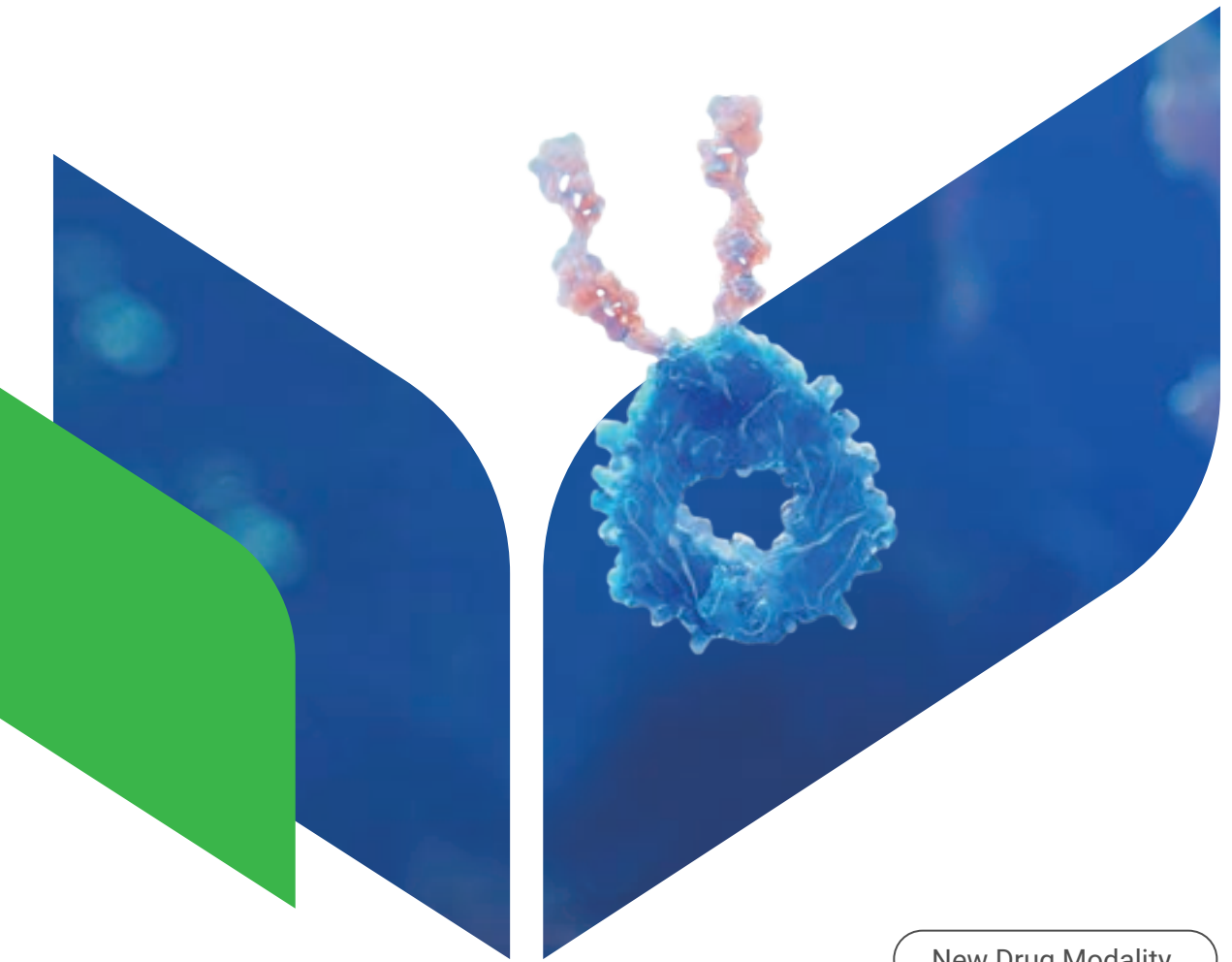
Laboratory Testing Division
Bioanalytical Services

