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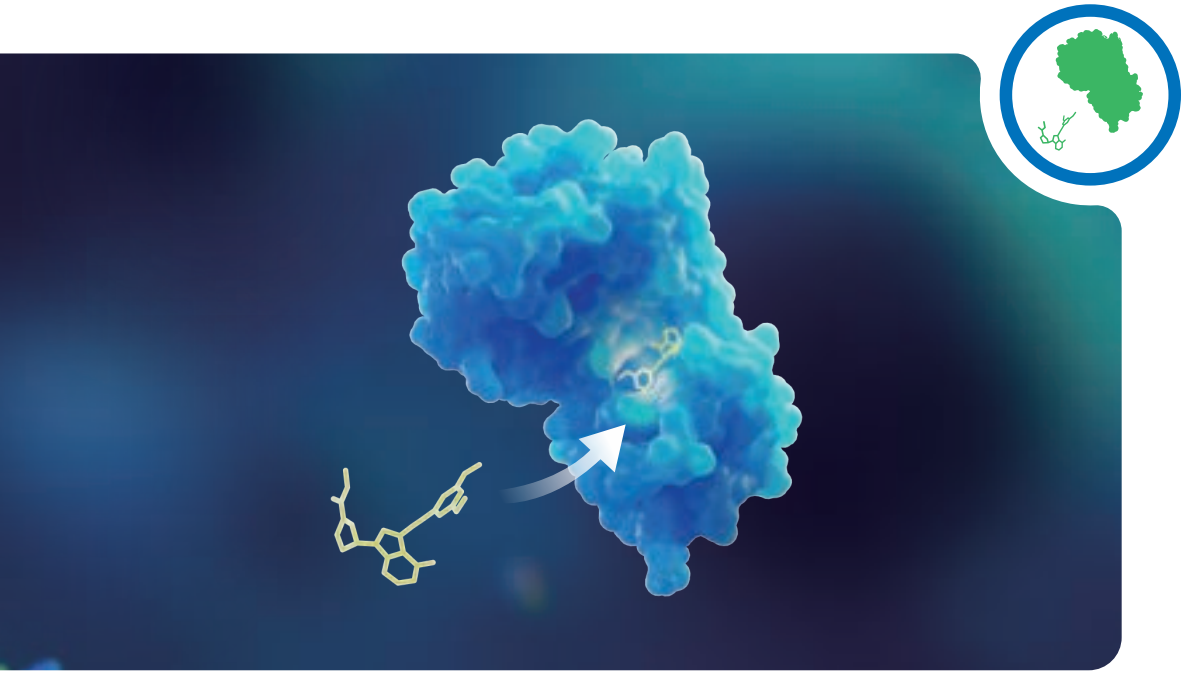
WuXi AppTec

