▪ MetID in human ▪ Radiolabeled AME study in human ▪ Metabolites in safety testing (MIST)

Key Features

Well established experimental system for MetID

Metabolite identification in various complex matrices includes *in vitro* incubations with liver/kidney/lung/intestinal microsomes, S9, lysosomes, recombinant enzymes, hepatocytes, tumor cells, plasma, and blood and *in vivo* samples of plasma, urine, feces, bile, and various tissues.

Wide range of compound types

Metabolite identification of test compounds with various structures and chemical properties, including regular small molecules, extremely polar and nonpolar small molecules, natural products, small molecular nucleotide compounds, PROTAC, polysaccharides, peptides, ADCs, oligonucleotides, PDCs, etc.

Customized experimental design for special needs

The customized experimental design shall be carried out according to the requirement, for example, derivatization to determine the compound structure, hydrogen/deuterium (H/D) exchange, and titanium trichloride (TiCl3) reduction, glutathione conjugation or acylglucuronidation for trapping the reactive metabolites, metabolite biosynthesis, and isolation.

Our Strengths

01 Efficiency

Thousands of metabolite identification projects/year

02 Full Service

Cover both non-radioactive and radioactive metabolite profiling and identification service, without method transferring and reducing additional time costs

03 Resource Collaboration

Work closely with WuXi AppTec's radioisotope chemical synthesis team to rapidly promote the progress of isotope projects

04 Excellence

The integrated quality control system is in place to monitor the quality of studies from the beginning of the experiment to the final report delivery

05 Highly Equipped Instrument

High-resolution mass spectrometry provide high accuracy data

06 Expert Team

▪ Expert with over 20 years' experience in medicinal chemistry reviews elucidation of metabolite structures
▪ A well-known expert on drug metabolism participates in study design and data interpretation

Instruments and Software

High Resolution Mass Spectrometry

Orbitrap Eclipse™ Tribrid™

Orbitrap Exploris™ 480

Q-Exactive™ HF

VION™ IMS QTof

Q-Exactive™Plus

Q-Exactive™

LTQ Orbitrap XL

Xevo®G2 QTof

Software for Data Process

- **Compound Discoverer**
- **Mass Frontier**
- **Metworks**
- **Metabolynx**
- **UNIFI**

For small molecule metabolite identification

- **BioPharma Finder**

For biological large molecule metabolite identification

Equipment for Radioactivity Detection

TopCount.NXT™

MicroBeta²®

Solid scintillation counter: off-line detection of radioactivity

Tri-Carb 3110TR

Liquid scintillation counter: total radioactivity detection

v.ARC™

Beta-RAM

On-line detection of radioactivity

Study Model of Metabolite Profiling and Identification (MetID)

Study	Study Objective	Study Model
In Vitro Met ID	Analyze metabolic soft spot to help optimization of lead compound structures	Metabolic soft spot analysis and identify
	Screen reactive metabolites to help optimization of lead compound structures	Reactive metabolite trapping (glutathione or cysteine)
	Compare *in vitro* metabolism across species to help the election of toxicological species	MetID in liver microsomes
		MetID in hepatocyte
		MetID in S9
		In vitro MetID in plasma or blood
In Vivo Met ID	Evaluate metabolite exposure and metabolic clearance pathways in animals	Met ID in animal samples (plasma/blood, urine, feces, bile, tissue, etc.)
	Evaluate metabolite exposure and metabolic clearance pathways in human	Met ID in human samples (plasma, urine, feces, etc.)
	Evaluate metabolite exposures in humans and animals to determine whether selected toxicological species are appropriate from the MIST perspective	Profiling and identification of metabolites in human and animal plasma samples collected in steady status
Radiolabeled *in vitro* metabolism and *in vivo* ADME study	Quantitative comparison of *in vitro* metabolism of radiolabeled compound cross-species	Identification of *in vitro* metabolites of a Radiolabeled compound
	Determine quantitatively and qualitatively metabolites and metabolic pathways of the radiolabeled compound in animals	Profiling and identification of Radiolabeled metabolites in animal plasma, urine, feces, and/or bile
	Determine quantitatively and qualitatively metabolites and metabolic pathways of a radiolabeled compound in humans	Profiling and identification of Radiolabeled metabolites in human plasma, urine, and feces

Metabolite Profiling and Identification

In Vitro MetID

Study Purpose

The pharmaceutical industry has widely used *in vitro* metabolite identification (MetID) assays since they are simple, rapid with high throughput. The *in vitro* MetID assays can be used to investigate the biotransformation of test compounds for predicting *in vivo* metabolism and save experimental animals.

In the early stage of drug development, the *in vitro* MetID assays are intended to identify "soft spots" for optimizing lead compounds and screen reactive metabolites to assess the potential toxicity of a lead compound. In the preclinical development stage, the *in vitro* MetID focuses on comparative metabolism in humans and animals, and results from the study can be used to support the selection of toxicological animal species. Information that identifies the major metabolites and associated metabolic pathways in *in vitro* can be used to design a metabolizing enzyme phenotyping experiment.

Platform Introduction

The WuXi AppTec DMPK MID team utilizes ultra-high performance liquid chromatography (UPLC) coupled with a photodiode array Detector (PDA) and high-resolution mass spectrometry (HRMS) to search and identify metabolites formed in incubation. Based on different study goals, three types of routine assay can be performed, including metabolic soft-spot analysis, reactive metabolite screening, and a metabolism comparison cross-species. The comprehensive incubation systems include but are not limited to liver microsomes, liver S9, hepatocytes, and blood/plasma. Meanwhile, some special types of assays can also be performed, such as acidified S9 and lysosomal systems used to investigate the payload-containing components released from an antibody drug conjugate (ADCs). In addition, some uncommon methods or approaches can be employed to confirm the metabolite structure, such as metabolite matching, hydrogen/deuterium (H/D) exchange, and titanium trichloride reduction.

Table 1. Common MetID Assays

Assay Type	Stage	Characteristics
Metabolic soft spot analysis	Early Screening	▪ Analysis and determination of the metabolic soft spots ▪ Liver microsomes/hepatocyte incubation systems with single or multiple species ▪ Focus on two or three major primary metabolites ▪ Sample incubation with a short time ▪ Fast turnaround time
Reactive metabolite screening (use glutathione or cysteine as a trapping agent)	Screening	▪ Rapid screening of compounds that can produce reactive metabolites ▪ Single or multiple species liver microsomes incubation system with GSH/Cys
MetID in single species and metabolism comparison cross-species	Screening/IND	▪ Detect major metabolites of compounds or compare metabolic profiles cross-species to support the toxicological animal species selection ▪ Metabolite profiling and major metabolite identification with a single species or multiple species ▪ Comprehensive incubation systems: liver microsomes, S9, hepatocytes, plasma

Table 2. Special MetID Assays

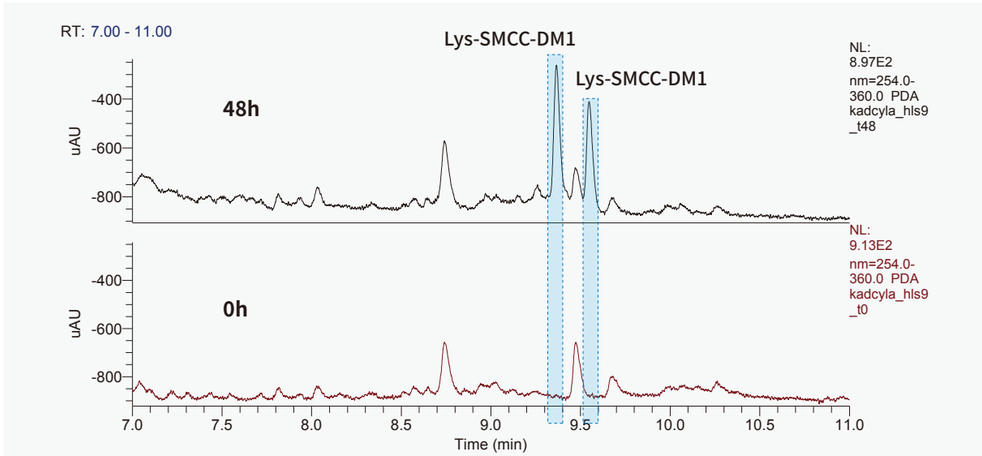
Type of Test Article	Study Model	Characteristics
Linear peptide and cyclic peptide	Met ID of peptides *in vitro* plasma/blood	▪ *In vitro* major metabolite profiling and identification ▪ Cross-species comparison of *in vitro* metabolite difference ▪ Single or multiple species: mouse, rat, dog, monkey, human, etc.
	Met ID of peptides in liver microsomes	
	Met ID of peptides in hepatocyte	
Antibody drug conjugate (ADC)	Met ID in acidified S9	▪ Acidified S9 with a similar function of lysosome can release payload-containing components from ADC [1] ▪ Identification of the major payload-related component released form ADC in S9 and comparison of metabolic differences across multiple species ▪ Single or multiple species: mouse, rat, dog, monkey, human
	Met ID in lysosomal	▪ Payload-containing components can be released from ADC in the lysosomes incubation system ▪ Identification of the major payload-related component released form ADC in lysosomes and comparison of metabolic differences across multiple species ▪ Single or multiple species: rat, monkey, human,
	Met ID in tumor cells	▪ Identification of the major payload-related component released form ADC in tumor cell
Oligonucleotide	Met ID in S9	▪ Major metabolite profiling and identification ▪ *In vitro* metabolism comparison cross-species
	Met ID in *in vitro* plasma	
Metabolites with exchangeable hydrogen	Hydrogen/deuterium exchange experiments	▪ Analysis and comparison of the MS data of targeted metabolites acquired under different mobile phases with and without deuterated reagents to determine how many exchangeable hydrogens they have and where these exchangeable hydrogens are locked
Metabolites with N-oxide	Reduction experiments by titanium trichloride	▪ Analysis and comparison of the MS data of the N-oxidation metabolite with and without treatment of titanium trichloride ▪ Confirm whether the metabolite is an *N*-oxidation metabolite

Case Study

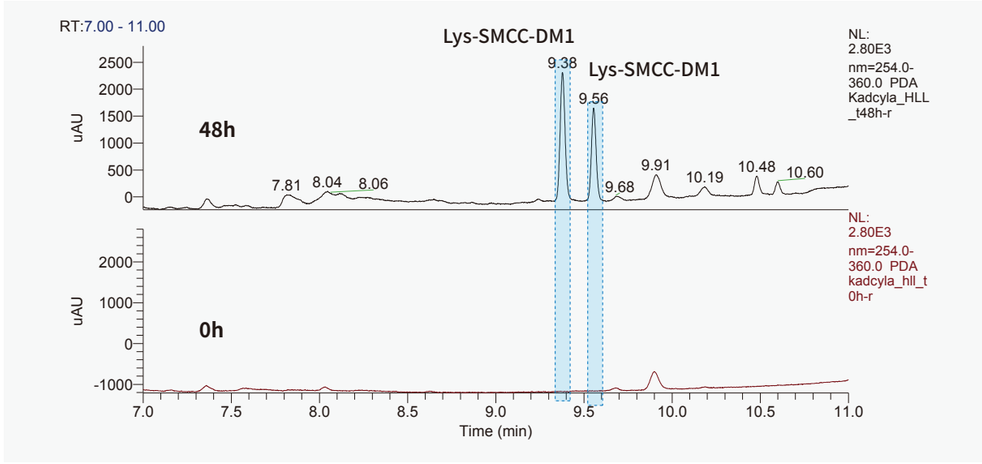
▶*In vitro* systems for investigating payload-containing components released from ADC

An ADC can be delivered into the cell either by antigen receptor-mediated internalization or by nonspecific endocytosis, followed by releasing the drug in the lysosomal compartment and resulting in the tumor cell apoptosis. To determine payload-containing components released from ADCs after entering tumor cells, *in vitro* acidified liver S9 and lysosomes systems of human and animal species, coupled with non-targeted HRMS data mining technologies, are established and applied to identify the major payload-related component released from ADCs. Those results can support the selection of toxicological animal species and analyte(s) for PK analysis.

