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Metabolite Profiling & Identification (MetID) Services

WuXi AppTec DMPK

Metabolite Profiling and Identification Services

We provide comprehensive metabolite profiling and structural characterization services, covering lead compound optimization in drug discovery stage to clinical development stage, including radiolabeled metabolite profiling and identification to support drug discovery and safety assessment. In addition, our extensive experience in the biosynthesis of metabolites also provides customers with more convenient ways to synthesize metabolites.

Our scientific team offers customized services to solve complex problems in metabolism studies, and our global research centers and departments collaborate efficiently to ensure a timely delivery of high-quality reports that meet the requirements of the regulatory authorities (NMPA, FDA, EMA, etc.). We provide flexible and customized metabolite profiling and identification solutions to meet customers' research needs.

WuXi AppTec DMPK Metabolite Identification (MetID) team has 3 research laboratories in China (Shanghai and Nanjing) and America (New Jersey). The team is equipped with various industry-leading high-resolution mass spectrometers (HRMS), such as quadrupole-time-of-flight MS (Q-Tof), quadrupole-orbitrap-linear ion trap MS (Orbitrap Tribrid), and metabolite screening and processing software. Furthermore, the team has extensive experience in metabolite identification of conventional small molecules and new modalities (PROTACs, Peptides, PDCs, ADCs, and Oligonucleotides). The team has completed more than 8,000 screening projects, over 500 IND filings, and more than 100 clinical safety assessment experiments.

MetID Services

Drug discovery stage	Metabolic soft spot screening	Reactive metabolite trapping
	In vitro species comparison	*In vitro* and *in vivo* correlation
Pre-clinical development -IND stage	*In vitro* species comparison	*In vivo* MID in rodent and non-rodent PK/TK studies
	^{3}H- or ^{14}C-ADME studies in animals (mouse, rat, dog, monkey)	
Clinical development -NDA stage	Phase I: MID in human SAD/MAD samples	Phase I: Metabolites in Safety Testing (MIST)
	Phase II or III: Radiolabeled human mass balance study and metabolite profiling and identification	

Note: SAD: Single Ascending Dose, MAD: Multiple Ascending Dose, MIST: Metabolites in Safety Testing.

Our Strengths

▶Professional team and state-of-the-art equipment

Our team has outstanding research capabilities in metabolite profiling and identification of conventional small molecules (including polar small molecules, nucleotides, chiral molecules, and prodrugs) and new modalities (such as Oligonucleotides, ADCs, Peptides, and PROTACs), with extensive experience in metabolite identification research in early-stage R&D, preclinical IND filing, and clinical stages. We have published dozens of SCI papers and have given many presentations in academic conferences.

20+

20+ years since our team was established

15+

15+ core members with over ten years of experience in metabolite profiling and identification

20+

20+ years of medicinal chemistry experience experts to participate in experimental design and data interpretation

HRMS platform and supporting software

The team is equipped with industry-leading high resolution mass spectrometry platforms, such as Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ and Waters® Vion™ IMS QTof. In addition, professional data processing softwares are available within the team, including Thermo Scientific™ Compound Discoverer™/Thermo Scientific™ BioPharma Finder™, UNIFI, and Mass-MetaSite (Molecular Discovery).

*PROTAC refers to Proteolysis-Targeting Chimera.

Thermo Orbitrap Eclipse™ Tribrid™

Thermo Orbitrap Exploris™ 480

Thermo Q-Exactive™ HF

Waters VION™ IMS QTof

Thermo Q-Exactive™Plus

Thermo Q-Exactive™

Thermo LTQ Orbitrap XL

Waters Xevo®G2 QTof

- Thermo Compound Discoverer
- Thermo Mass Frontier
- Thermo Metworks
- Waters Metabolynx
- Waters UNIFI
- Molecular Discovery Mass-MetaSite
- Thermo BioPharma Finder

For screening and structural elucidation of smalll molecule metabolites

For screening and structural elucidation of large molecule metabolites

▶Fast delivery time

No waiting time

With three research centers worldwide and high throughput, studies can be scheduled without delay and conducted as soon as the arrival of the samples/compounds.

Short turnaround time

A variety of assay types are available for selection depending on the study purpose, and projects can be flexibly arranged with dedicated personnel, thus allowing the study to be conducted promptly and efficiently.

