Case Study

Bioanalytical Lab

Hybrid LBA LC-MS/MS Supporting Preclinical and Clinical Pharmacokinetics Bioanalysis of ADC for Total Antibody and Total ADC

Pharmacokinetic (PK) bioanalysis is an essential part of drug development. Traditionally, ligand binding assays (LBA) have been used to support the quantitative analysis of biotherapeutics and biomarkers in PK bioanalysis. For more than a decade, the Hybrid LBA LC-MS/MS method has been employed for this purpose, offering unique advantages and extensive applications in clinical PK bioanalysis.

PPD™ Laboratory services bioanalytical lab in Suzhou has successfully supported multiple Hybrid LBA LC-MS/MS quantitative assay projects for biotherapeutics and biomarkers in various preclinical and clinical PK studies. For antibody drug conjugate (ADC) bioanalysis, customers only need to provide the ADC drug, and PPD will provide all other necessary reagents.

The Hybrid LBA LC-MS/MS method supports the analysis of total antibodies and total ADCs in the following ways:

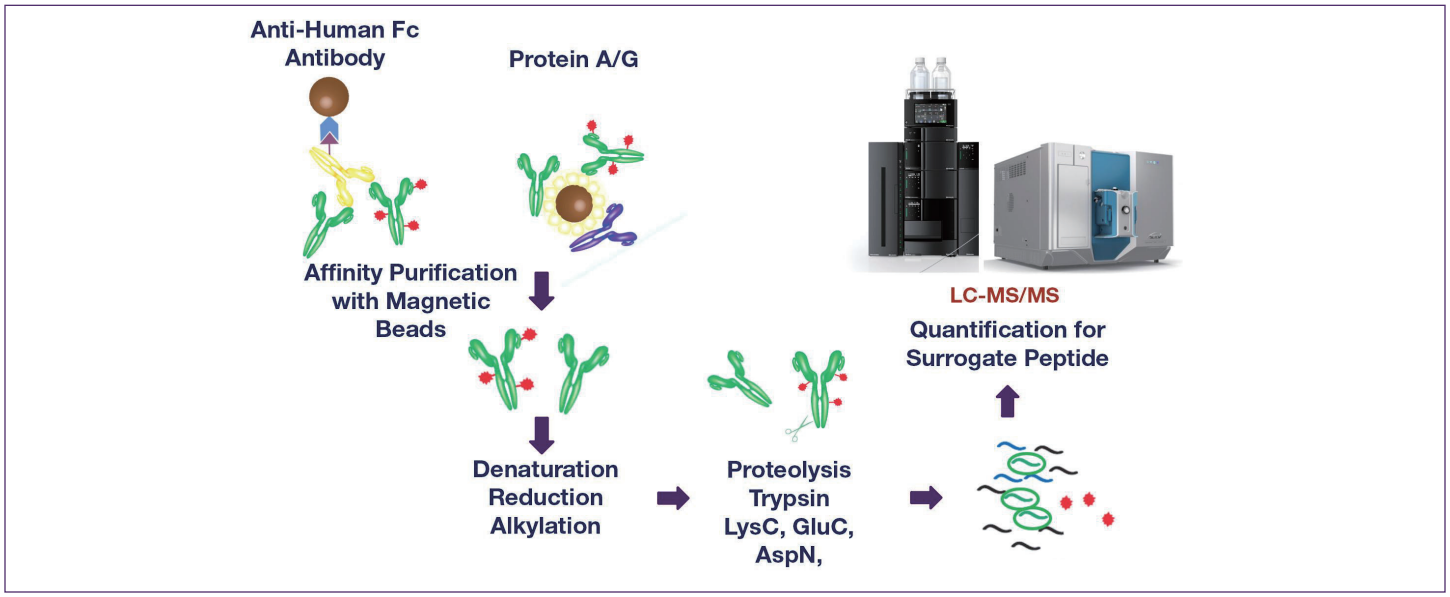
- **No specific antibodies are required from the client.**
- **The method is highly specific.**
- **The method has a short development cycle.**
- **The quantitative method features a wide linear range.**
- **For preclinical programs, a suite of methods can support multi-species PK testing. Antibody substitution and linker modification do not affect the method's performance.**

Two case studies are highlighted below. When specific antibodies for the planned LBA assay were not available within a short time frame, the customer chose the Hybrid LBA LC-MS/MS method to support their preclinical and clinical studies.

Case 1: Preclinical Pharmacokinetics Bioanalysis of Total Antibody and Total ADC from ADC

A generic approach can be applied for the quantification of different mAbs across various pre-clinical species (such as mouse, rat, rabbit, pig, dog, and monkey plasma or serum). The immunoaffinity approach, using magnetic beads coated with anti-human Fc antibody, is employed to enrich samples from monkey plasma. Since ADCs are too large for practical direct quantitative analysis using LC/MS/MS technology, separate the samples into two portions. One portion is used for the Total Antibody assay and is digested with trypsin, while the other portion is used for the Total ADC assay and is hydrolyzed.