In vitro MetID case study of ADC:



Metabolite profile (LC-UV) of T-DM1 (Kadcyla, ADC) in human acidified liver S9 after 48h incubation



Metabolite profile (LC-UV) of T-DM1 (Kadcyla, ADC) in human lysosomal after 48 h incubation

Metabolite Profiling and Identification

In Vivo MetID

Study Purpose

Metabolite profiling and identification studies in whole blood or plasma of animals can provide information on exposure of drug-related components (and/or) the metabolite profiles of drugs. This can be used to evaluate the effect on bioavailability arising from absorption or metabolism and to guide subsequent structural optimization and formulation adjustment.

The metabolite profiling and identification study in animal excreta (including bile, urine, and feces) can provide information on the drug's metabolic and clearance pathways. The correlation between *in vitro* and *in vivo* metabolism of the drug can be analyzed when compared with results from *in vitro* metabolism studies in liver microsomes and hepatocytes, supporting the prediction of metabolism and disposition in humans based on *in vitro* metabolic pathways.

The first-in-human (FIH) studies will provide the earliest information of drug exposure to humans. Also, metabolite profiles in human excreta, together with mass balance data, can provide information on major metabolic and clearance pathways of drugs in humans. The metabolite profiling and identification of human plasma from the multiple ascending dose (MAD) experiment can determine how many metabolites are in the plasma and identify their structures and relative abundances. Further study is conducted to evaluate whether major human plasma metabolites are present in animal circulation with exposure levels equal to or higher than those in humans, which is very important to ensure toxicity of major human plasma is tested in the toxicological species.

Platform Introduction

The *in vivo* MetID platform in WuXi AppTec uses ultra-high performance liquid chromatography (UPLC) coupled with photodiode array detector (PDA) and high-resolution mass spectrometry (HRMS) such as Q-TOF and Orbitrap to acquire accurate mass spectral data efficiently. Using HRMS and multiple data processing tools, MetID results are generated with great confidence, which are used to support safety assessment, clinical studies, and regulatory submissions. The established *in vivo* MetID platform has performed metabolite profiling and identification of lead compounds and drug candidates with a variety of structures, including but not limited to conventional small molecules, high-polar and non-polar small molecules, natural products, nucleotides, PROTAC, polysaccharides, peptides, ADCs, PDCs, and oligonucleotides. Sample matrices include whole blood, plasma, urine, feces, bile, and tissues.

Feature Introduction

Metabolite in Safety Testing[2]

After the drug enters the human body, the produced metabolites are all exogenous substances for the human body. When the exposure to a metabolite at a steady state exceeds 10% of the total exposures of all drug-related components in humans, the safety of this metabolite should be evaluated in toxicological studies. Drug metabolites in plasma can be searched and identified, and relative abundances of the detected metabolites obtained using high-resolution mass spectrometry combined with relevant data processing software. For major metabolites, further comparison of its exposure to toxicological species should be determined after multiple-dosed toxicological species. Results from the study can ensure if the selected toxicological species are appropriate.

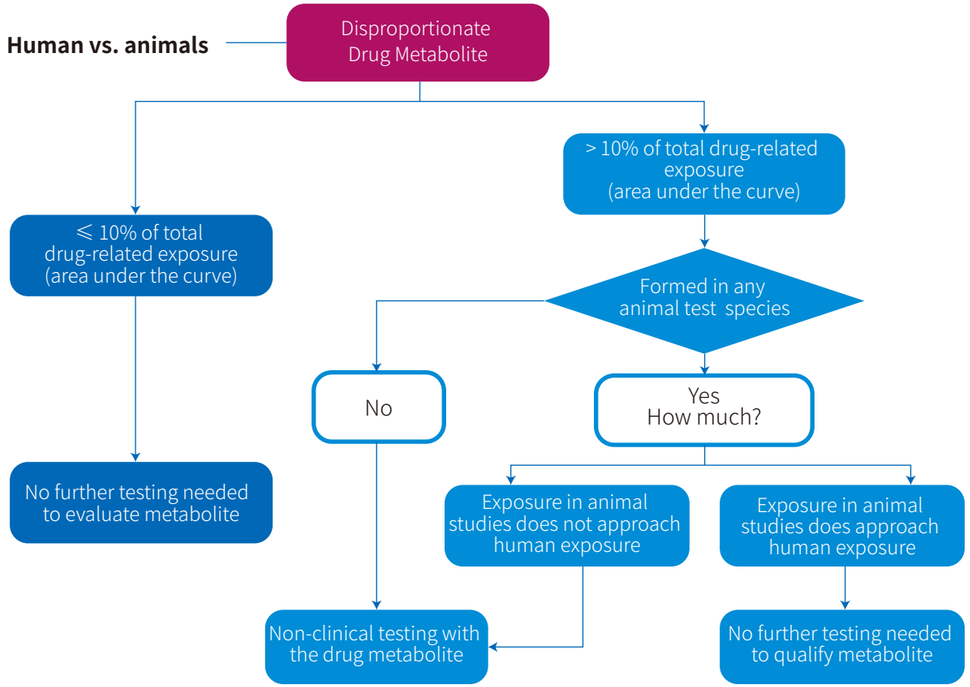


Table 1. Representative Assays

Assay Type	Stage	Characteristics
MetID in Animal Circulation	Screening /IND	▪ Species: mouse, rat, dog, monkey, etc. ▪ Plasma AUC pool [3] or/and equivalent volume pool ▪ LC-UV-HRMS system, analysis, and identification of metabolites ▪ Determining the relative abundance of parent and metabolites in plasma
MetID in Animal Tissues or Excreta	Screening /IND	▪ One or more matrices: urine, feces, bile, or tissue ▪ Pooled sample in equal proportion ▪ LC-UV-HRMS system, analysis, and identification of metabolites ▪ Propose Major Metabolic Pathways and Excretion Pathways
MetID in Human Circulation	NDA	▪ Plasma AUC pool [3] or/and equivalent volume pool ▪ LC-UV-HRMS system, analysis, and identification of metabolites ▪ Determining the relative abundance of parent and metabolites in plasma
MetID in Human Excreta	NDA	▪ One or more matrices: urine, feces, etc. ▪ Pooled sample in equal proportion or volume ▪ LC-UV-HRMS system, analysis, and identification of metabolites ▪ Proposing Major Metabolic Pathways and Excretion Pathways
Metabolite in Safety Testing (MIST)	NDA	▪ AUC[3] pooled multiple ascending dosed human plasma in a constant state ▪ AUC[3] pooled multiple ascending dosed animal plasma in a constant state ▪ LC-UV-HRMS system, analysis, and identification of metabolites ▪ Exposure of major metabolites with relative abundance higher than 10% in the human body will be compared with metabolites in plasma of toxicological species

Metabolite Profiling and Identification

Radiolabeled MetID

Study Purpose

Radioisotope tracing technology combined with an LC-HRMS analysis platform can be used to quantify and identify *in vitro* Radiolabeled metabolites (animals and humans), helping determine *in vitro* metabolic pathways.

Radiolabeled absorption, distribution, metabolism, and excretion (ADME) of candidate compounds in animals and humans can be performed using radioactivity analysis together with LC-HRMS. These studies can determine mass balance and extraction and metabolite profiles and structures in plasma, urine, feces, and/or bile, and drug clearance pathways in *in vivo* metabolite profiles. The combination of mass balance, excretion routes, metabolite profiles and identification, a major metabolic pathway, and the clearance pathway of a drug in animals and humans can be accurately determined. These results can further guide the follow-up studies, such as metabolizing enzyme phenotyping and/or transporter substrate analysis, clinical DDI experiments, and special population experiments (hepatic or renal impairment, gene polymorphism).

Platform Introduction

WuXi AppTec DMPK can provide Radiolabeled MetID of [^{14}C] and [^{3}H] labeled compounds *in vitro*, in animals and humans. Experimental models include but are not limited to metabolic stability and metabolite identification studies *in vitro* (plasma, liver S9, liver microsomes, or hepatocytes from multiple species) and *in vivo* (mice, rats, dogs, monkeys, and humans). The radiolabeled metabolite identification platform has been applied to various compound types, including conventional small molecules, high-polar and non-polar small molecules, natural products, nucleotides, PROTAC, peptides, ADCs, PDCs, and oligonucleotide. Matrix types include blood, plasma, urine, feces, bile, tissues.

Feature Introduction

Radioisotope tracer technology has unique advantages in MetID studies. Based on radioactive metabolite profiles, the distribution of each metabolite can be clearly and accurately observed. Apart from searching for metabolites, [^{14}C] isotopes can also help the identification of metabolites. Mass spectrometry or UV detection may differ between MS/UV response and the actual amount of metabolites due to their ionization efficiency or UV absorption. The radioactivity determination can intuitively and accurately quantify and characterize metabolites (see the following Figure).

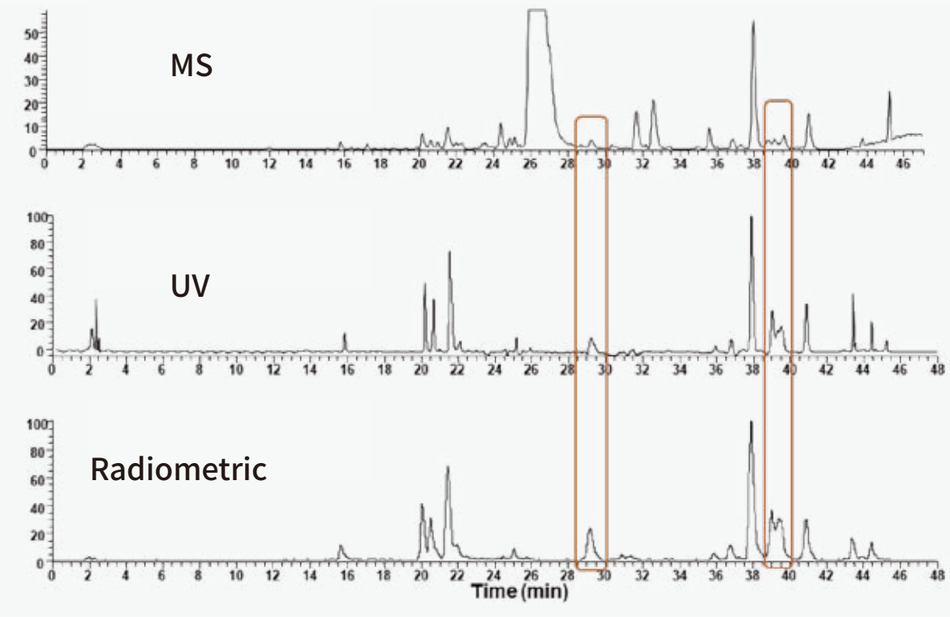


Table 1. Representative Radiolabeled Assays

Assay Type	Stage	Characteristics
In vitro MetID for cross-species comparison	IND	▪ Single or multiple species: mouse, rat, dog, monkey, human, etc. ▪ Matrix: plasma, liver microsomes, hepatocytes, liver S9, etc. ▪ Radioactive detector coupled with LC-MS ▪ Provide the *in vitro* metabolism difference between humans and animals
MetID in Animal Circulation	IND	▪ Species: mouse, rat, dog, monkey, etc. ▪ Matrix: plasma ▪ Radioactive detector coupled with LC-MS ▪ Evaluate whether there are major metabolites in the circulation of animals
MetID in Animal Tissues or Excreta	IND	▪ Species: mouse, rat, dog, monkey, etc. ▪ One or more matrices: urine, feces, bile, or tissue, etc. ▪ Pooled by an equal proportion ▪ Radioactive detector coupled with LC-MS ▪ Propose the major metabolic and clearance pathway in animals
MetID in Human Circulation	NDA	▪ Pooled by AUC method [3] or an equal volume ▪ Radioactive detector coupled with LC-MS ▪ Evaluate whether there are major metabolites in the circulation of human
MetID in Human Excreta	NDA	▪ One or more matrices: urine, feces, etc. ▪ Pooled by equal proportion or equal volume/weight ▪ Radioactive detector coupled with LC-MS ▪ Propose the major metabolic and clearance pathway in human

REFERENCES

1. Eugenia Kraynov, et al., Current Approaches for Absorption, Distribution, Metabolism, and Excretion Characterization of Antibody-Drug Conjugates: An Industry White Paper, Drug Metabolism and Disposition, 44: 617-623, May 2016

2. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), Safety Testing of Drug Metabolites Guidance for Industry, Pharmacology/Toxicology, March 2020, Revision 2

3. Cornelis E. C. A. Hop, et al, Plasma-Pooling Methods To Increase Throughput for *in Vivo* Pharmacokinetic Screening, Journal of Pharmaceutical Sciences, Vol. 87, No. 7, July 1998

04 Radiolabeled *In Vivo* PK/ADME Study

Lead Optimization (LO)

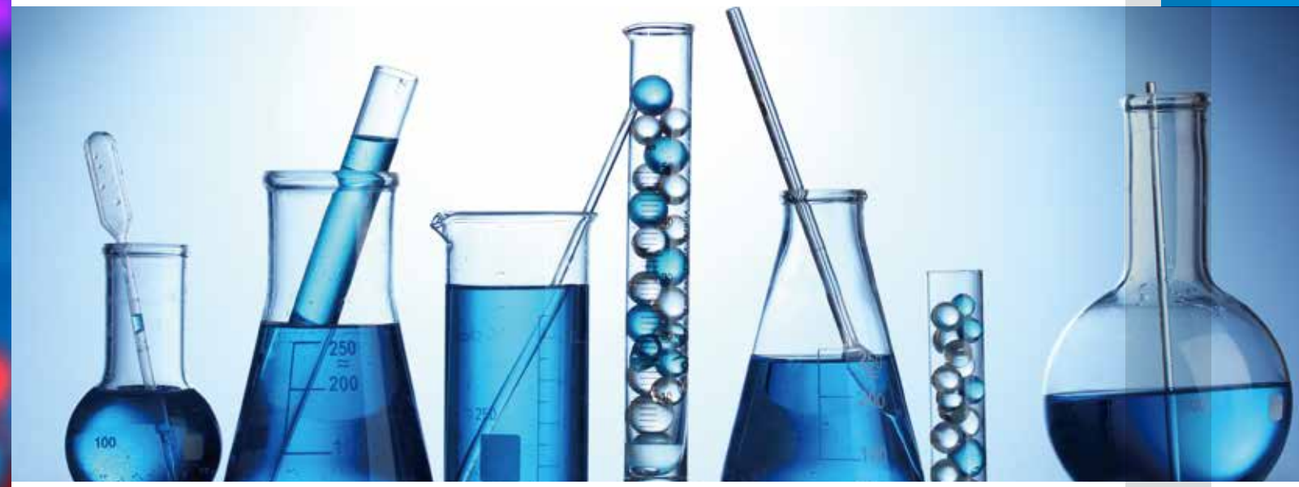
If the test article is found easy to bind and challenging to extract in specific test systems (cells, plasma, and other biological matrices), ^{14}C- and ^{3}H- labeled test articles can be used to reduce the difficulty of sample treatment and eliminate the effect of low recoveries. It is recommended to use radiolabeled test articles for endogenous substances to avoid endogenous interference and obtain accurate exogenous data.

Pre-Clinical Candidate (PCC)

A following administration of the radiolabeled test article is typically performed when the test article has a low bioavailability. It is determined to be due to poor absorption or rapid metabolism by comparing the post-labeled results (parent and metabolites) and pre-labeled (only parent) results. The results can help to optimize the chemical designs.

Investigational New Drug (IND)

Radiolabeled test articles can solve the low recovery issue in the mass balance studies. The Quantitative Whole-Body Autoradiography study (QWBA) can obtain complete and detailed tissue distribution information by scanning the animals' whole body. This can be used to predict the drug efficacy and accumulation degree comprehensively to guide clinical medication.



Our Radiolabled Research Platform

Key Features

▶Dares to Develop

^{14}C- and ^{3}H- radiolabeled test articles are applied in preclinical and clinical studies to meet NMPA and FDA submission requirements and successfully fill the research field gap in China.

▶Strive For Excellence

By optimizing the collection and detection methods, the total recoveries of most radiolabeled test articles can exceed 90% in the mass balance studies. For those below 90% (rare), the ^{14}C CO_2 collection apparatus can help to increase the recovery rate and achieve the excretion route.

▶Customized Design

We can combine the non-labeled and radiolabeled methods to provide a personalized study design for customers based on the characteristics of test articles. This includes and is not limited to *in vitro* plasma protein binding studies, *in vitro* metabolite profiling in various incubation systems (plasma, S9, liver microsomes, hepatocytes), and *in vitro* CYP/UGT enzymes phenotyping studies.

Our Strengths

Experienced and Comprehensive

WuXi AppTec has over ten years and more than 300 IND and NDA submission experience, we provide radio-related *in vitro* and *in vivo* preclinical and clinical analysis studies.

Quick Turnaround Time

By collaborating with multiple hospitals, the shortest time to submit mass balance results can be 24-48 hours. Preliminary data can help determine whether the experimental expectation is achieved or the clinical volunteers can be out of the group.

01 02 03

Well Controlled Timeline and Cost

^{14}C-labeled test articles can be provided by the client or synthesized directly in WuXi AppTec. We reduce the cost, shorten shipping time, and design reasonable labeling sites based on chemical structure, synthetic process, and early-stage metabolite identification results.

Routine Assays of Radiolabeled Test Articles

Classification	Purpose/Characteristic	Assay Type
Non-Clinical *In Vivo* PK/ADME	Obtain excretion recovery of total radioactivity, determine excretion rate, and major excretion route	Mass Balance Study (fecal, urinary, and biliary excretion)
	Investigate the distribution of total radioactivity in specific tissues	Tissue Distribution Study (dissection method)
	Determine plasma concentration-time curve and ratio of blood to plasma	Blood/Plasma Pharmacokinetic Study
	Achieve metabolite profiling and metabolite identification of radiolabeled test articles	Refer to page 85 for details
Quantitative Whole-body Autoradiography (QWBA) * Figure 1	Investigate the distribution of total radioactivity throughout the body, obtain complete and detailed tissue distribution results, and assess the safe dosage of radioactivity in the human body	Tissue Distribution Study (QWBA method)
Clinical *In Vivo* PK/AME	Obtain excretion recovery of total radioactivity, determine excretion rate and major excretion route	Mass Balance Study in Human
	Obtain plasma concentration-time curve and ratio of blood to plasma	Blood/Plasma Pharmacokinetic Study in Human
	Achieve metabolite profiling and metabolite identification of radiolabeled test articles	Refer to page 85 for details

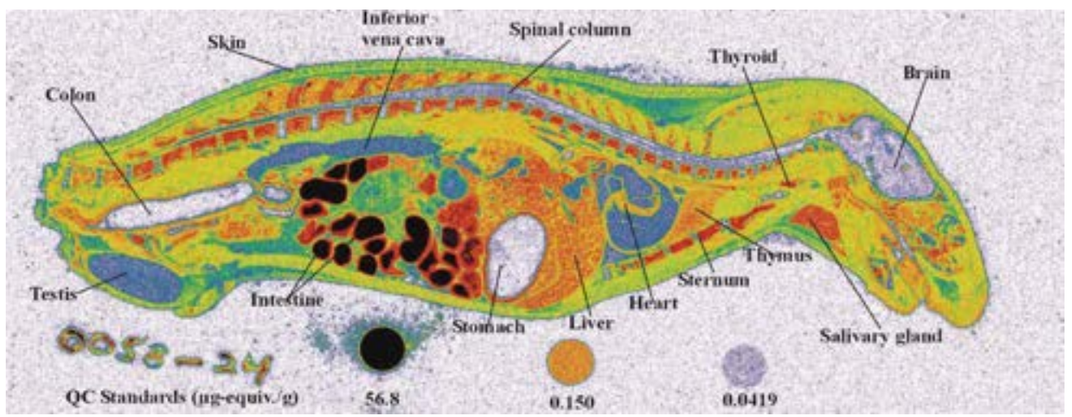


Figure 1: Representative Quantitative Whole-body Autoradiography (QWBA) Images following Administration of ^{14}C- labeled Test Article to Rats

Radiolabeled *In Vivo* PK/ADME Study

Radiolabeled Non-Clinical *In Vivo* PK/ADME

Study Purpose

After animals are administered with the radiolabeled test articles, the following studies will be conducted to

1) Investigate the recovery rate and mass balance of the total radioactivity in the urine and feces,
2) Investigate the tissue distribution of the total radioactivity,
3) Determine the concentration-time profiles of the plasma/blood,
4) Identify the metabolite profiles and major metabolites of plasma, bile, urine, and feces collected from animals (refer to the metabolite profiling and identification studies).

Platform Introduction

Various species (mouse, rat, minipig, dog, and monkey) models can be provided for *in vivo* studies with radiolabeled test articles.

Animals will be transferred to the corresponding metabolic cages. Urine, feces, bile, plasma, and tissue samples will be collected at specific time points after administration. The total radioactivity in the various matrices will be analyzed by liquid scintillation counter to investigate the pharmacokinetics of radiolabeled test articles.

The types of test articles include but are not limited to small molecules, peptides, ADC, and oligonucleotides.

Metabolic cages for rats

Metabolic cages for dogs

Table 1 Representative Assay Types

Study Type	Study Phase	Species	Matrix	Study Description
Mass balance	IND	Mouse, rat, minipig, beagle dog, cynomol-gus monkey	Feces, urine	Urine, feces, cage rinse/wash, and carcass samples will be collected and analyzed by liquid scintillation counter to provide the results of excretion rate and mass balance after administration of [^{14}C] or [^{3}H] test articles to animals.
Biliary excretion	IND	Mouse, rat, beagle dog, cynomolgus monkey	Bile, feces, urine	Bile, urine, feces, and cage rinse/wash will be collected and analyzed by liquid scintillation counter to provide the results of biliary excretion rate and mass balance after administration of [^{14}C] or [^{3}H] test articles to BDC animals.
Tissue distribution (dissection method)	IND	Mouse, rat	Tissue, blood, plasma	Tissues, blood, and plasma will be collected at the specified time points and analyzed to investigate the distribution and elimination in all selected tissues.
Blood/plasma concentration-ti me profiles	IND	Mouse, rat minipig, beagle dog, cynomolgus monkey	Blood, plasma	Blood/plasma will be collected at different time points and analyzed to determine the pharmacokinetics parameters of the total radioactivity.

▶Non-routine Experiment/ Special Experiment

$^{14}CO_2$ Collection study

If the recovery of the total radioactivity in mass balance is less than 90%, it is recommended to conduct a $^{14}CO_2$ collection study.

The animals will be placed in sealed glass cages and the air flow in the cages guided by an air pump. After passing through the CO_2 absorber, the total radioactive recovery of $^{14}CO_2$-related gas from an absorber will be analyzed and calculated to determine whether the test articles can be excreted via the respiratory route.

$^{14}CO_2$metabolic cage

Radiolabeled *In Vivo* PK/ADME Study

Quantitative Whole-body Autoradiography (QWBA)

Study Purpose

This study aims to determine the tissue distribution of radiolabeled test articles derived radioactivity using the quantitative whole-body autoradiography (QWBA) method. Based on the exposure and half-life in rodent tissues, the distribution results can provide the estimated effective dose of radioactivity to human subjects.

Platform Introduction

The radioactivity experimental platform of DMPK can provide a variety of species (CD-1 mouse, C57 mouse, SD rat, LE rat, NHP, small dog, etc.) models for QWBA study.

One animal/gender will be euthanized at a specific time point post dosing. All animals will be euthanized, and the carcasses will be immediately frozen. Quantitative whole-body autoradiography (QWBA) is applied to determine total radioactivity in various tissues (the table below) and in the area of interest (AOI).

Whole-body sections of the carcass in the CMC block will be performed in the large cryostat microtome and exposure to a ^{14}C sensitive phosphor imaging plate. Selected tissues and AOI will be analyzed using quantitative whole-body autoradiography (QWBA) techniques and within AIDA software using a region sampling tool.