Automated and accelerated process

To improve efficiency and accuracy, we use the TECAN Freedom EVO® semi-automated incubation platform, data processing software which assists the rapid metabolite screening and structural elucidation, and a semi-automated platform for report generation.

▶Comprehensive IP protection measures

Minimum authorization management

Minimal authorization.
Cascading approval.

Data backup

Off-site data backup.
Periodic data review to check tape readability.

Thorough SOP system

Adherence to compliance is crucial for enterprise development. We strictly follow compliance rules throughout the entire process, with zero tolerance for any leakage of confidential information.

Multiple internal checks

Independence of the compliance management department.
Double verification within the team.

Data reliability

Information and data follow ALCOA principles (Attributable, Legible, Contemporaneous, Original, Accurate) .

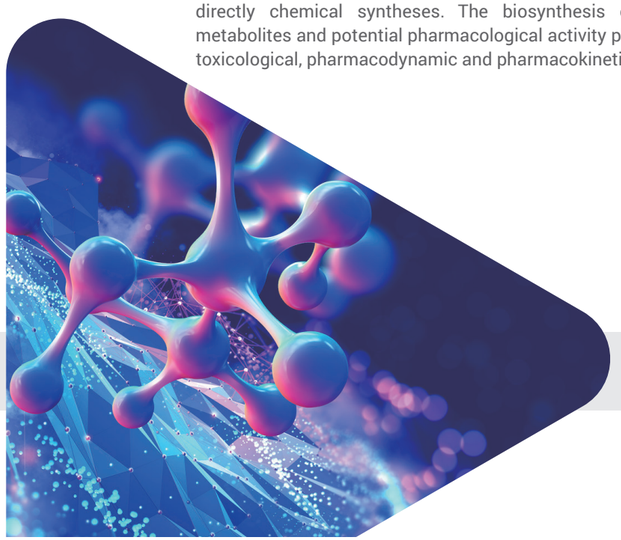
▶High-quality report delivery

- Comprehensive QC process for data and reporting, covering all key aspects of the report
- Two levels of report review to ensure the accuracy and scientific validity of metabolite elucidation and data interpretation
- Semi-automated report writing to avoid human error

▶Customized study design

Our team has extensive experiences in various customized studies, e.g. the chemical-assisted methods such as hydrogen/deuterium exchange experiment and titanium trichloride reduction experiment for the identification of special metabolites, the special incubation system and *in vitro* metabolic systems containing specific enzyme inhibitors, the biosynthesis of target metabolites *in vitro* or *in vivo*, and covalent binding recognition of small molecules to target sites by covalent inhibitors, etc.

The biosynthesis of target metabolites refers to metabolite standards obtained by *in vitro* incubation of liver microsomes, liver S9, or hepatocyte, or isolation and purification of *in vivo* plasma, urine, bile, and fecal samples from animals after dosing. Generally, it is very difficult to obtain metabolite standards through conventional chemical syntheses due to the challenges in predicting the precise structures of metabolites and directly chemical syntheses. The biosynthesis of disproportionate metabolites and potential pharmacological activity plays a key role in the toxicological, pharmacodynamic and pharmacokinetic (PK) studies.



Radiolabeled Technique Platform for Metabolite Identification

▶Capabilities for conducting radiolabeled ADME studies

The team has extensive experiences in ^{14}C- and ^{3}H-labled ADME studies in pre-clinical testing animals (mice, rats, dogs, and monkeys) and has carried out radiolabeled ADME studies for more than 400 compounds, supporting regulatory NMPA and FDA filings.

So far, we have conducted human radiolabeled MetID studies with dozens of compounds to acquire comprehensive information of human absorption, metabolism, and excretion. We have provided critical information for drug-drug interaction (DDI) studies and clinical safety assessments, facilitating the successful approval of many drugs.