WuXi AppTec

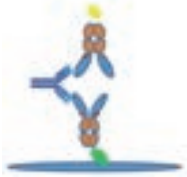
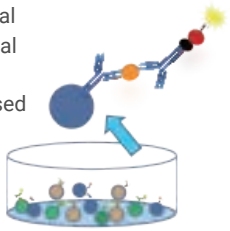
Fusion Protein & Peptide-Drug Conjugate (PDC)

Bioanalysis

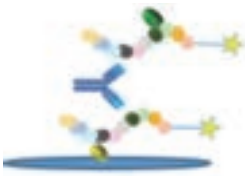
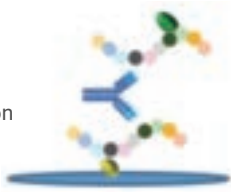
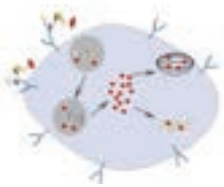


New Drug Modality

Bioanalytical Solutions for Fusion Protein

Research Contents		Analyte	Platform	Experiment Design
Pharmacokinetics/ Toxicokinetics	**PK/ TK**	Fusion Protein	ELISA/MSD	**Key reagents:** Monoclonal antibody targeting functional protein, antigen, biotinylated monoclonal antibody targeting the complementarity-determining region, SA-HRP 
Immunogenicity	**ADA**	Fusion Protein Antibody	MSD	Whole molecule confirmatory, functional domains confirmatory 
	NAb	Neutralizing Antibody	Cell-based assay (recommended) or non-cell-based assay	Fusion proteins typically bind to target receptors, activate cellular pathways, and enhance cell killing. Neutralizing antibodies block the binding of drugs to cell surface receptors, thus inhibiting the cytotoxic effects of the drugs. 
Pharmacodynamics/ Biomarker	**RO**	Receptor Occupancy	FACS	The binding of antibody components to target receptors is a crucial step for fusion proteins to exert their effects. Evaluating the receptor occupancy of drug targets using appropriate cell populations and detection strategies provides data support for drug efficacy and dosage studies. 
	Biomarker	Cytokines Pharmaco-dynamic Biomarkers	ELISA/MSD/ Luminex/ELLA/ Simoa/Gyros/ SMCxPRO, etc.	Biomarkers are objective indicators that can measure and evaluate normal biological processes, pathological processes, or responses to drug interventions. They are widely used in clinical settings as tools for diagnosis, prognosis, prediction, drug efficacy, safety, and monitoring. 

Bioanalytical Solutions for Peptide-Drug Conjugates (PDCs)

Research Contents		Analyte	Platform	Experimental Design
Pharmacokinetics/ Toxicokinetics	PK/ TK	Intact PDC	LCMS	Pretreatment: Protein precipitation/SPE  ~10 ng/ml (LLOQ)
		Naked Peptide	LCMS	Pretreatment: Protein precipitation/SPE  ~10 ng/ml (LLOQ)
		Free Payload	LCMS	Pretreatment: Protein precipitation  ~1 ng/ml (LLOQ)
Immunogenicity	ADA	Intact PDC	MSD/ELISA	Anti-PDC antibody detection  Sensitivity：＜100 ng/ml
		Naked Peptide	MSD/ELISA	Anti-peptide antibody detection  Sensitivity：＜100 ng/ml
	NAb	Neutralizing Antibody	Cell-based assay (recommended) or non-cell-based assay	The peptide portion binds to the target receptor, and through receptor-mediated endocytosis transfers the PDC into the cell, thereby releasing the payload and exerting its cytotoxic effects. Neutralizing antibodies block the binding of the drug to the cell surface receptors, thereby inhibiting the cytotoxic effects of the drug. 