Cryostat Microtome (Leica)

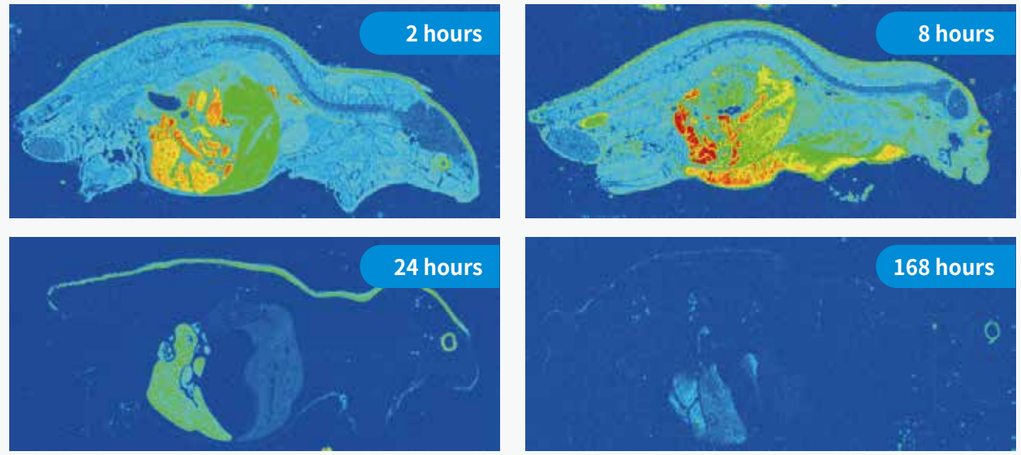
Imager (Amersham Typhoon RGB)

Tissue Group	Tissue Name
Vascular	Plasma (LSC)
	Aorta
	Blood (cardiac)
Central Nervous System	Brain (whole)
	Cerebellum
	Brainstem
	Spinal cord
Ocular	Eye (whole)
	Eye (ciliary body)
	Eye (cornea)
	Eye (iris)
	Eye (sclera)
	Eye (vitreous humor)
	Lens
	Uveal tract
Dermal	Skin; non-pigmented skin
	Skin; pigmented skin
Endocrine	Adrenal gland
	Adrenal cortex
	Adrenal medulla
	Pituitary gland
	Thyroid gland
Fatty	Brown fat
	White fat (inguinal)

Tissue Group	Tissue Name
Gastrointestinal (GI)	Oral mucosa
	Tongue
	Esophagus wall
	Stomach wall (glandular)
	Stomach wall (non-glandular)
	Small intestine wall
	Large intestine wall
	Cecum mucosa
Metabolic /Excretory	Kidney
	Kidney medulla
	Kidney cortex
	Bile (in duct)
	Liver
	Urinary bladder wall
Muscular	Heart
	Muscle (femoral)
	Diaphragm
Reproductive	Epididymis
	Prostate
	Seminal vesicle
	Testis

Tissue Group	Tissue Name
Respiratory	Lung
	Nasal turbinates
	Trachea
Secretory	Lachrymal gland
	Ex-orbital lachrymal gland
	Intra-orbital lachrymal gland
	Harderian gland
	Pancreas
	Salivary gland
Skeletal	Bone (femur)
	Bone marrow (femur)
GI Tract Contents	Stomach contents
	Small intestine contents
	Large intestine contents
	Cecum contents
Lymphatic	Lymph node (cervical)
	Spleen
	Thymus

Representative Quantitative Whole-body Autoradiography Images at Various Time Points following Oral Administration of [^{14}C] Test Article to Male LE Rats



Radiolabeled *In Vivo* PK/ADME Study
Radiolabeled Clinical *In Vivo* PK/AME

Study Purpose

To conduct the single-center, single-dose, open-label mass balance clinical trial using radioactive radiolabeling technology the human [^{14}C]-AME study monitors the *in vivo* absorption, metabolism, and excretion process after administration of [^{14}C] test article formulation. The main research scope includes:

1) Determining the radiolabeled test article's mass balance results and human main excretion route,

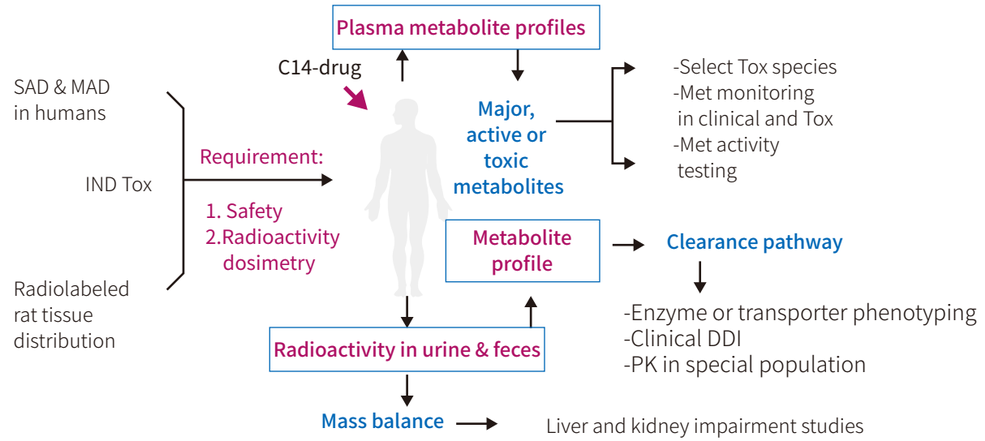
2) Analyzing the ratio of whole blood to plasma and the pharmacokinetics of total radioactivity in plasma,

3) Identifying the major metabolites in urine, feces, and plasma.

Platform Introduction

The human [^{14}C]-AME study could evaluate the overall pharmacokinetic characteristics of the test article, including absorption, excretion route (mass balance), and metabolite identification. The study can also provide the basis for clinical PK and DDI experimental design and guidance for drug instruction. The WuXi AppTec's DMPK platform cooperates with the Clinical Center to conduct the human [^{14}C]-AME clinical study.

The human [^{14}C] -AME study is usually carried out after the phase I clinical escalation experiment. The safe and reliable human radioactive dose is estimated based on the results of single-dose or multiple-dose escalation experiments and the QWBA experiment [3]. Subjects are given a dose of [^{14}C] test article, and urine, feces, plasma are collected. Total radioactivity (TRA) in urine and feces is measured to obtain the mass balance results. In addition, metabolite profiling is performed on selected plasma, urine, and feces samples. Possible metabolic pathways and major metabolites of the test article are provided, and the toxicity or activity of the major metabolites should be tested. The comprehensive results can guide further clinical study design to ensure test articles can be used safely and effectively in patients.



The DMPK platform has cooperated with multiple clinical centers to conduct human mass balance studies in recent years. Clinical metabolite identification studies supported several innovative drugs to be successfully promoted and marketed.

Representative Assay Types

Assay Type	Species	Test Content	Description
Urine Adhesion	Human	Urine	Investigate the adhesion of the test article to the collection container in urine.
Radioactive Clinical Formulation	Human	Preparation of Radioactive Clinical Formulations	Prepare the dosing formulation, investigate the homogeneity and stability of the dose formula. Determine the residual radioactivity of empty vials after administration to calculate the actual dose.
Human Mass Balance Study	Human	Feces, urine, plasma,	Determine the total radioactivity in whole blood, plasma, urine, and feces. Obtain the urinary and fecal excretion rate and mass balance results. Calculate pharmacokinetic parameters and the ratio of whole blood to plasma.
LC-MS/MS Analytical Method Development and Validation in Plasma	Human	Plasma	Quantitatively analyze the test articles and major metabolites (if any) in human plasma. The data will be further used to guide radiolabeled/unlabeled clinical studies.

REFERENCES

1. Ronald, E. and David C., 2013. Radiolabeled mass-balance excretion and metabolism studies in laboratory animals: a commentary on why they are still necessary. Xenobiotica. 43(2), p.219-225

2. Shin-ichiro, N. and Shin-ya H., 1988. Quantitative analysis of whole body autoradiogram using a computer-assisted image analyzer. Drug Metabolism and Pharmacokinetics, 3(2), p.121-135

3. Gabrielsson, J. and Weiner D., 2001. Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications, 4th edition, Swedish Pharmaceutical Press. p.207

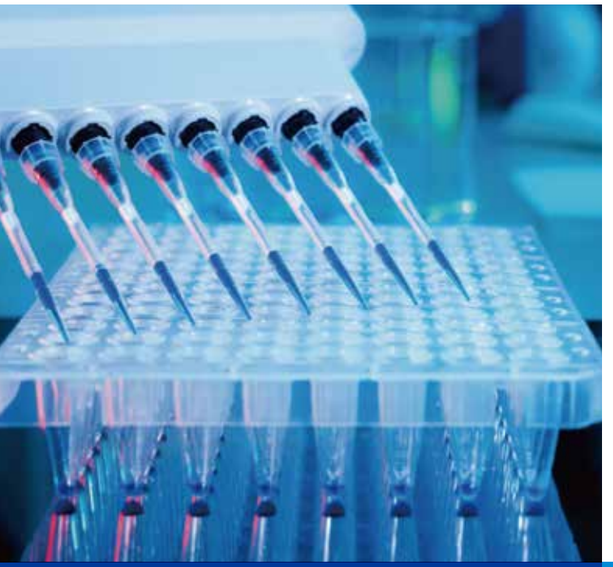
05 Non-GLP Bioanalysis

It is necessary to study a drug mechanism and evaluate its efficacy to achieve safe, rational, and effective drug use in the drug development process. This evaluation includes pharmacokinetics, pharmacodynamics, and toxicology. An effective analytical methodology is required to monitor drugs, their metabolites, and changes in biomarkers to qualitatively and quantitatively elucidate the drug transformation process and changes in the body under the influence of drugs. Bioanalysis is an effective detection method that accurately determines the concentrations of drugs, metabolites, and biomarkers in biological matrices (such as serum, plasma, urine, cells, and various tissues). Bioanalysis can provide safety and efficacy data for drugs, help researchers accurately evaluate the experimental results of pharmacology, pharmacokinetics, toxicokinetics, and bioequivalence, and make critical decisions. Bioanalysis has become an indispensable part of new drug research.

Our Non-GLP Bioanalysis Platform

Since 2006, we have focused on preclinical bioanalytical services and established a long-term mutual trust collaboration model with pharmaceutical companies worldwide. With an experienced professional analysis team, advanced analytical instruments, whole-process electronic management, and reliable quality control systems, we continuously empower our customers with high-efficiency and high-quality bioanalytical services from screening stages to a clinical trial application that includes drug discovery, *in vitro* and *in vivo* pharmacokinetics, pharmacodynamics, and toxicology. We provide high-quality customized services for the personalized needs of customers in large and small molecule drugs, biomarkers, immunogenicity, and cell-based assays.

- ***In vitro* sample bioanalysis:** We support ADME *in vitro* experiments with various high throughput autosamplers such as ADDA (Apricot Designs Dual Arms). The analytical speed is as fast as 12 seconds/sample.
- ***In vivo* sample bioanalysis:** We have considerable experience in the bioanalysis of conventional small molecule chemical drugs. We developed bioanalytical solutions for other types of molecules, including polar compounds, unstable compounds, chiral compounds, biomarkers, PROTACs, peptides, proteins, nucleoside triphosphates, and oligonucleotides. We have also established bioanalytical solutions for special dosing methods such as liposomes, PEGylated drugs, and antibody-drug conjugates (ADCs).
- **Special matrices and microsampling analysis:** We have developed bioanalytical methods for samples obtained from more than 70 biological matrices and special sample collection methods. Sample pretreatment and bioanalysis methods have been established for matrices such as cerebrospinal fluid, microdialysate, PBMC, and ophthalmic tissues with limited sample volume and demanding lower limit of quantification (LLOQ) requirements. We have a complete set of procedures from sample collection to bioanalysis for special sample collection methods such as dry blood spot, dry plasma spot, capillary sampling, and volumetric absorptive microsampling (VAMS).
- **Instrument platform:** The qualitative and quantitative bioanalysis of drug candidates, metabolites, biomarkers, and large molecule anti-drug antibodies in *in vivo* and *in vitro* samples is achieved through industry-leading liquid chromatography mass spectrometry (LC-MS) analysis platform, ligand binding assay (LBA) platform, and related technologies to support the research and optimization of drugs and formulations.