Scheme of the workflow for the testing of total antibody from ADC



Scheme of the workflow for the testing of total antibody from ADC

Analyte	Total antibody from ADC
Matrix	Monkey Plasma Dipotassium EDTA
Extraction Type	Immunocapture with Streptavidin Magnetic Beads and Biotinylated Anti-human Fc Antibody
Sample Volume (µL)	20.0 µL
Sample Storage Temperature	-80 °C
Regression, Weighting	Linear, 1/concentration^2
Standard Curve Concentrations	100 to 50000 ng/mL
Platform	Shimadzu LC-40, SCIEX 7500

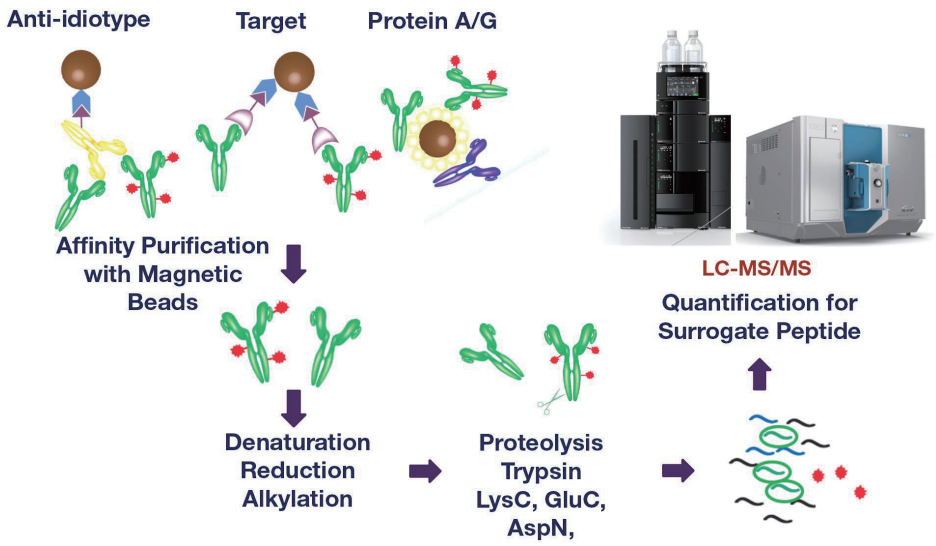
Method summary table for testing of total ADC from ADC

Analyte	Total ADC from ADC
Matrix	Monkey Plasma Dipotassium EDTA
Extraction Type	Immunocapture with Streptavidin Magnetic Beads and Biotinylated Anti-human Fc Antibody
Sample Volume (µL)	20.0 µL
Sample Storage Temperature	-80 °C
Regression, Weighting	Linear, 1/concentration^2
Standard Curve Concentrations	100 to 50000 ng/mL
Platform	Shimadzu LC-40, SCIEX 7500

Case 2: Clinical Pharmacokinetics Bioanalysis of Total Antibody and Total ADC from ADC

For clinical studies, specific antibodies and/or unique surrogate peptides should be used (such as in human plasma and serum). The immunoaffinity approach is used to enrich the ADC drug from human plasma using streptavidin magnetic beads and biotinylated target protein. The bound proteins are subjected to proteolysis using trypsin following standard protein denaturation, reduction, and alkylation processing steps. As a result of the digestion, characteristic peptide fragments originating from the light and heavy chains of the complementarity determining region (CDR) are produced by this procedure.

Scheme of the workflow for the testing of total antibody from ADC



Scheme of the workflow for the testing of total antibody from ADC

Analyte	Total antibody from ADC
Matrix	Human Plasma Dipotassium EDTA
Extraction Type	Immunocapture with Streptavidin Magnetic Beads and Biotinylated Target Protein
Sample Volume (µL)	25.0 µL
Sample Storage Temperature	-80 °C
Regression, Weighting	Linear, 1/concentration^2
Standard Curve Concentrations	100 to 20000 ng/mL
Platform	Shimadzu LC-40, SCIEX 7500

Method summary table for testing of total ADC from ADC

Analyte	Total ADC from ADC
Internal Standard (IS)	IS (SIL payload)
Matrix	Human Plasma Dipotassium EDTA
Extraction Type	Immunocapture and digestion
Sample Volume (µL)	25.0 µL
Sample Storage Temperature	-80 °C
Regression, Weighting	Quadratic, 1/concentration2
Standard Curve Concentrations	100 to 50000 ng/mL
Platform	Shimadzu LC-40, SCIEX 7500

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