Bioanalytical Challenges and Experience with Fusion Proteins

Challenges	
Fusion Protein	▪ Instability of fusion proteins in human biological matrix ▪ Specificity ▪ Sensitivity
Anti-Fusion Protein and Anti-Functional Protein Antibody	▪ Drug resistance ▪ Anti-functional protein antibody: low immunogenicity
Neutralizing Antibody	▪ Drug resistance ▪ Robustness ▪ Sensitivity
Receptor Occupancy	▪ Detection strategy ▪ Target cell population selection ▪ Drug toxicity
Biomarkers	▪ Sensitivity ▪ Robustness ▪ Multiple-plex detection

Bioanalysis Experience with Fusion Proteins	
Functional Protein	**Fusion Partner**
▪ IL-2 ▪ EPO ▪ C5 ▪ NGF ▪ CD24 ▪ PTH ▪ CD-1 ▪ GLP-1 ▪ FSH ▪ IL-12/15 ▪ FGF21 ▪ GCSF ▪ Annexin V ▪ Anti-platelet Thrombolytic Agent	▪ Fc ▪ Hybrid Fc ▪ FH

Bioanalytical Challenges and Experience with Peptide-Drug Conjugates (PDCs)

Challenges	
Intact PDC	▪ Small molecule toxins are pH-sensitive ▪ Poor stability ▪ Sensitivity
Naked Peptide	▪ Poor stability ▪ Non-specific adsorption ▪ Protein binding ▪ Specificity
Free Payload	▪ Toxin releases ▪ Toxin converts into isomers ▪ Sensitivity
NAb	▪ Drug resistance ▪ Robustness ▪ Sensitivity

Bioanalysis Experience with PDCs	
PDC	**Payload**
Six PDC projects	MMAE, Paclitaxel, Memantine

Fusion Protein Case Study

Background:

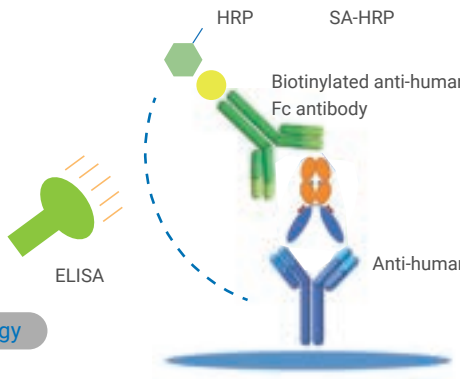
Taking GLP-1 Fc fusion protein as an example, this medication specifically binds to the GLP-1 receptor (GLP-1R), activating it to promote insulin secretion. With its proven ability to lower fasting blood glucose levels, GLP-1 fusion protein holds significant potential for diabetes treatment.

Study Design:

We provide customizable services in support of clinical applications in accordance with all major global regulatory agencies.

- **PK:** Quantitation of GLP-1 fusion protein in human serum by ELISA.
- **ADA:** Quantitation of anti-GLP-1 fusion protein antibody in human serum by electrochemiluminescent (ECL) immunoassay.
- **NAb:** Quantitation of anti-GLP-1 fusion protein antibody in human serum by cell-based method.