We provide customers with high-quality data on millions of *in vitro* and *in vivo* samples each year based on highly optimized experimental procedures, robust high-throughput technologies, and good laboratory management systems. The comprehensive application of sample pretreatment automation and electronic notebook systems enables the visual tracking of the whole process and ensures a short bioanalysis turnaround time (TAT) for customers. For example, the TAT of a conventional small molecule screening project is less than 24 hours for *in vitro* bioanalysis and 48 to 72 hours for *in vivo* bioanalysis. In the meantime, we follow bioanalytical regulations and industry standards to ensure the robustness of analytical methods and the reliability of our data. By helping customers overcome complex technical challenges of bioanalysis and providing specialized analytical services, we strive to empower our customers to quickly develop new drugs that can change and save lives.

Key Features

▶Deep in technology:

Each analytical technology platform has a dedicated team

Dedicated analytical teams have been established for the multiple analytical technology platforms in the preclinical drug development process, including *in vitro* high-throughput screening team, *in vivo* screening team, biomarker team, (investigational new drug) IND, and large molecule bioanalysis team. Each team constantly strives for perfection in their respective focus areas and delivers high-quality data efficiently. At the same time, our professional operation team, instrument maintenance team, report, and QC team work jointly to ensure the delivery of high-quality data and smooth progress of the projects.

▶Excellent in analysis:

High-caliber new analytical capability building research team

We continuously monitor the development of new modalities, various cutting-edge advancements, and new bioanalytical technologies, and we have formed a dedicated bioanalytical research and development team. This team, led by ~10 Ph.D.'s from prestigious universities, has developed several high-quality bioanalysis platforms for new modalities such as peptides, proteins, and oligonucleotides. Since its inception, the team has published five papers, 26 conference posters, and two patents. The team members have won many awards, including the European Bioanalytical Forum Bioanalysis Rising Star Award, the Clinical and Pharmaceutical Solutions through Analysis Forum Excellent Young Scientist Award, and multiple customer awards.

Our Strengths

01 Seamless upstream and downstream transitions

- Analytical method transfers between *in vitro* and *in vivo* phases and between non-GLP and GLP bioanalysis phases
- One-stop analysis

02 Comprehensive services

- From screening to IND filing
- Multiple analyte types: conventional small molecules, PROTACs, biomarkers, peptides, proteins, oligonucleotides.
- PK and ADA experiments for large molecules
- Over 70 matrices

03 High-throughput and automated instrument platforms

- Automated sample pretreatment systems
- Industry-leading analytical platforms
- High-throughput analytical platforms (12 sec/injection)

04 High-quality data for IND filing

- PK and ADA experiments
- Following IND filing criteria of NMPA, FDA, EMA
- Zero findings through customer and regulatory audits

05 Experienced research staffs

- > 60% hold Master's or Ph.D. degrees
- > 20% have more than ten years of industrial experience

Non-GLP Bioanalysis

Scope of Bioanalytical Services

The WuXi AppTec DMPK Non-GLP Bioanalysis Team continuously focuses on various new modalities, new formulations, and microsampling techniques. We have extensive experience in many routine and challenging bioanalytical projects. Table 1 highlights some uncommon analytes and bioanalytical methods used. Table 2 summarizes some non-routine sample collection techniques and their corresponding bioanalytical methods, and Table 3 lists some representative biological matrices.

Table 1. Selected uncommon analytes and their methods

Analyte	Features	Description
PROTAC	▪ 500 Da < molecular weight < 2,000 Da ▪ Peak splitting due to multiple chiral centers ▪ Endogenous interference ▪ Stability issue ▪ Solubility issue ▪ Low extraction recovery	▪ Alcoholic organic mobile phase ▪ Optimized LC gradient ▪ Rapid preparation at low temperature ▪ Use fresh-collected blank matrix ▪ Add desorbent ▪ Completed > 3,000 PROTAC method development and sample analysis
Oligonucleotide	▪ Average molecular weight ~10,000 Da ▪ Non-specific binding issue ▪ Chemical modification and conjugation of a delivery system ▪ High protein binding ▪ Stability issue ▪ Poor retention and sensitivity with conventional chromatographic method	▪ Liquid-liquid extraction/solid-phase extraction ▪ Ion-pair method/non-ion-pair method ▪ LC-MS/MS, liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), and hybridization-based liquid chromatography coupled to fluorescence detection (LC-FLD) ▪ Bioanalysis of ASO and siRNA ▪ Bioanalysis of oligonucleotides with various delivery systems including liposomes ▪ Bioanalysis of oligonucleotides in more than 20 biological matrices ▪ LLOQ in the biological matrix as low as ng/mL level
Peptide	▪ Molecular weight < 5,800 Da ▪ Non-specific binding issue ▪ Low extraction recovery ▪ Stability issue ▪ Sensitivity issue	▪ High-acidic mobile phase ▪ Add desorbent ▪ Solid-phase extraction (SPE) by µElution ▪ Derivatization method to improve stability and sensitivity ▪ LLOQ as low as pg/mL level in biological matrix ▪ > 10 years of bioanalytical experience
Protein	▪ Molecular weight > 5,800 Da ▪ Stability issue ▪ Non-specific binding issue ▪ Matrix interference ▪ Specificity issue	▪ LBA: · Detection by ELISA or MSD · Need for suitable capture/detection reagents · Usually determined by a double-antibody sandwich assay or indirect method · The MSD method offers better sensitivity and wider linear range than ELISA ▪ LC-MS: · Bioanalysis based on LC-MS/MS and LC-HRMS · Qualitative and quantitative analysis on intact, subunit, and surrogate peptide levels · Immunoaffinity purification
Anti-drug antibody (ADA)	▪ Complex composition ▪ Polyclonal positive control ▪ False positive / False negative ▪ Multiple lots of blank matrices	▪ ELISA or MSD ▪ Test article labeled as capture/detection reagents ▪ Different methods of acid dissociation to facilitate ADA detection ▪ Establish screening/confirmatory cut-point ▪ Screening, confirmatory, and titer assays for positive samples
Antibody-drug conjugate (ADC)	▪ Handling of highly toxic compounds ▪ Special sample pretreatment ▪ Challenging intact protein analysis ▪ Special important characteristic: DAR value	▪ Class II biosafety cabinet and corresponding safety protection guidance ▪ Payload/linker of non-antibody moiety and surrogate peptide generated after enzymatic digestion analysis by LC-MS/MS ▪ LBA based total and conjugated antibody bioanalysis ▪ Qualitative/semi-quantitative analysis of intact ADC and subunits based on immunopurification (IP)-LC-HRMS ▪ Drug-to-antibody ratio (DAR) value characterization
PEGylated drug	▪ Heterogeneous molecular weight ▪ Difficult to find parent ion (Q1) in mass spectrum	▪ Q1 is obtained by in-source fragmentation ▪ Non-conventional columns
Small molecule biomarker	▪ Endogenous ▪ Highly polar or non-polar ▪ Separation of isomers ▪ Matrix effect ▪ A blank matrix that is difficult to obtain ▪ Stability issue ▪ Sensitivity issue	▪ Surrogate analyte/matrix ▪ Stable isotope-labeled internal standard ▪ Solid-phase extraction pretreatment ▪ Derivatization pretreatment ▪ Single point method for rapid screening ▪ LC-HRMS analysis

Analyte	Features	Description
Large molecule biomarker	▪ Endogenous ▪ Difficult to obtain reference material and internal standard ▪ Severe matrix effect ▪ No suitable blank matrix	▪ LC-MS method: Quantification is performed on the intact protein level after immunoprecipitation or surrogate peptides after enzymatic digestion; stable isotope-labeled proteins/peptides are probably required ▪ LBA method: Recombinant/purified protein is required for quantification by ELISA/MSD platform ▪ Alternative matrix screening
Nucleoside triphosphate	▪ Extremely high polarity ▪ Low ionization efficiency in mass spectrometry ▪ Stability issue	▪ HILIC chromatography ▪ Ion-pair chromatography ▪ Special sample collection and processing method
Cassette dosing pharmacokinetics	▪ Low dosage (method sensitivity needs to be improved) ▪ Assessment of interferences between compounds	▪ LLOQ at pg/mL level in a biological matrix can be reached
Chiral compound	▪ Screening of chiral columns ▪ Separation issue	▪ Chiral column library ▪ Fast screening of chiral columns
Liposomes	▪ Stability issue ▪ Separation of unencapsulated and encapsulated forms	▪ Solid-phase extraction ▪ Ultrafiltration ▪ Develop specific separation protocols and stabilizers based on analyte and formulation characteristics

HP D300e

Table 2 Examples of microsampling and analysis techniques

Technology	Features
Dry blood/plasma spots	▪ Convenient for sample transportation and storage ▪ Suitable for a sampling of unstable and hydrolyzable compounds ▪ Extraction of test articles with aqueous or organic solvents
Microdialysis	▪ Limited sample volume ▪ Very low sample concentration ▪ LLOQ as low as pg/mL level for a neurotransmitter in cerebrospinal fluid
Quantitative capillary sampling	▪ Simplify the blood sample collection step in the animal experiment
sVAMS (volumetric absorptive microsampling)	▪ Facilitate sample transportation and storage ▪ Simplify blood sample collection step in animal experiment

Table 3 Examples of various biological matrices involved in drug development

Species	Liquid Matrix	Tissue		
Mouse	Plasma	Brain	Muscle	Fat
Rat	Serum	Liver	Bone marrow	Hair
Rabbit	Whole blood	Spleen	Spinal cord	Testis
Pig	Urine	Lung	Skin	Ovary
Dog	Bile	Kidney	Nerve	Uterus
Nonhuman primate	Cerebrospinal fluid	Heart	Lympho	Prostate
	Aqueous humor	Stomach	Pancreas	Thyroid
	Tear	Intestine	Joint	Ocular tissue
	Microdialysate	Bone		

Non-GLP Bioanalysis

Type of Bioanalytical Assays and Acceptance Criteria

WuXi AppTec DMPK Non-GLP Bioanalysis Team provides customers with solid bioanalytical services, covering screening to clinical trial application for discovery stage drug development. Table 4 and Table 5 list some bioanalytical assay types and criteria for LC-MS and LBA analysis platforms at different stages.

Table 4 Selected types of bioanalytical assays and acceptance criteria of LC-MS analytical platform

Assay Type	Description	Experimental content	Acceptance criteria
Screening Phase	▪ Semi-quantitative analysis ▪ Sample from single or cassette dosing study ▪ All biological matrices ▪ All preclinical species	▪ Method development and analysis of biological samples in liver microsomes, hepatocytes, plasma, serum, simulated intestinal fluid, simulated gastric fluid, Caco2 cells, MDR1 cells, and other models	▪ The peak height of the analyte does not exceed the saturation limit of a mass spectrometer. ▪ The signal-to-noise ratio of the analyte is no less than 3. ▪ The MS response of the analyte in the double blank or single blank sample is not higher than that of the unknown sample with the lowest concentration.
	▪ Quantitative analysis ▪ Sample from single or cassette dosing study ▪ All biological matrices ▪ All preclinical species	▪ Method development ▪ Specificity (including analytes and internal standards) ▪ Carryover ▪ Stability (if required) ▪ Sample analysis ▪ Standard curve and LLOQ ▪ Dilution integrity ▪ Number of quality control samples (low, medium, and high) ⩾ 5% number of unknown samples	▪ Specificity < 50% of the LLOQ ▪ Bias ⩽ ± 20% for biofluid matrix ▪ Bias ⩽ ± 25% for homogenates of a solid matrix ▪ Carryover ⩽ LLOQ
IND Filing	▪ All biological matrices ▪ All preclinical species ▪ Support IND filling for NMPA, ▪ FDA and EMA Chinese and/or English written report	Full validation (including at least the following eight items): ▪ Linearity (regression model) ▪ Within-run and between-run precision and accuracy ▪ Specificity (Selectivity) and interference ▪ Matrix effect ▪ Extraction recovery ▪ Dilution integrity ▪ Carryover ▪ System suitability	▪ Linearity: at least six non-zero calibrator levels covering the quantitation range. ▪ The back-calculated concentration should be within ± 15% of the nominal value (± 20% at LLOQ). ▪ Accuracy: ± 15% of nominal concentrations; except ± 20% at LLOQ. ▪ Precision: ± 15% CV, except ± 20% CV at LLOQ ▪ Selectivity: ⩽ 20% of the LLOQ in 6 lots of matrix ▪ Interference: internal standard interference of the analyte to be ⩽ 20% of LLOQ. ▪ Internal standard interference ⩽ 5% of LLOQ. ▪ Matrix effect: % CV of the internal standard-normalized matrix factor ⩽ 15%. ▪ Extraction recovery: results should be precise and reproducible, % CV ⩽ 15%. Precision (% CV) ⩽ 15% Carryover: ⩽ 20% LLOQ
		Partial validation (the following eight tests are optional): ▪ Linearity (Regression model) ▪ Within-run and between-run precision and accuracy ▪ Specificity (Selectivity) and interference ▪ Matrix effect ▪ Extraction recovery ▪ Dilution integrity ▪ Carryover ▪ System suitability	
		▪ Stability evaluation (optional following six stability tests) ▪ Stability of analytes in stock and working solutions ▪ Short-term stability of analytes in a biological matrix at room temperature or on wet ice ▪ Long-term stability of analytes in a biological matrix at -80 °C ▪ Stability of analytes in the biological matrix after freeze/thaw cycles ▪ Stability of analytes in a processed sample (autosampler)	▪ Stability of stock solution and working solution: deviation from the control solution ⩽ 10.0% and % CV ⩽ 10.0%. ▪ Other stability: 2 concentration levels, LQC and HQC in triplicate at each level. ▪ Accuracy ⩽ ±15.0% from the respective nominal values; the precision ⩽ 15.0%.