▶The platform of radioactivity detecting instruments

Solid scintillation Counter: Topcount
For offline radioactivity detector

Solid scintillation Counter: Microbeta
For offline radioactivity detector

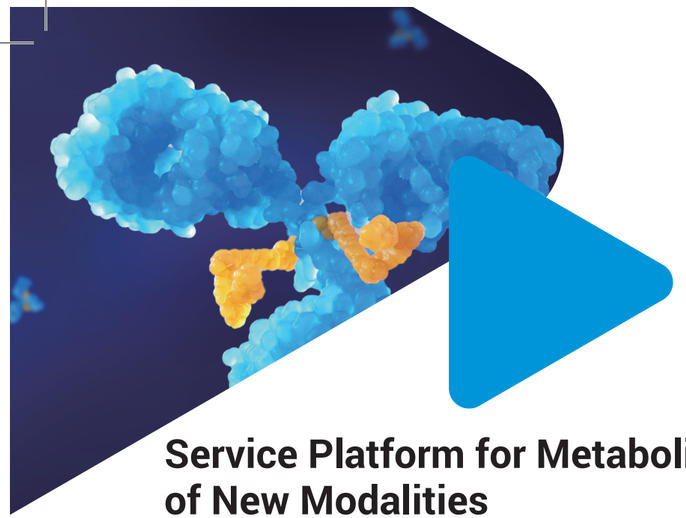
Liquid Scintillation Counter : Tricarb
For total radioactivity

Online Radioactivity Detector: v. ARC
For online radioactivity detector

Online Radioactivity Detector: Beta-RAM
For online radioactivity detector

▶Radiolabeled ADME study description

Type	Level	Species	Matrixes	Description	Submitted results
Metabolite identification in animal and human *in vitro*	IND	Mice, rats, dogs, minipigs, monkeys, humans	Plasma, microsomes, hepatocytes, liver S9	▪ Sample incubation and processing; ▪ Metabolite radioprofiling using online detector and/or off-line detector; ▪ Metabolite identification and characterization by LC-HRMS/MS	▪ Distribution of metabolites; ▪ Structure interpretation on major metabolites; ▪ Cross species comparison; ▪ Proposed metabolic pathways
Metabolite identification in animals *in vivo*	IND	Rats, mice, dogs, monkeys	Plasma, urine, bile, feces	▪ 1- 4 matrices (plasma, urine, feces, bile); ▪ Sample pooling and processing; ▪ Metabolite radioprofiling using online detector and/or off-line detector; ▪ Metabolite identification and characterization by LC-HRMS/MS	▪ Distribution of metabolites in plasma; ▪ % Dose of metabolites in urine, bile and feces; ▪ Structure interpretation on all quantifiable metabolites; ▪ Proposed metabolic pathways
Metabolite identification in human *in vivo*	NDA	Humans	Plasma, urine, feces	▪ Sample pooling and processing; ▪ AUC pooling for plasma, equal % volume/weight pooling for urine and feces; ▪ Metabolite radioprofiling using online detector and/or off-line detector; ▪ Metabolite identification and characterization by LC-HRMS/MS	▪ Distribution of metabolites in plasma; ▪ % Dose of metabolites in urine and feces; ▪ Structure interpretation on all quantifiable metabolites; ▪ Proposed metabolic pathways



Service Platform for Metabolite Identification of New Modalities

▶Extensive projects experience

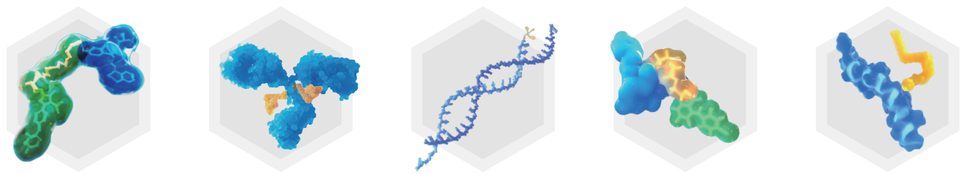


Table 1. Comprehensive research system based on molecular types

New modality type	Study type	Sample source	Biological matrixes	Analysis of content
Oligonucleotide	Metabolite identification	*In vitro*	Plasma/Serum, S9, tissue homogenates, hepatocytes, lysosomes	Parent drug, metabolites
		In vivo	Plasma, urine, feces, bile, target tissue	Parent drug, metabolites
	Sequence characterization	Standard compound	/	Sequence mapping analysis
PROTAC	Metabolite identification	*In vitro*	Plasma, liver microsomes, hepatocytes	Parent drug, metabolites
		In vivo	Plasma, urine, feces, bile	Parent drug, metabolites
ADC	Metabolite identification	*In vitro*	Plasma, S9, lysosome, tumor cells	Payload-related species
		In vivo	Plasma	Payload-related species
	DAR value determination	*In vitro* and *In vivo*	Plasma	DAR species
Peptide	Metabolite identification	*In vitro*	Plasma, liver microsomes, hepatocytes, kidney S9, kidney homogenate	Parent drug, metabolites
		In vivo	Plasma, urine, feces	Parent drug, metabolites
PDC	Metabolite identification	*In vitro*	Plasma/whole blood, hepatocytes/S9, kidney homogenate, tumor cells	Parent drug, metabolites
		In vivo	Plasma, urine, feces, tissues	Parent drug, metabolites