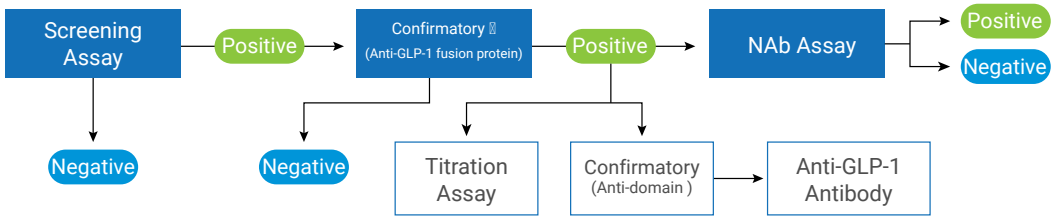
GLP-1
Fusion Protein
PK



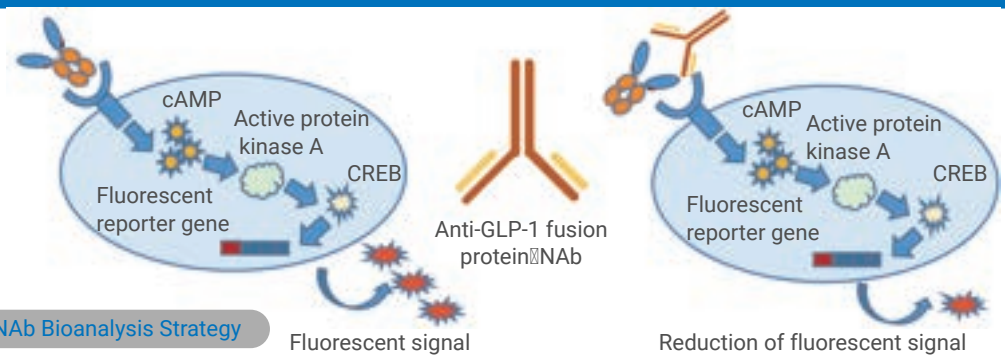
PK
GLP-1 Fusion Protein

Accuracy and precision
Selectivity
Dilutional linearity
Hook effect
Specificity
Stability
Robustness

PK Bioanalysis Strategy



ADA Bioanalysis Strategy



NAb Bioanalysis Strategy

Bioanalytical Services

Global Integrated Bioanalytical Platform

WuXi AppTec Bioanalytical Services operates globally and has leading integrated laboratories in China and the U.S. We adhere to GLP and GCP principles and strictly follow high industry regulatory standards. Our comprehensive platform, equipped with advanced instruments and staffed by experienced experts, provides professional one-stop services in preclinical/clinical mass spectrometry, immunochemistry, molecular/cellular, and central laboratory services to global clients.

Shanghai, China
Headquarters
Bioanalytical Services (Clinical)
Central Lab

Suzhou, China
Bioanalytical Services (Preclinical)
Antibody Service

New Jersey, USA
Bioanalytical Services (Preclinical/Clinical)
Central Lab

Chengdu, China
Bioanalytical Services (Preclinical)

Nantong, China
Bioanalytical Services (Preclinical)

18+ Years lab operations in China and the U.S.
24,300 m² lab space

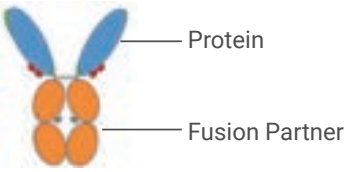
5,000+ small molecule validated methods
2,800+ large molecule validated methods
2M+ samples analyzed per year

Global Regulatory Standards

Successfully passed global regulatory inspections, comply with GLP, GCP, CAP quality standards
200+ China NMPA
30+ International regulatory agencies (U.S. FDA, OECD, EMA, PMDA, CAP)

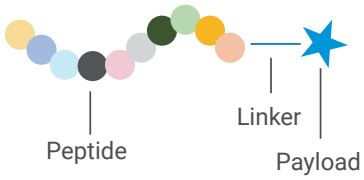
Fusion Protein is a novel protein generated by merging a biologically active protein with another naturally occurring protein (fusion partner) through techniques such as advanced engineering.

We have supported more than 30 preclinical and clinical projects to help sponsors expedite their global IND, NDA, and BLA drug applications.



Peptide-Drug Conjugates (PDCs) are formed by chemically linking a biologically active small molecule drug to a peptide. The peptide acts as a carrier to target and transport the small molecule drug to the desired cells.

With state-of-the-art instrument platforms and extensive experience in PDC methodology, we are capable of swiftly and efficiently tackling challenging issues encountered during PDC method development.



Peptide-Drug Conjugate (PDC) Case Study 1

Background:

Consider a PDC (Peptide-Drug Conjugate) drug in Phase I clinical trials. The peptide component is engineered to bind specifically to the tumor targets currently under investigation. Once bound, the small molecule toxin component penetrates the tumor cells, inducing cell death. With the overexpression of this target across multiple cancerous tissues, PDC drugs targeting this molecule hold significant promise for the treatment of a wide range of solid tumors, marking a potential breakthrough in cancer treatment.