Table 5 Selected types of bioanalytical assays and acceptance criteria of LBA analysis platform

Assay Type	Description	Experimental content	Acceptance criteria
Screening Phase	▪ Pharmacokinetics (PK) ▪ Quantitative analysis of each sample ▪ All biological matrices ▪ All preclinical animal species	▪ Analytical methods ▪ Standard curves ▪ ULOQ & LLOQ ▪ Stability (if required) ▪ Sample analysis ▪ Quality control (QC) samples (low, medium, high). Total Number ⩾ 5% of all samples	▪ %CV ⩽ 20% for at least six non-zero points (ULOQ and LLOQ ⩽ 25.0%), average back-calculated concentrations (except anchor point) within ±20% bias of theoretical value (ULOQ and LLOQ within ±25%). ▪ At least 2/3 of the QC samples %CV ⩽ 20%, the average back-calculated concentration is within ±20% bias of the theoretical value, and at least 50% replicates of each concentration level meet the above acceptance criteria.
	▪ Anti-Drug antibody ▪ Semi-quantitative analysis of each sample ▪ All biological matrices ▪ All preclinical animal species	▪ Analysis methods ▪ Screening cut-point ▪ Confirmatory cut-point ▪ Sensitivity ▪ Drug tolerance ▪ Stability (if required) ▪ Sample analysis (screening, confirmatory, and titration)	▪ Screening cut-point factor (SCPF) and confirmatory cut-point (CCP) are obtained with appropriate statistical methods. ▪ Screening assay acceptance criteria: %CV of signal response of positive control (PC) ⩽ 20%, %CV of signal response of negative control (NC) ⩽ 25%, signal response of QC samples must meet: HPC > sLPC ⩾ SCP > NC. ▪ Confirmatory assay acceptance criteria: %CV of signal response of PC ⩽ 20%, %CV of signal response of NC ⩽ 25%, inhibition rate of QC must meet: HPC, cLPC ⩾ CCP > NC. ▪ The general process of immunogenicity sample analysis has three steps: screening assay, confirmatory assay, and titration assay. The screening assay detects the presence of potential positive samples; the confirmatory test is to confirm the possible positive samples, and the titration assay is performed if the sample is confirmed as positive.

Assay Type	Description	Experimental content	Acceptance criteria
IND Filing	▪ All biological matrices ▪ All preclinical animal species ▪ NMPA, FDA, and EMA IND filing requirements ▪ Chinese and/or English word reports	▪ PK method validation (at least the following eight validation parts) ▪ Assay range ▪ Intra- and inter-batch precision, accuracy ▪ Hook effect and dilution linearity ▪ Selectivity (matrix effect and hemolysis assessment) ▪ Specificity ▪ Robustness ▪ Stability ▪ Parallelism (NMPA) PK partial method validation (optional for the above 8 parts)	▪ %CV ⩽ 20% for at least 6 non-zero points (ULOQ and LLOQ ⩽ 25.0%) ▪ Average back-calculated concentrations (except anchor point) within ±20% bias of theoretical value (ULOQ and LLOQ within ±25%) ▪ At least 2/3 of the QC samples %CV ⩽ 20%, the average back-calculated concentration is within ±20% bias of the theoretical value, and at least 50% of replicates of each concentration level meet the above acceptance criteria ▪ Sensitivity：LLOQ ▪ Each concentration will be analyzed in five replicates for dilution linearity evaluation. At least 4/5 replicates per concentration within ±20.0% bias and %CV ⩽ 20.0% ▪ At least ten individual blank matrices will be evaluated for selectivity or matrix effects, with at least 80% meeting %CV ⩽ 20% (or 25%) and the average back-calculated concentration within ±20% (or 25%) bias of the theoretical value ▪ Specificity assessment with at least 2/3 of the samples within ± 25% bias of the theoretical value and %CV ⩽ 25.0% ▪ Fine-tuning specific method parameters to evaluate the assay results to verify the robustness of the method ▪ Parallelism assessment with at least 2/3 of the samples %CV ⩽ 30%
		▪ ADA method validation (at least the following nine validation parts) ▪ Calculate assay cut-points (screening cut-point and confirmatory cut-point) ▪ Titration assay ▪ Sensitivity ▪ Intra- and inter-batch precision ▪ Robustness ▪ Drug tolerance ▪ Hook effect ▪ Selectivity (matrix effect and hemolysis assessment) ▪ Stability ADA partial method validation (optional for the above nine parts)	▪ Screening cut-point factor (SCPF) and confirmatory cut-point (CCP) were obtained using appropriate statistical methods ▪ Screening assay acceptance criteria: %CV of signal response of positive control (PC) ⩽ 20%, %CV of signal response of negative control (NC) ⩽ 25%, signal response of QC samples must meet: HPC > sLPC ⩾ SCP > NC ▪ Confirmatory assay acceptance criteria: %CV of signal response of PC ⩽ 20%, %CV of signal response of NC ⩽ 25%, inhibition rate of QC must meet: HPC, cLPC ⩾ CCP > NC ▪ The general process of immunogenicity sample analysis includes three steps: screening assay, confirmatory assay, and titration assay. The screening assay detects the presence of potential positive samples; the confirmatory test is to confirm the possible positive samples, and the titration assay is performed if the sample is confirmed as positive
		▪ Stability evaluation (6 following parts are optional) ▪ Stability of analytes in biological matrices at room temperature ▪ Stability of analytes in biological matrices at 2 °C - 8°C ▪ Long-term stability of analytes in biological matrices at ⩽ -60°C ▪ Freeze-thaw cycle stability of analytes in biological matrices	▪ Two concentration levels per time point, three replicates ▪ Stability samples, the bias from the theoretical value should be within ±20%, and %CV ⩽ 20%

Non-GLP Bioanalysis

Laboratory Space and Instrumentation

The Non-GLP Bioanalysis Team has three sites in China. They include our Shanghai, Suzhou, and Nanjing sites. All the laboratories have instrument rooms and sample processing rooms. Our team has established a variety of advanced LC-MS platforms to meet customers' analytical needs at different stages. We have a wide range of mass spectrometers, such as multiple generations of Sciex triple quadrupole mass spectrometers, Waters XeVo G2 QToF, Vion QToF, and ThermoFisher Orbitrap Q-Exactive™ Plus, Q-Exactive™ HF, and Eclipse systems. These mass spectrometry systems are coupled with ultrahigh performance liquid chromatography systems such as Waters UPLC and Shimadzu UHPLC. For the screening stage *in vitro* sample bioanalysis, high throughput autosamplers such as CTC and ADDA are utilized. SpectraMax M5/M5e instruments from Molecular Devices and QuickPlex SQ120 instrument from MSD are applied for the LBA analysis of large molecules. In addition, we have multiple automated sample preparation systems such as the HP D300e instrument and Hamilton Microlab STAR™ liquid handler workstation to support fast sample preparation.

Figure 1. Non-GLP bioanalytical instrument room

Figure 2. Sample preparation laboratory equipped with yellow light lamps to avoid photodegradation of the compounds

Waters ACQUITY UPLC

Shimadzu Nexera UHPLC LC-30A

Figure 3. Selected high-performance liquid chromatography systems of Non-GLP bioanalysis laboratory

CTC Analytics AG PAL3 Autosampler

Apricot Designs Dual Arm

Figure 4. Selected high throughput autosampler systems of Non-GLP bioanalysis laboratory

Sciex Triple Quad™ 6500+

Sciex Triple Quad™ 7500

Figure 5. Selected triple-quad mass spectrometry systems of Non-GLP bioanalysis laboratory

Waters Vion™ QTof

ThermoScientific Orbitrap Q-Exactive™ HF

Figure 6. Selected high-resolution mass spectrometry systems of Non-GLP bioanalysis laboratory

Molecular Devices SpectraMax M5e

MESO QuickPlex SQ 120

Figure 7. Selected ligand binding assay instrument platforms of Non-GLP bioanalysis laboratory

HP D300e

Hamilton Microlab STAR™

Figure 8. Selected automation systems of Non-GLP bioanalysis laboratory

REFERENCES

1. Li W. et al. Handbook of LC-MS Bioanalysis: Best Practices, Experimental Protocols, and Regulations (WILEY, 2013)

2. Jemal M. et al. LC-MS development strategies for quantitative bioanalysis. Curr Drug Metab. 7. 491-502 (2006)

3. Shou W. et al. Recent development in software and automation tools for high-throughput discovery bioanalysis. Bioanalysis. 4.1097-1109 (2012)

4. Janiszewski J. et al. Development of a high-speed, multiplexed sample-delivery instrument for LC-MS/MS bioanalysis. 4.1039-1056 (2012)

5. FDA: Guidance for Industry, Bioanalytical Validation Method (2018)

6. EMA: ICH Guideline M10 on Bioanalytical Method Validation (2019 Draft)

7. OECD: Principles of Good Laboratory Practice (1997)

8. FDA: Immunogenicity Testing of Protein Therapeutic Products-Developing and Validating Assays for Anti-Drug Antibody Detection Guidance for Industry (2019)

9. EMA: Guideline on Immunogenicity Assessment of Therapeutic Proteins (2017)

10. EMA: Guideline on Immunogenicity Assessment of Monoclonal Antibodies Intended for *In Vivo* Clinical (2012)

11. China Food and Drug Administration (now National Medical Products Administration): Good Laboratory Practice for Nonclinical Laboratory Studies (Order No. 34) (2017)

12. China Food and Drug Administration (now National Medical Products Administration): Guideline for Method Validation of Quantitative Analysis of Biological Samples (2015)

06 Drug Metabolism and Pharmacokinetics of New Drug Modalities

With the deepening understanding of disease pathogenesis and drug mechanism of action, continuous innovation and breakthroughs occur in drug research and development (R&D). Novel therapeutic approaches and new drug modalities continue to emerge and occupy an increasingly important position. New drug modalities (such as therapeutic proteins, peptides, oligonucleotides, antibody-drug conjugates (ADC), proteolysis-targeting chimeras (PROTACs), etc.) are significantly different from conventional small molecule drugs in both structural characteristics and pharmacokinetic behaviors. Each type of new drug modality has its unique research strategies. Higher requirements are put forward for the corresponding analytic ability. The way appropriate pharmacokinetic studies and druggability evaluation are conducted for each new drug modality is critical for researchers in the field.

With visionary thinking, our team of scientists continues to improve research capabilities and broaden the scope of platform research. We have established pharmacokinetic research strategies and test service systems for each type of new drug modality. Our capabilities on new drug modalities help our clients and global partners move toward success.

Our dedicated R&D team established a comprehensive set of pharmacokinetic research strategies and test systems from *in vitro* to *in vivo* for different new drug modalities. With more than 10 PhDs as core members, the R&D team includes scientific consultants and experts in different fields covering *in vitro* and *in vivo* ADME, metabolite identification, QWBA and isotope mass balance, and bioanalysis.