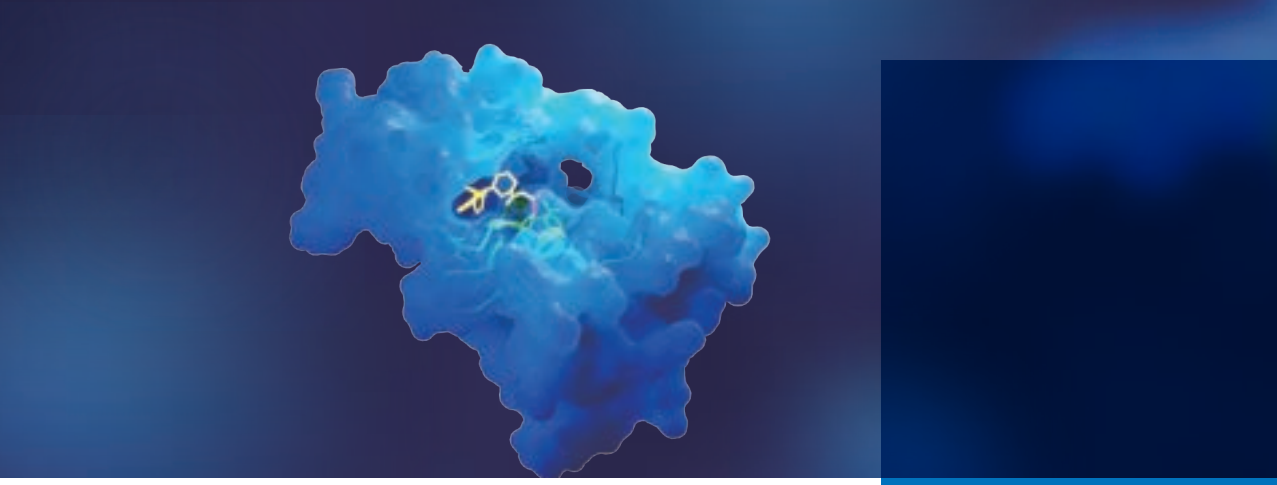
PRECLINICAL DRUG DEVELOPMENT TESTING FOR

COVALENT DRUGS

Shorten the Research & Development Cycle with WuXi AppTec DMPK

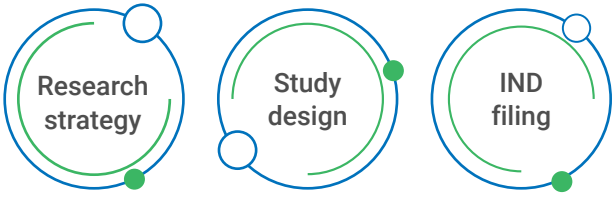


New Modalities Series | Covalent Drugs



Unique Pharmacokinetics Evaluation System for Covalent Drugs

Covalent drugs pose unique challenges, including complex pharmacokinetics, diverse metabolism, and off-target toxicity, requiring specialized expertise. To address these challenges, WuXi AppTec DMPK has developed tailored research platforms and integrated pharmacokinetic strategies that ensure reliable data. We bring extensive experience in bioanalysis, in vitro ADME, metabolite identification, and radiolabeled ADME to support successful preclinical development.



01 High-resolution mass spectrometry (HRMS) platform

02 Various *in vitro* ADME assays

03 *In vivo* and *in vitro* metabolite identification

04 Radiolabeled compound synthesis and ADME studies

WuXi AppTec DMPK has supported covalent drug projects for over 100 clients, successfully advancdozens of covalent drugs into clinical trials. The DMPK study protocols are tailored to your projects, combined with appropriate analytical methods to accelerate the development and regulatory filing of covalent drugs.

Pharmacokinetic Challenges of Covalent Drugs

Binding to Serum Albumin: Covalent drugs may bind to serum albumin reversibly or irreversibly, complicating distribution and elimination studies. High reactivity with plasma proteins makes measuring free drug concentrations challenging.

Complex Metabolism: Covalent drugs can form adducts with target proteins, glutathione (GSH), or other residues, leading to diverse and complex metabolic pathways.

Off-Target Toxicity: Unintended covalent binding raises potential safety concerns, making toxicity evaluation critical during preclinical development.

Key Capabilities and Platforms

Well-established high-resolution mass spectrometry (HRMS) platform

Confirm the covalent binding rate and binding site

Various *in vitro* ADME assays

Comprehensively assess of ***in vitro*** distribution and metabolism of covalent drugs

Diverse *in vivo* and *in vitro* metabolism models and MetID methods

Determine the metabolites and elimination pathway of covalent drugs

Radiolabeled compound synthesis and ADME studies

Clarify the covalent protein binding fraction of the covalent drug and its active metabolites, and identify the binding mode and binding site

High-Resolution Mass Spectrometry (HRMS)

Mass spectrometry is widely used in covalent drug discovery. When compounds covalently bind to a target protein, the molecular weight of the protein increases. The precise molecular weight can then be determined, and the irreversible covalent bond can be monitored using HRMS. The method is simple to develop, high throughput, and has good reproducibility. WuXi AppTec DMPK provides high-throughput screening and characterization services based on HRMS, including conventional covalent drugs, molecular glues, PROTACs (Proteolysis-Targeting Chimeras), etc.

Case study

The mass shift can be clearly observed after drug covalent binding to the target protein. The covalent binding can be monitored by HRMS and the binding ratio can be obtained by calculation. The specific binding site is determined by peptide mapping.