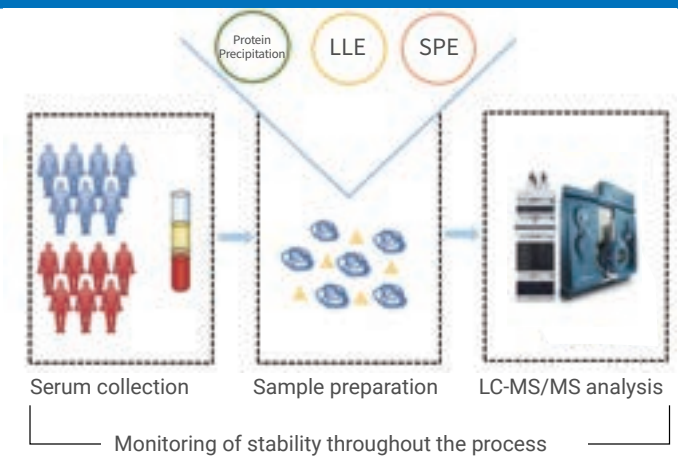
Study Design:

We provided customized IND submission support services in accordance with global regulatory guidance.

- **PK bioanalysis of PDC payload:** Quantitation of payload in human/mouse serum by LC-MS/MS.
- **PK bioanalysis of intact PDC:** Quantitation of intact PDC in human/mouse serum by LC-MS/MS.

Intact PDC PK
Payload PK

Accuracy and precision
Selectivity
Recovery rate
Specificity
Hemolysis and Lipemia
Interference
Stability
Residue
Dilution reliability
Injection reproducibility



Peptide-Drug Conjugate (PDC) Case Study 2

Background:

A robust bioanalytical method must be developed to accurately quantify the concentration of a PDC (Peptide-Drug Conjugate) drug in rat plasma post-administration, encompassing both the peptide and the free payload. With a molecular weight exceeding 4500 daltons, this PDC drug features a peptide linked to a cytotoxic compound via a cleavable linker. Here, we discuss the bioanalytical strategy and the challenges in analyzing the peptide and the free payload in rat plasma.

Study Design:

During method development, we successfully tackled the PDC drug's stability and non-specific adsorption issues in plasma, along with sensitivity challenges related to multiply charged species, low recovery, and systemic carryover. Using protein precipitation, we established an LC-MS/MS analysis method for quantifying the PDC drug in rat plasma (with stabilizers).

- The LC-MS/MS method demonstrates excellent linearity, with a detection range of 5.00 to 2500 ng/mL and an R2 value greater than 0.997.
- In accuracy and precision analyses, the average accuracy of each QC sample concentration level is within ±15.0% (with the LLOQ sample within ±20.0%). Precision values (%CV) for each QC sample concentration level do not exceed 15.0% (and do not exceed 20.0% for LLOQ).
- Adding stabilizers to rat plasma has proven effective in ensuring the stability of the PDC during sample processing. Our method validation and sample analysis have further confirmed the robustness and high reproducibility of the established method.

Our Advantages

Global Integrated Platform: We provide one-stop services to global sponsors, from preclinical to clinical stages, supported by multiple BAS sites worldwide.

With our high-sensitivity platforms and extensive experience in PDC method development, we are capable of quickly and efficiently resolving challenging issues encountered during PDC method development.

Experience with more than 30 fusion protein projects, including IL-2, EPO, C5, NGF, CD24, PTH, CD-1, GLP-1, IL-12, FGF21, and more.

Customized Services: Our sponsor-centric professional project management team delivers tailored services to meet your specific needs.

Statistical Services: Our immunogenicity cut-point determination offers expedited data delivery, ensuring you quickly get accurate, reliable results. Our services are grounded in scientific rigor, providing enhanced accuracy and dependable outcomes.

Experienced Team: Our core technical team members boast over 10 years of experience, with our management team having more than 20 years.

Common Industry References

1. 9012 Guidelines for Validation of Quantitative Analytical Method of Biological Samples, Chinese Pharmacopoeia
2. Bioanalytical Method Validation and Study Sample Analysis M10, International Council for Harmonisation
3. Bioanalytical Method Validation, U.S. Food and Drug Administration
4. Guideline on Bioanalytical Method Validation, European Medicines Agency (EMA)
5. Immunogenicity Testing of Therapeutic Protein Products—Developing and Validating Assays for Anti-Drug Antibody Detection, U.S. Food and Drug Administration

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