For years, we have collaborated with well-known pharmaceutical and biotech companies focusing on new drug modalities. Extensive experience is achieved by testing massive amounts of new modality molecules annually.

Our scientists continuously share DMPK knowledge and experience of new drug modalities to the field, publishing scientific articles in peer-reviewed journals, presenting research in international academic conferences, and completing multiple patent applications.

Drug Metabolism and Pharmacokinetics of New Drug Modalities
Therapeutic Proteins

Therapeutic protein drugs are a class of biological products composed of polymer substances with molecular weights of more than ten kDa, mainly including monoclonal antibody (mAb), polyclonal antibody (PcAb), bispecific antibody (BsAb), and Fc fusion protein. This class of drugs has characteristics of high activity, high specificity, and low toxicity. It is widely used in the fields of cancer, autoimmunity, anti-virus, and cardiovascular diseases.

In 1986, the world's first murine monoclonal antibody drug, the anti-CD3 monoclonal antibody Muromonab-CD3 for the treatment of organ transplant rejection, was approved by the US Food and Drug Administration (FDA), starting the introduction of therapeutic protein drugs [1]. In the past five years, protein drugs have rapidly developed. The United States and European Union have established a leading position in the number of the first approval new antibody drugs. As of December 2020, the cumulative number of new antibody drugs first approved by the FDA and the European Medicines Agency (EMA) reached a respective 98 and 85. The market size reached $142.7 billion in 2019 and $150 billion in 2020. Globally, therapeutic protein drugs have become one of the fastest-growing segments of the pharmaceutical industry. With the continuous development of biotechnology, the market prospects of these drugs will become more and more promising.

Therapeutic protein drugs have ADMET properties that are different from small molecules, as described below:

Table 1 Comparison of different properties of traditional small molecule drugs and therapeutic protein drugs

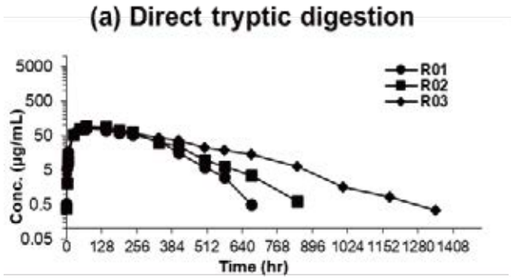
	Traditional small molecule drugs	Therapeutic protein drugs
Molecular Weight (Da)	Typically ⩽ 1 kDa	Typically > 10 kDa
Biological activity and specificity	Low	High
Immunogenicity	None	Yes
Purity and Cost	High purity and low cost	Low purity and high cost
Half-life	Short	Long
Oral Bioavailability	Wide range (related to the compound intrinsic properties)	Generally low
Administration Route	PO	IV, SC, IM
Distribution	Wide range of Vd values Possibly transporter-mediated distribution	Vd is generally low and approximates plasma/serum volume
Metabolism & Elimination	Phase I/II enzyme metabolism; biliary and renal excretion	Pinocytosis or receptor-mediated intracellular catabolism
PK Linearity	Linear in low dose and possibly non-linear in high dose	Linear in high dose and possibly non-linear in low dose
PK Analyte	Parent drug and major active metabolites	Antibody [2, 3]
Analytical Method	LC-MS/MS	LC-MS, MSD, ELISA, etc. [2, 3]

WuXi AppTec DMPK has extensive experience in providing high-quality services to more than 600 worldwide clients and global DMPK submission strategies in the field of therapeutic proteins [4, 5]. We have established comprehensive therapeutic protein druggability evaluation platforms with secure animal resources and competitive project turnaround times.

Immunoassays are generally used for sample analysis of therapeutic proteins. This method has the advantages of high sensitivity and simple operation and the disadvantages of narrow linear range, matrix interference, detection antibody requirements, long purchase of analytical reagents, and method development cycles. Relying on leading analytical capabilities and years of successful experience, the DMPK department optimizes varieties of biological sample analysis strategies for therapeutic protein drugs.

- DMPK researchers have successfully developed analytical methods based on liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS) and liquid chromatography-high-resolution mass spectrometry with similar lower limits of quantification as an enzyme-linked immunosorbent assay (ELISA), higher specificity, shorter method development, and sample analysis cycles.
- DMPK has vast experience in the MSD analytical method, with higher sensitivity and wider linear range than ELISA.
- DMPK works seamlessly with the WuXi AppTec Hits department to prepare the detection antibody at the early stage of a project to ensure a shorter analysis period.
- DMPK works closely with WuXi AppTec BAS department to provide comprehensive flow cytometry services for the receptor occupancy (RO), immunophenotyping (IPT) to support preclinical studies for therapeutic protein drugs.

Case sharing: SD rats were subcutaneously injected with 10 mg/kg of trastuzumab, and serum was collected at different time points for sample analysis. Direct tryptic digestion was compared with protein A affinity purification (Magnetic beads IP) followed by tryptic digestion for sample pretreatment. Then LC-MS/MS was used to detect the characteristic peptides. The results were as follows:



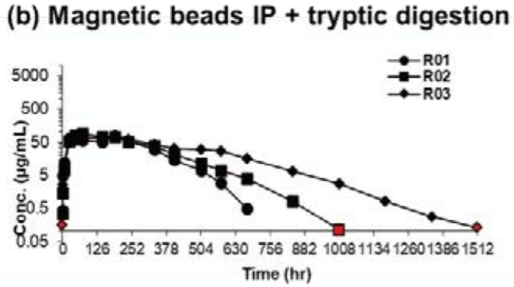


Figure a, Plot of trastuzumab drug concentration-time curves using trypsin direct digestion method

Figure b, Plot of trastuzumab drug concentration-time curves using protein A affinity purification and tryptic digestion method

The pharmacokinetic parameters were calculated using the data from the characteristic peptide method and compared with those obtained by the ELISA method from the literature.

Table 2 The pharmacokinetic parameters of trastuzumab in the serum of SD rats analyzed by different sample analysis methods

	Trypsin direct digestion	Protein A affinity purification + trypsin digestion	ELISA *
Pharmacokinetics parameters	Mean ± SD	Mean ± SD	Mean ± SD
C_{max} (μg/mL)	86.1±5.70	81.3±8.40	72.8±3.88
T_{max} (h)	64.0±13.9	104.0±77.1	60±13.9
$AUC_{0\text{-last}}$ (μg·h/mL)	27600±5170	26135±6361	26900±5020

(* Source of ELISA result: Qu X, Hou S, McCann M, etc. Pharmacokinetic Analysis of the mAb Adalimumab by ELISA and Hybrid LBA/LC/MS: A Comparison Study Featuring the SCIEX BioBA Solution, 2017)

Conclusion from the results:

1.The affinity purification method can obtain better sensitivity, significantly improve the signal-to-noise ratio, and obtain a lower limit of quantification.

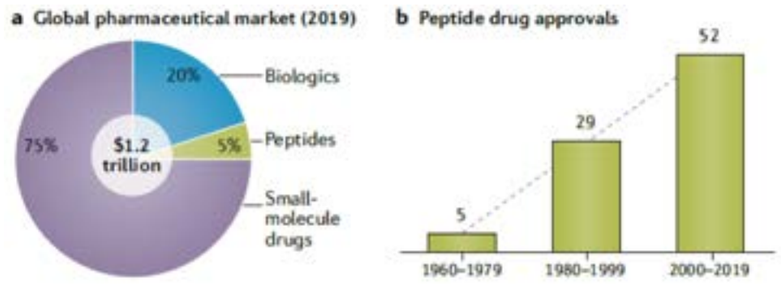
2.There was no systematic bias between the LC-MS results and those obtained by ELISA.

Drug Metabolism and Pharmacokinetics of New Drug Modalities

Peptides

Peptides are α-amino acid chains linked by peptide bonds. Those with the range of 10 to 40 amino acids are called polypeptides [6] and generally have molecular weights below ten kDa. Peptides play an essential role in the signal transduction process in the form of hormones, cytokines, and antimicrobials. Peptides have great affinity and selectivity with therapeutic targets, low toxicity, and the properties of small and large molecules. They offer unique advantages for satisfying substantial unmet medical needs.

Insulin was the first therapeutic peptide drug that was marketed in 1922. With the rapid progress of molecular biology and biotechnology in the second half of the twentieth century, more than 80 peptide drugs were marketed by 2020 to treat diseases such as diabetes, tumors, osteoporosis, multiple sclerosis, HIV, and chronic pain. In 2019, peptide drugs accounted for 5% of the global pharmaceutical market, with sales exceeding $50 billion and an average market growth rate of 7.7% [7].



The main challenges of developing peptide drugs are their intrinsic low permeability properties and instability in the gastrointestinal tract and plasma. Marketed peptide drugs are primarily administered by parenteral routes, such as intramuscular and subcutaneous injection. Medicinal chemists hope to improve metabolic stability and bioavailability through chemical modifications that include replacements with D-amino acids and artificial amino acids. They also want to add fatty chains, PEGylation, cyclization, N-methylation, and N-terminal acetylation [8]. Some new approaches are also used, such as nanoparticles, long-acting microsphere products, inhalants that effectively prolong the half-life of peptide drugs in the body.

Appropriate and in-depth preclinical DMPK assessments are essential for the clinical success of peptide compounds. The main ADMET characteristics of peptide compounds with solutions provided by WuXi AppTec DMPK are summarized as follows:

- Peptides tend to have poor permeability because of their large molecular weight. They are mainly absorbed through capillary blood vessels or lymphatic vessels. The larger the molecular weight, the greater the proportion of lymphatic absorption. Passive diffusion is the main absorption pathway, and bioavailability is generally low. A flip-flop phenomenon is often observed in *in vivo* PK due to slow absorption. Appropriate absorption enhancers such as surfactants, bile salts, phospholipids, fatty acids, glycerides, etc. can help improve the absorption [9]. The WuXi AppTec DMPK provides different *in vitro* models (PAMPA, MDR1-MDCK, Caco-2, PEPT1, PEPT2, and other tailored permeability and transporter models) to assess the permeability of peptides. With an extensive formulation screening experience and vehicle panel, we provide appropriate vehicles to promote the *in vivo* absorption of peptides.

- Peptides are susceptible to enzymatic metabolism and pH-mediated hydrolysis. They are liable to tissue degradation throughout the body, such as plasma, liver, gastrointestinal tract, and kidney brushing border membrane. We have a complete list of *in vitro* stability test systems, including plasma, simulated gastrointestinal fluid, microsomes of various tissues (such as liver, intestine, and kidney), S9, cytosol, and tissue homogenate. We are developing a new brush border membrane vesicle (BBMV) platform to meet more in-depth research needs.

- Peptides tend to have issues of non-specific adsorption and instability in *in vitro* test systems. DMPK laboratory has a low adsorption consumables and enzyme inhibitors panel to help solve these problems.

- Most peptides are readily excreted from the body through urine. Enhanced binding to plasma proteins through chemical modification can reduce renal clearance. We can research various species such as rats, mice, dogs, and monkeys to conduct *in vivo* renal clearance and mass balance experiments to determine the main clearance pathways and guide further optimization.

- Appropriate processing and preservation methods should be adopted to ensure the stability of peptide samples in *in vivo* experiments. In addition, non-specific adsorption, poor recovery, and residue carryover are issues often encountered during the bioanalysis. WuXi AppTec DMPK has more than 10 years of successful experience in peptide biological sample method development and analysis with the lower limit of quantification down to pg/mL for various endogenous peptides and modified peptide compounds.

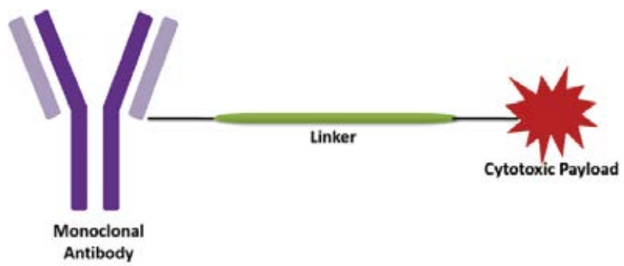
- Peptides may cause potential immunogenicity. Preclinical anti-drug antibody (ADA) testing was performed for most of the marketed and researched peptides. In WuXi AppTec, DMPK, ELISA, and MSD are used for ADA detection. Well-established screening, validation, and titration methods can meet the requirements of global submission.