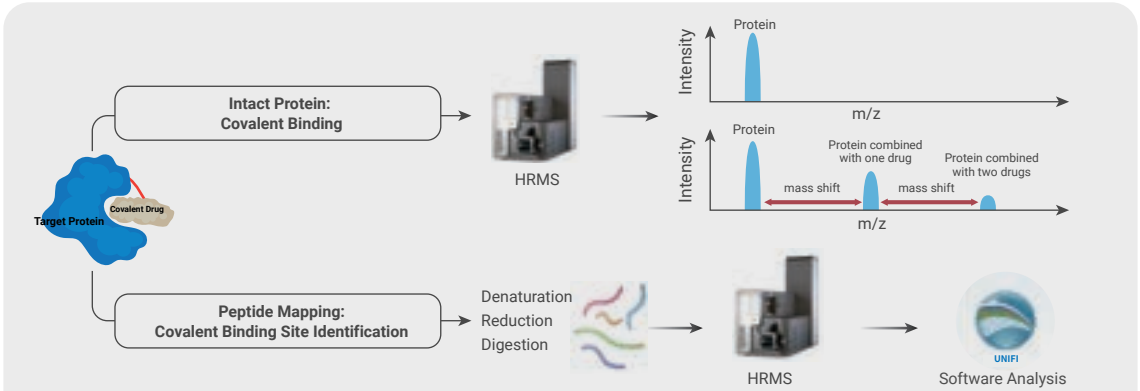


Figure 1. Monitor covalent binding and identify binding site by HRMS

In Vitro Distribution and Metabolism

Due to the potential reaction between the warhead and plasma proteins, conventional methods may present low reliability of the f_u value. By employing optimized conditions/methods such as lower temperature, plasma dilution, ultracentrifugation, or flux dialysis, the plasma protein binding of covalent drugs with low plasma stability could be determined to obtain a more reliable f_u value. Additional assay types have been established to further characterize the ***in vitro*** distribution and metabolism of covalent drugs, such as HSA binding, HSA stability, GSH stability in buffer, and GSH stability mediated by GST enzymes.

Case study

Table 1. Comparison of f_u for four covalent drugs in human plasma: data from WuXi AppTec DMPK vs. reported values

Covalent drugs	f_u obtained in WuXi AppTec DMPK	f_u reported in the literature
Afatinib	0.11	0.095
Ibrutinib	0.025	0.027
Osimertinib	0.055[a],0.032[b]	0.050[c],0.030[d]
Aspirin	0.48	0.42

[a]flux dialysis method at 37 °C. [b]ultracentrifugation at 4 °C.[c]Pharmacology reivew. [d]J Pharm Sci. 2020;109(10):3181-3189.

Metabolite Identification

The elimination pathways of covalent drugs are often complex. In addition to forming covalent bonds with cysteine residues of target proteins, these drugs may also react with glutathione (GSH) or other amino acid residues, leading to diverse metabolic products.

Incubation system	Objective
Liver microsomes with GSH	Determine the metabolites of covalent drugs, including GSH conjugates with electrophilic warheads or reactive metabolites.
Liver S9/cytosol	Determine the conjugates from electrophilic reaction mediated by glutathione transferase.
Hepatocytes	Determine the metabolites of covalent drugs in the presence of Phase I and Phase II enzymes.
Plasma or serum albumin	Evaluate the covalent binding of covalent drugs with serum albumin.

Case study

The following figures show the metabolite profiles of Osimertinib in liver microsome systems with/without GSH (Figures 2-A and 2-B), by comparing with phosphate buffer containing GSH (Figure 2-C). The GSH adduct of warhead (M4), the oxidative products mediated by CYP enzymes (M3 and M5-M9), and the reactive intermediate captured by GSH (M2) were identified.

