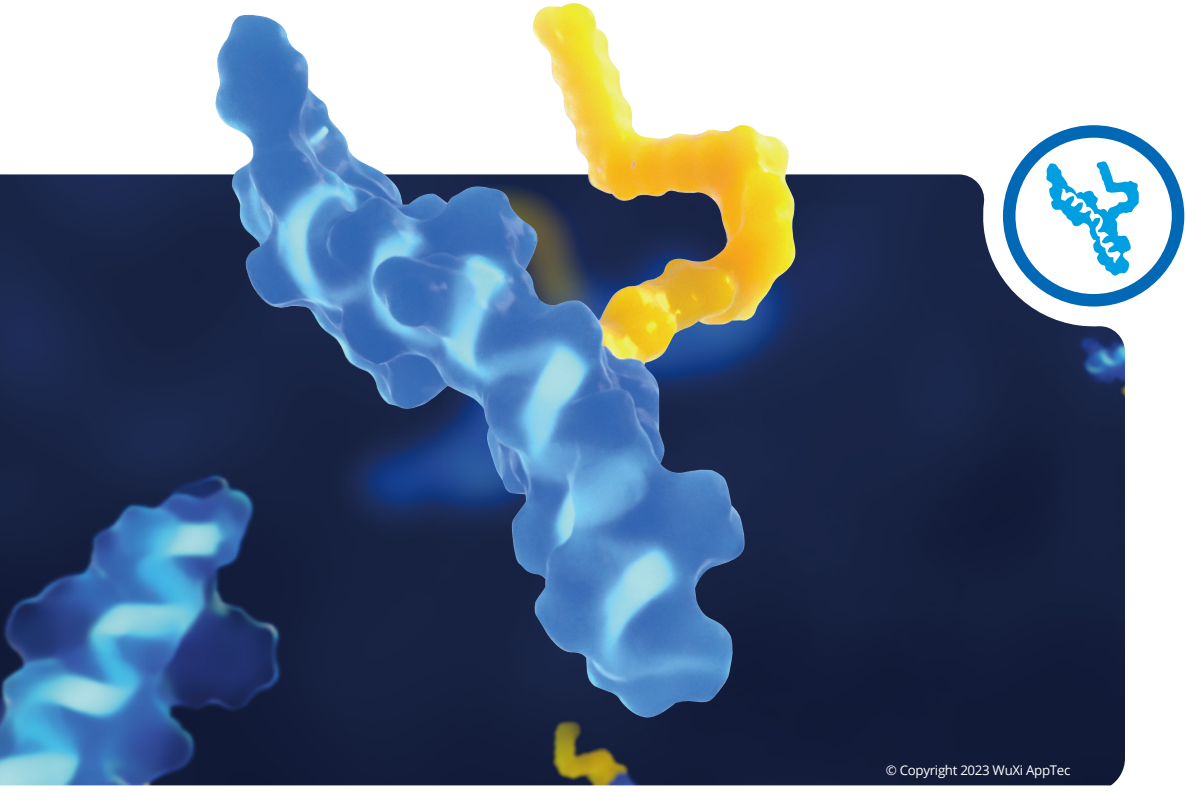
药明康德
WuXi AppTec

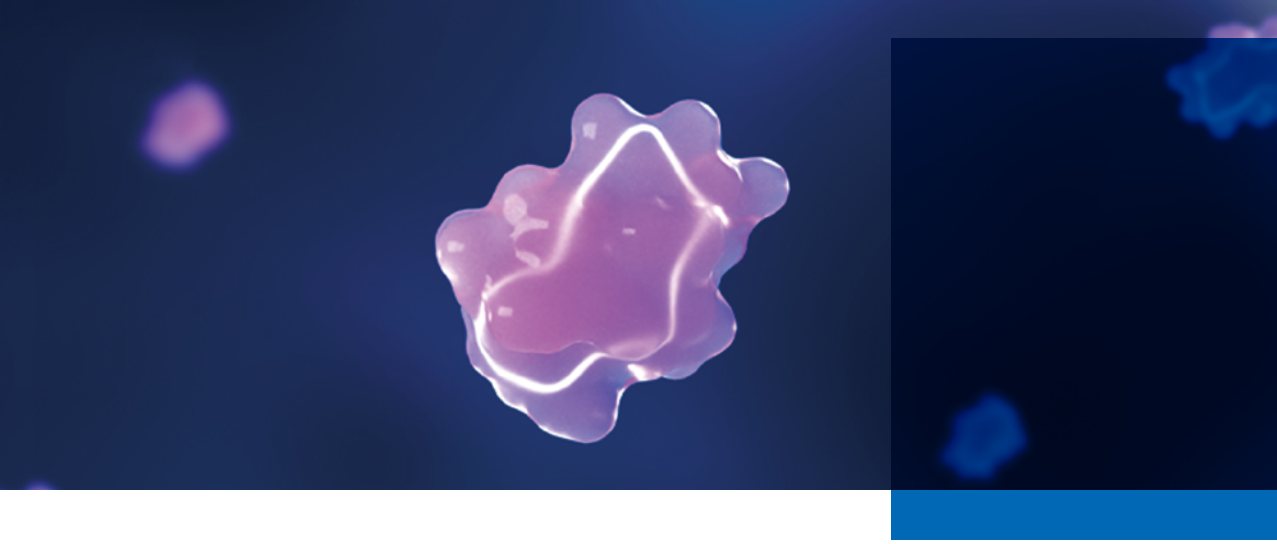
PRECLINICAL DRUG DEVELOPMENT TESTING FOR

PEPTIDE

Shorten the Peptide Development Cycle
with WuXi AppTec **DMPK** Services



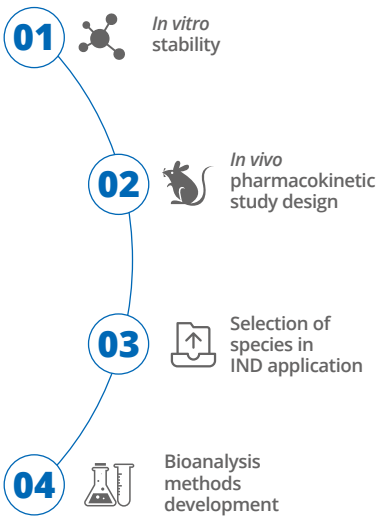
New Modalities Series | Peptide

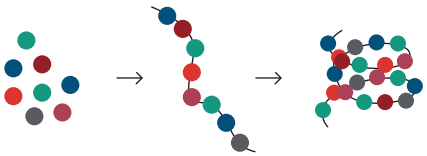


Unique Pharmacokinetics Evaluation System for **Peptide Drugs**

Peptides — a unique class of pharmaceutical compounds composed of a series of well-ordered amino acids usually with molecular weights of 500-5,000 Da — have become a popular area of drug development. However, due to their structures and properties, **peptides** pose many challenges to **peptide** drug development, including low dosage, non-specific adsorption, low stability, high protein binding ratio, poor membrane permeability, complicated metabolic pathways, and a short half-life.

WuXi AppTec's Drug Metabolism and Pharmacokinetics (**DMPK**) Service Department has built a well-established druggability evaluation system for **peptides** based on the team's experience in **peptide** research. The system provides a complete solution for **DMPK** studies of **peptides**, covering stability and metabolic soft spot research at the screening stage, evaluation of *in vivo* pharmacokinetics and species selection at the preclinical candidate stage, and radiolabeled **ADME** experiments at the IND application and clinical trial stage. With extensive experience in strategic research, experimental design, sample analysis, problem-solving, and data interpretation, we're now able to deliver fast, high-quality, and effective **peptide** research services to our partners and ensure smooth project implementation.





We have supported the screening and evaluation of tens of thousands of **peptide** molecules as well as the IND applications of dozens of **peptide** molecules. In this process, we have accumulated extensive experience and formed a specific methodology for pharmacokinetic research related to various **peptide** compounds. We are confident that we can help our clients develop new **peptides** better and faster.

Peptide Pharmacokinetic Research Services

Absorption

- Permeability assessment through *in vitro* permeation model
- Study on absorption through *in vivo* PK
- Formulation screening

Distribution

- Plasma protein/albumin binding ratio (ultracentrifugation)
- Tissue distribution or **QWBA** method to study drug distribution

Metabolism

- Whole blood/plasma stability
- Liver and kidney S9/homogenate stability
- Study on metabolic transformation through metabolite identification

Excretion

- Study on excretion pathways through *in vivo* excretion experiments

Drug-drug Interactions (DDI)

(For Peptides with Low Molecular Weight)

- Well-established DDI assessment based on drug metabolic enzymes
- Well-established DDI assessment based on transporters