WuXi AppTec DMPK has supported hundreds of research projects of peptide compounds over the years. Experience has been gathered in research strategies, experimental design, sample analysis, issue solving, and data interpretation, which is how we offer clients a comprehensive, efficient, and one-stop research solution.

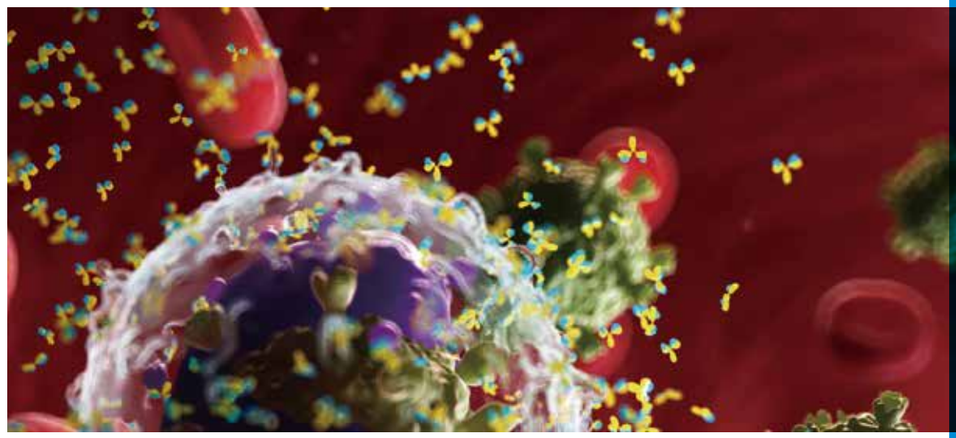
Drug Metabolism and Pharmacokinetics of New Drug Modalities

Antibody-Drug Conjugates (ADCs)

Traditional chemotherapy and monoclonal antibody drugs have been widely used in cancer therapy. However, most chemotherapeutic drugs that lack targeting to tumor cells will kill normal cells and cause side effects. A Monoclonal antibody (mAb) can specifically target tumor cells but has no cytotoxicity. Antibody-drug conjugates (ADCs) represent emerging biotherapeutics that are composed of toxic small molecules (payload) conjugated to the mAb via a linker (see the illustration below). The antibody part of an ADC binds specifically to the antigen expressed on the surface of target cells, which leads to the distribution of ADC to target tissues followed by ADC internalization and payload release within target cells. Theoretically, due to the lack of targeting to normal cells, the toxicity of ADC to normal cells is relatively low. ADC combines the advantages of high specificity and cytotoxicity and is regarded as a promising anticancer drug. The development of ADC drugs has attracted much attention, but the real success in clinical application began with the first ADC drug, MYLOTARG (Gemtuzumab Ozogamicin), approved by FDA in May 2000. In recent years, with the development of conjugation technology and the discovery of new payloads, ADC drug development made rapid progress. As of June 2021, a total of 13 ADC drugs have been launched. Eight of those ADC drugs were launched in the past two years. More than 80 ADC drugs are in different clinical development stages.



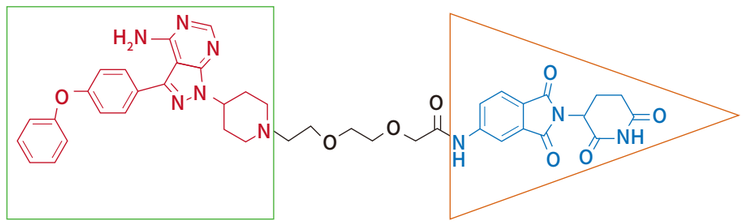
There is currently no guidance for authorities to follow in the DMPK evaluation of ADC drugs, which brings challenges for its development. The DMPK evaluation of ADC drugs not only needs to investigate the absorption, distribution, metabolism, and excretion (ADME) process of the intact ADC. It also needs to conduct selective pharmacokinetic studies for the toxic payload. The release of toxic payload in target cells is highly related to efficacy and toxicity. Investigations on the release mechanism (the release of payload from ADC in plasma/serum, S9, and tumor cells) and structure characterization of payload-related species are essential. Based on the abundant successful experience in supporting ADC discovery and development projects, the WuXi AppTec DMPK established a set of DMPK evaluation strategies and test systems for ADC drugs. We have expertise in the structural characterization and quantitative analysis of payload-related species, the development of high sensitivity bioanalysis methods for ADC, total antibody and conjugated payload, and analysis of drug antibody ratio (DAR). We support the development of ADC drugs from early discovery to the clinical development stage.



Drug Metabolism and Pharmacokinetics of New Drug Modalities
Proteolysis-Targeting Chimeras (PROTACs)

Biologics have rapidly developed in recent years, while the research and development (R&D) of small molecule drugs that once served as the main body seems to be no longer with "halo." The emergence of proteolysis-targeting chimeras (PROTACs) provides a new opportunity for small molecule drug R&D.

PROTACs apply a natural proteolysis mechanism, the ubiquitin proteasome degradation pathway, which relies on bringing the critical enzyme of ubiquitination, the E3 ligase, closer to the target protein, inducing the E3 ligase to attach a ubiquitin tag to the target protein, which in turn promotes the degradation of the target protein. Therefore, the structure of PROTAC is equivalent to a combination of three small molecules: one ligand binds E3 ligases, the other ligand binds target proteins, and a linker for conjugating the two ligands.



PROTAC Construction Example

PROTACs continuously reduce target protein levels because they can be reused after completing the task. The unique mechanism action gives PROTACs many advantages, mainly because they have excellent development potential in the degradation of undruggable targets and overcoming drug resistance. However, compared with other types of drug molecules, PROTACs face the following DMPK challenges:

- First, their unique structure always leads to large molecular weights, contrary to the classical Lipinski's rule of five. PROTAC compounds tend to have poor aqueous solubility and cellular permeability, leading to unsatisfactory oral bioavailability. WuXi AppTec DMPK has developed a set of formulation screening strategies for insoluble PROTACs to enhance absorption by formulation optimization means.
- Second, for traditional small molecule inhibitors, the higher the exposure, the better the efficacy. Multiple studies with PROTACs have shown that functional ternary complexes can be formed at appropriate or low concentrations, but a hook effect will be observed at high concentrations. A hook effect is unfunctional, but competitive target protein-PROTAC or E3 ligase-PROTAC binary complexes are formed, resulting in reduced drug efficacy or toxic reactions. Therefore, the optimal dose setting plays an important role here.
- Third, with the properties of high protein binding and possible instability in tested matrices, the binding ratio of PROTAC compounds cannot be determined with the conventional equilibrium dialysis method. Given this, tailored experimental methods are established by using diluted plasma and ultracentrifugation to get reliable results.
- Fourth, due to the unique structure of PROTACs, less metabolite generation is favored to avoid unexpected protein degradation or toxic reactions. Metabolite monitoring becomes a crucial part of the preclinical screening stage.

WuXi AppTec DMPK has established a general system for DMPK evaluation of PROTAC compounds, covering **the DMPK research strategies, preclinical formulation development, *in vitro* and *in vivo* experimental condition setting, biological sample analysis capacity and capability, *in vitro* and *in vivo* metabolite identification.** This system evaluates the permeability, *in vivo* absorption, stability, and metabolism of PROTAC compounds. We also provide customized research strategies to meet different needs to help customers promote sound PROTAC candidates.

Drug Metabolism and Pharmacokinetics of New Drug Modalities
Oligonucleotides

There are several types of oligonucleotide drugs, including antisense oligonucleotide (ASO), small interfering RNA (siRNA), microRNA (miRNA), and aptamer. Oligonucleotide drugs have become candidates for the treatment of various diseases due to their high efficiency, low toxicity, and low potential in producing drug resistance. With more companies entering the field, the indications of oligonucleotide drugs have gradually expanded and now cover rare diseases, cancer, infectious diseases, and neurodegenerative diseases. As of December 2020, 13 oligonucleotide drugs had been approved by FDA. According to Technavio data, from 2019 to 2024, the global oligonucleotide market size is expected to grow by approximately $9 billion. From 2018 to 2022, the compound annual growth rate of the global oligonucleotide market size is expected to be 10%.

Oligonucleotides can achieve high selectivity by pairing with DNA, mRNA, or pre-mRNA through the Watson-Crick base complementary pairing principle. They precisely inhibit specific genes and silence incorrectly coded genes, thereby preventing unwanted proteins' expression. As a new drug modality, oligonucleotide drugs are polar and charged and usually require chemical modifications (e.g., GalNAc) and drug delivery systems to improve drug properties. With DMPK properties significantly different from small chemical molecules and monoclonal antibody drugs, oligonucleotides pose high drug research and development challenges.

A robust bioanalytical method is essential in the pharmacokinetic and toxicokinetic study of an oligonucleotide drug. Based on the oligonucleotide structures and different quantitative requirements, WuXi AppTec DMPK provides various world-leading analytical platforms, including liquid chromatography-tandem mass spectrometry and liquid chromatography-high-resolution accurate mass spectrometry, molecular hybridization-based liquid chromatography-fluorescence chromatography, and ligand binding technology. To date, we have developed the bioanalytical methods for various ASOs and siRNAs in different matrices, including biological fluids and more than 20 biological tissues such as the brain, liver, and kidney. These methods are for the bioanalysis of multi-species *in vitro* and *in vivo* biological samples, supporting the early drug discovery, IND application to various stages of clinical studies.

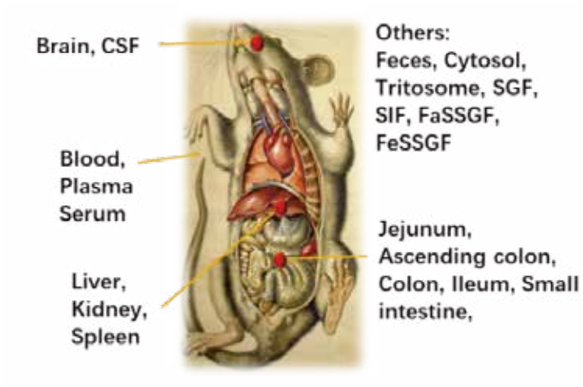


Figure: the matrices in which oligonucleotide drugs have been analyzed

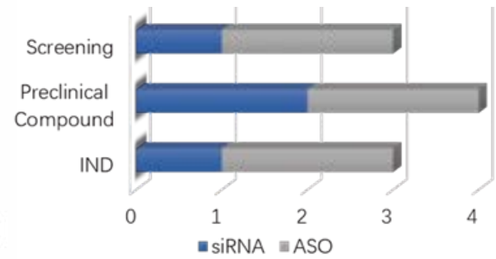


Figure: statistics of oligonucleotide drug projects supported by WuXi AppTec DMPK in 2020

REFERENCES

1. Ruei-Min Lu, Yu-Chyi Hwang, and I-Ju Liu, etc. Development of therapeutic antibodies for the treatment of diseases. Journal of Biomedical Science, 2020, 27:1

2. FDA: Immunogenicity Testing of Protein Therapeutic Products-Developing and Validating Assays for Anti-Drug Antibody Detection Guidance for Industry, 2019

3. EMA: Guideline on Immunogenicity Assessment of Therapeutic Proteins, 2017

4. ICH: S6 (R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, 2011

5. China Food and Drug Administration: General Principles for Technical Review of Non-clinical Safety of Therapeutic Biological Products, 2007

6. ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin, Guidance for Industry, Food and Drug Administration

7. Muttenthaler, M., King, G.F., Adams, D.J. et al. Trends in peptide drug discovery. Nat Rev Drug Discov (2021)

8. Lau, Jolene L., and M. K. Dunn. " Therapeutic peptides: Historical perspectives, current development trends, and future directions. " Bioorganic & medicinal chemistry 26 10 (2018): 2700-2707

9. Di, L. Strategic Approaches to Optimizing Peptide ADME Properties. AAPS J 17, 134 143 (2015)

Our Values

Integrity & Dedication, Working Together & Sharing Success; Doing the Right Thing, Doing It Right.

WuXi AppTec