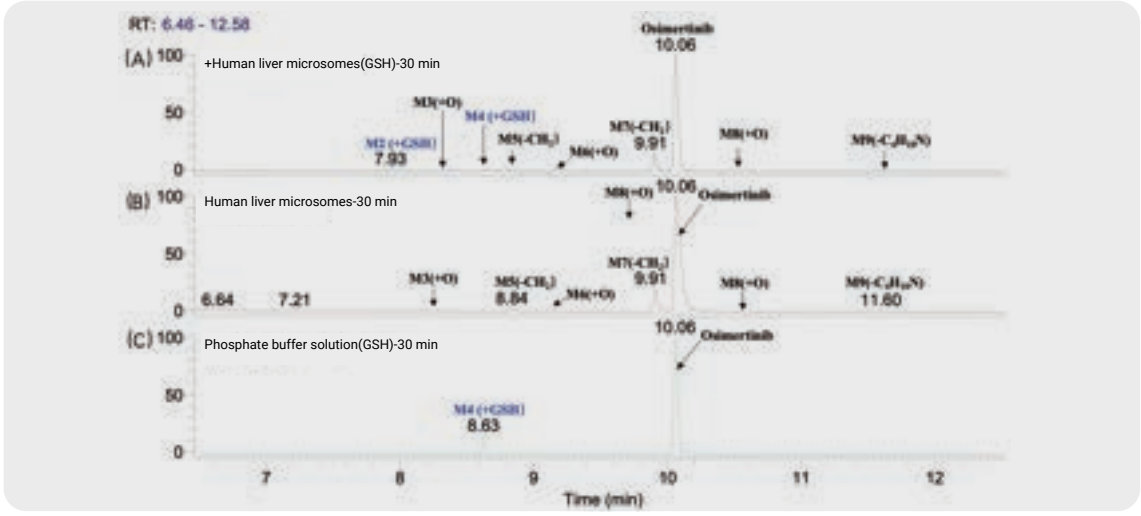


Figure 2. The metabolite profile of Osimertinib in liver microsomes with/without GSH

Radiolabeled Compound Synthesis and ADME Studies

At WuXi AppTec DMPK, we use radioactive labeling tracing technology to determine the covalent binding sites and ratios of the parent drug, metabolites, or reactive metabolite intermediates with proteins. Combined with HRMS and enzyme digestion, we can identify potential binding sites and proteins, including those of the parent drug or metabolites, as well as the protein type and binding site. Additionally, we synthesize 14C or 3H radiolabeled test compounds, selecting the optimal labeling position based on the chemical structure, synthetic procedures, and results from previous MetID studies using cold test compounds.

Case study

Following a single oral administration of a radiolabeled compound in rats, plasma is collected, and proteins are precipitated using organic reagents. The total radioactivity of both the extracted and unextracted portions can be measured, allowing for the accurate determination and analysis of covalent drugs and their metabolites, as well as the binding sites to proteins.

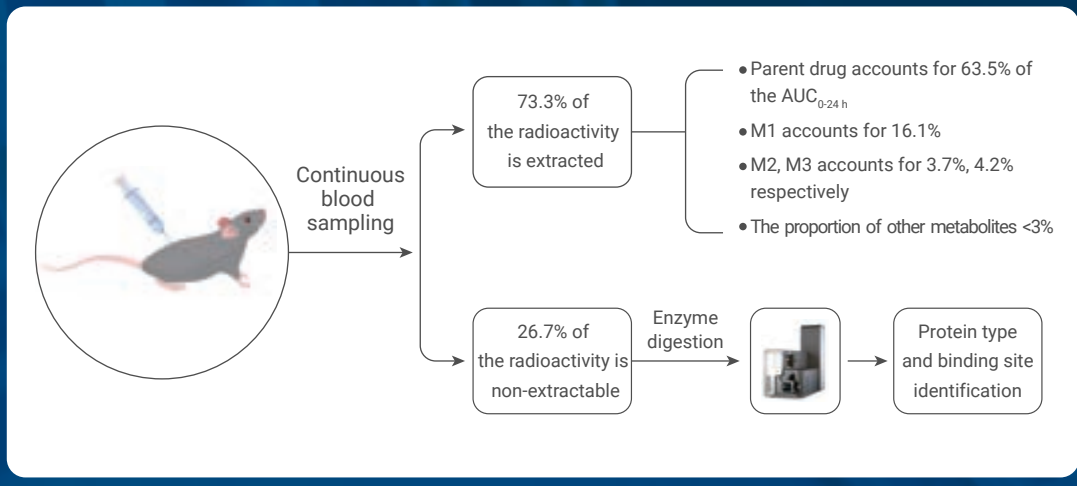


Figure 3. The radioactive isotope platform is used to identify covalent drugs and binding sites and ratios in plasma ***in vivo***

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