Pharmacokinetic Research Content

	SCREENING	PRECLINICAL CANDIDATE	IND
ADME	▪ Whole blood/plasma stability ▪ Liver and kidney S9/homogenate stability ▪ Hepatocytes metabolic stability ▪ *In vitro* permeability (MDR1-MDCK) ▪ Plasma protein/albumin binding (ultracentrifugation) ▪ Simulated gastrointestinal fluid stability (for oral **peptides**)	▪ *In vitro* permeability (Caco2) ▪ Metabolite identification (hepatocytes and liver S9) ▪ *In vitro* metabolite identification	▪ Whole blood/plasma stability ▪ Liver and kidney S9/homogenate stability ▪ Hepatocytes metabolic stability ▪ Metabolite identification (hepatocytes and liver S9) ▪ *In vitro* metabolite identification ▪ *In vitro* permeation (Caco2) ▪ Plasma protein binding (ultracentrifugation) ▪ Metabolites identification of *in vivo* samples
DDI	**Peptides** with Low Molecular Weight: ▪ Study on inhibition of cytochrome P450 enzymes	**Peptides** with Low Molecular Weight: ▪ Study on inhibition of efflux transporters ▪ Study on substrates of efflux transporters	**Peptides** with Low Molecular Weight: ▪ Study on inhibition and induction of cytochrome P450 enzymes ▪ Study on inhibition and substrates of efflux and uptake transporters
PK	▪ PK study in rodents ▪ PK or biomarker detection in PD research	▪ Formulation screening ▪ Preliminary experiment of tissue distribution in rodents ▪ Preliminary experiment of excretion of urine, feces, and bile in rodents ▪ PK study in non-rodents	▪ Development and validation of bioanalysis methods (including ADA) ▪ Tissue distribution study in rodents ▪ Excretion study of urine, feces, and bile in rodents ▪ PK study in rodents and non-rodents ▪ *In vivo* radiolabeled **ADME** and **QWBA** experiments

Challenges in **Peptide** Pharmacokinetic Studies

High Difficulty

- **Peptide** molecules are physiologically active. Therefore, the dosage is small and the drug concentration *in vivo* is rather low. Additionally, there are a large number of endogenous interfering substances in organisms. Both the specificity and sensitivity of the analysis method must be high.
- Multiple charged ions are likely to be generated in the mass spectrometry ion source by **peptide** molecules. Also, ions of different valence states are dispersed to a certain extent, making it difficult to determine the optimal ion pair.
- **Peptide** molecules also have some other common problems such as non-specific adsorption, low stability, and a high protein binding ratio, posing serious challenges for sample processing and detection.
- **Peptides** cannot easily cross various bio-membrane barriers in the body as they are highly polarized and poorly permeable. Thus, the oral bioavailability of **peptides** needs to be improved.
- **Peptides** have poor metabolic stability, wide metabolic pathways, and short half-life periods.

High Significance

- Screen out stable **peptides**, enhance *in vivo* exposure, and prolong half-life.
- Improve the absorption and intracellular concentration by studying the mechanism of permeation and transport of **peptides**.
- Assess the immunogenicity of **peptides** and determine their impact on PK.

Key Study Capabilities

- Comprehensive characterization of metabolic stability and soft spots of **peptides**.
- High-sensitivity quantitative analysis of **peptides** of various molecular weights and types.
- Study on mass balance and tissue distribution of **peptides** through radiolabeled **ADME** and **QWBA** platforms.

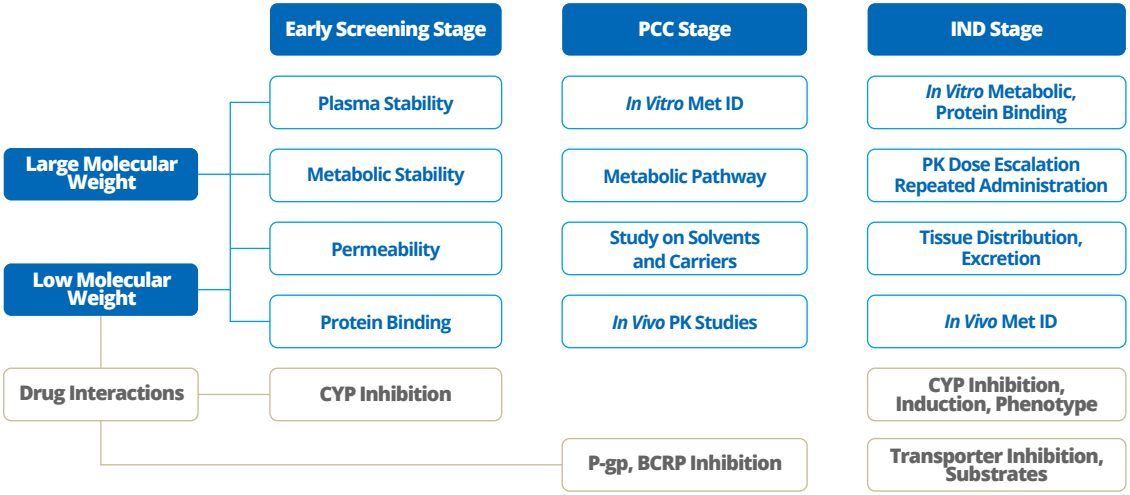
Pharmacokinetic Research Strategies for **Peptides**

Preclinical research on **peptides** mainly focuses on **stability, permeation, delivery carrier, and tissue distribution studies.**

Early screening stage: Mainly investigating the *in vitro* properties of **peptide** molecules (including **plasma stability, metabolic stability, permeation, and protein binding**) and guideing molecular structure optimization. The interactions and enzyme phenotyping of **peptides** with low molecular weight are evaluated as well.