Case Study

▶Case study of *in vitro* metabolite identification of oligonucleotides

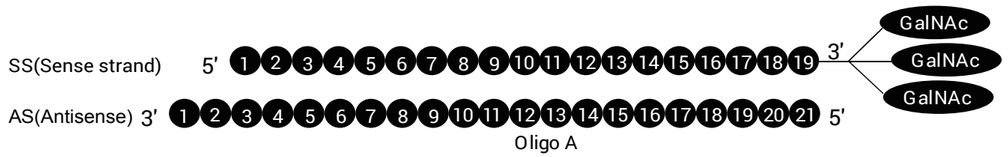


Figure 1. Structure of GalNAc-conjugated siRNAs

Oligonucleotides are usually metabolized by endonuclease and exonuclease rather than CYPs and phase Ⅱ metabolizing enzymes in the liver. Therefore, the corresponding *in vitro* incubation system should be selected for the metabolic study of oligonucleotide. For example, *in vitro* acidic liver S9 and liver homogenate incubation systems can be employed to effectively evaluate the metabolism of liver-targeted oligonucleotide compounds.

As for the analytical technology, liquid-liquid extraction and solid-phase extraction are commonly used to improve extraction recovery and reduce matrix interference. Moreover, a dedicated ion-pair chromatography coupled with high-resolution mass spectrometer (HRMS) (Thermo Q-Exactive™ Plus) system is employed to perform metabolite profiling of oligonucleotides, and professional software, such as Biopharma Finder ™, is used for data processing and metabolite analysis.

Oligo A (siRNA) was studied using an acidified liver S9 of mouse, rat, dog, monkey, and human for incubation of 48 hours. The results show that Oligo A was mainly metabolized through the hydrolysis of nucleic acid chains (exonuclease) and N-acetylgalactose (N-acetylaminoglucosidase) in liver S9. The *in vitro* and *in vivo* metabolite information of Oligo A is illustrated as follows:

Table 2. Metabolites of Oligo A *in vitro* liver S9 and *in vivo* liver

Code	Metabolic change	*In vitro* liver S9					In monkey
		Mouse	Rat	Dog	Monkey	Human	Liver
M1	SS_3'n-1	ND	ND	★	ND	ND	ND
M2	SS-(3GalNAc_Linker)	ND	ND	ND	★	★	★
M3	SS-GalNAc & 5' n-1)	ND	ND	★	★	★	ND
SS	Unchanged SS	★	★	★	★	★	★
M4	SS-GalNAc	★	★	★	★	★	ND
M5	SS-2GalNAc	★	★	★	ND	★	ND
M6	SS-3GalNAc	★	★	★	★	★	★
M7	AS_3' n-2	ND	ND	ND	ND	ND	★
M8	AS_3'n-1	★	★	★	★	★	★
AS	Unchanged AS	★	★	★	★	★	★

★: Detected; ND: Not detected

In addition, an approved drug siRNA-001 was incubated with *in vitro* acidified liver S9, and HRMS data were processed by the specific LC-HRMS system. The Full-scan mass spectrogram (MS), the MS/MS, and fragment elucidation of representative metabolite (AS_3'n-1) are shown below:

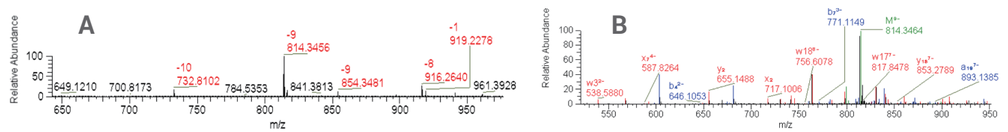


Figure 2. Full-scan MS, MS/MS and fragmentation elucidation of siRNA-001 metabolite AS_3 'n-1 (B)