PCC stage: This stage focuses on **the metabolic differences of species** and the ***in vivo* PK study** of **peptides**. It aims to select the appropriate species for relevant research by considering both pharmacodynamics and toxicology. The study of various delivery carriers makes it possible to achieve the desired *in vivo* exposure characteristics.

IND stage: In this stage, single **dose escalation** and **repeated administration PK** in animals will be studied. **The excretion pathway (mass balance), tissue distribution, and *in vivo* metabolites identification also need be evaluated.**



Our Strengths

Committed to Your Program

We have a specialized and dedicated service model. Each client will be connected to a dedicated study director who will provide comprehensive management services for the pharmacokinetic project from drug discovery to the clinical phase.

Extensive Experience and Short Turnaround Time

We have more than 10 years of experience in **peptide** research, with the annual study of 1,000+ **peptide** molecules, a variety of mature solutions, and a short experiment cycle.

Comprehensive Capabilities and High-Quality Delivery

With an experienced **peptide** research team and cutting-edge instruments and equipment, WuXi AppTec **DMPK** is equipped with comprehensive **peptide** study and analysis capabilities to ensure the delivery of high-quality *in vivo* and *in vitro* data.

Customized Study Design

We're familiar with multiple types of studies and offer customized study design services according to the special properties of a client's molecules.

Sufficient Research Resources

We have an orderly supply of primate resources to ensure project implementation and shorten the delivery cycle.

Case Analysis: Case Study Analysis of Exenatide by LC-MS/MS

Background: As a synthetic product of Exendin-4, which is similar to glucagon-like **peptide-1** (GLP-1), Exenatide consists of 39 amino acids and is mainly used in the treatment of type 2 diabetes. In this case, the PK in rats was studied. Since the administration dosage designed in this experiment was quite low, the plasma concentration was predicted to be low accordingly, so the detection sensitivity was required to be high (20 ~ 30 pg/mL). In addition, blood biochemical analysis was also required for this project, leaving a minuscule sample volume for PK detection. This posed a significant challenge to method development.

Problems and Solutions:

- Using special low-adsorption consumables and reagents, the amount of sample adsorbed by the container was greatly reduced.
- As for the nonspecific binding or co-precipitation between the sample and protein, the sample was alkalinized to improve the recovery rate.
- Solid phase extraction (SPE) was adopted to enrich and purify the plasma sample, which eliminated the endogenous interference and further improved the recovery rate.

The lower limit of quantitation (LLOQ) of this procedure reached ~ 20.9 pg/mL. This result was comparable to that of the RIA procedure reported in the literature.

Advantages:

- Low cost (no need to purchase commercial kits)
- High throughput (automated workstation for 96-well plates)
- Small sample amount (takes only 50-100 µl of a sample, unlike other procedures that require samples to be hundreds of microliters of a sample)
- High specificity

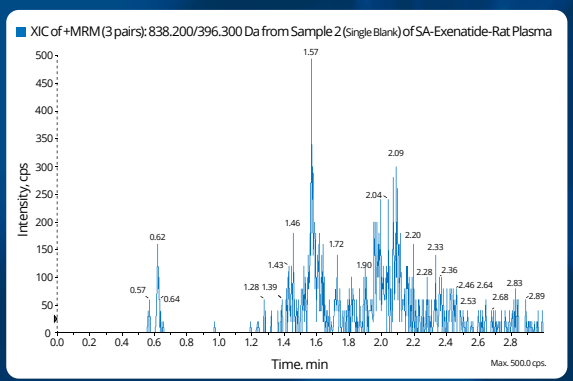


Fig. (a). LC-MS spectrum of single blank sample

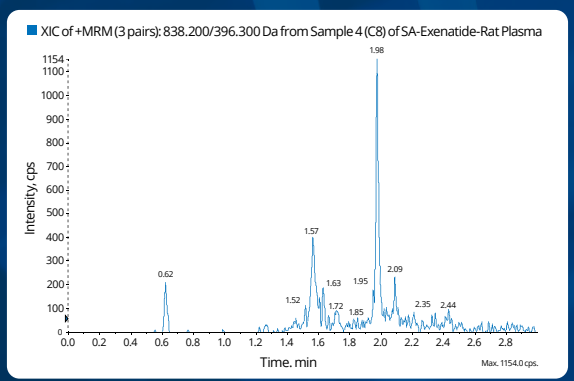


Fig. (b). LC-MS spectrum of LLOQ (20.9 pg/mL)

References

[1] Ai G, Zhen Z, Shan C, Che J, Hou Y, and Cheng Y. Single- and multiple-dose pharmacokinetics of exendin-4 in rhesus monkeys. *International Journal of Pharmaceutics*, 2008, 353:56-64.