

LC-MS/MS MEASUREMENT OF INFLIXIMAB, ADALIMUMAB, USTEKINUMAB, VEDOLIZUMAB AND ANTI DRUG ANTIBODIES USING MABXMISE KITS ON XEVO TQ ABSOLUTE XR MS FOR CLINICAL RESEARCH



G. Hammond¹, D. Fielitz², D. Lebert³, G. Bosch⁴
¹Waters Corporation, Wilmslow, United Kingdom, ²Waters Corporation, Eschborn, Germany . ³Promise Proteomics, Grenoble, France, ⁴Waters S.A.S - St Quentin en Yvelines, France

BACKGROUND

Quantitative measurement of therapeutic monoclonal antibodies and antidrug antibodies is valuable for research on inflammation related biologics. Immunoassay based approaches can be affected by matrix interferences and limited multiplexing, motivating peptide level LC-MS/MS methods with internal standards for improved analytical specificity and reproducibility. This clinical research study describes a research use only workflow incorporating standardized sample preparation kits and tandem quadrupole mass spectrometry for the measurement of four monoclonal antibodies and two antidrug antibodies.

METHODS

Materials

- ITDM2: Multiplex mAb quantification kit (Infliximab, Adalimumab, Ustekinumab, Vedolizumab)
- IADA1: Multiplex anti-drug antibody kit (anti-Infliximab, anti-Adalimumab)

LC-MS/MS Parameters

- ACQUITY™ Premier System
- Waters Peptide CSH™ C18 Column (100 × 2.1 mm, 1.7 μm, 130 Å)
- water/acetonitrile/formic acid gradient
- LC run time was 2.1 minutes
- ESI +ve MRM mode detection on a Waters Xevo™ TQ Absolute XR Mass Spectrometer; capillary 1.0 kV, desolvation temperature 650 °C, and desolvation gas flow 1100 L/h.

Extraction

CALs, QCs, and serum samples were processed using mAbXmise™ kits according to the manufacturer's protocol. Following internal standard addition, monoclonal antibodies were selectively extracted and enriched, matrix components removed, and proteins digested overnight with CutXmise protease at 37 °C to generate signature peptides for analysis (see Figure 1).

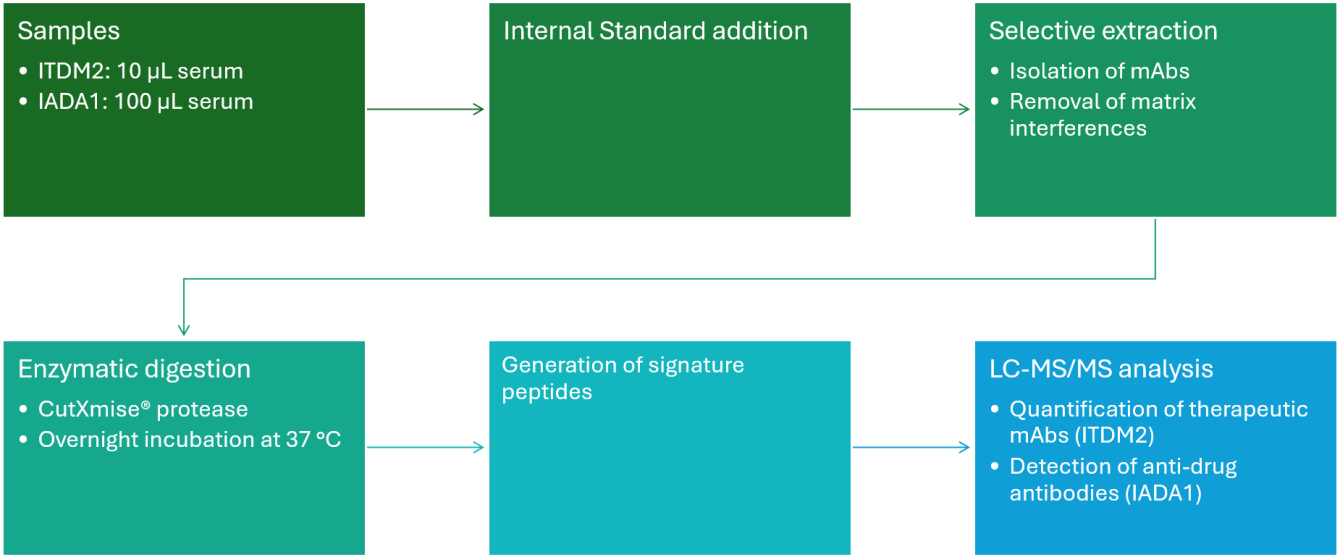


Figure 1. Extraction workflow

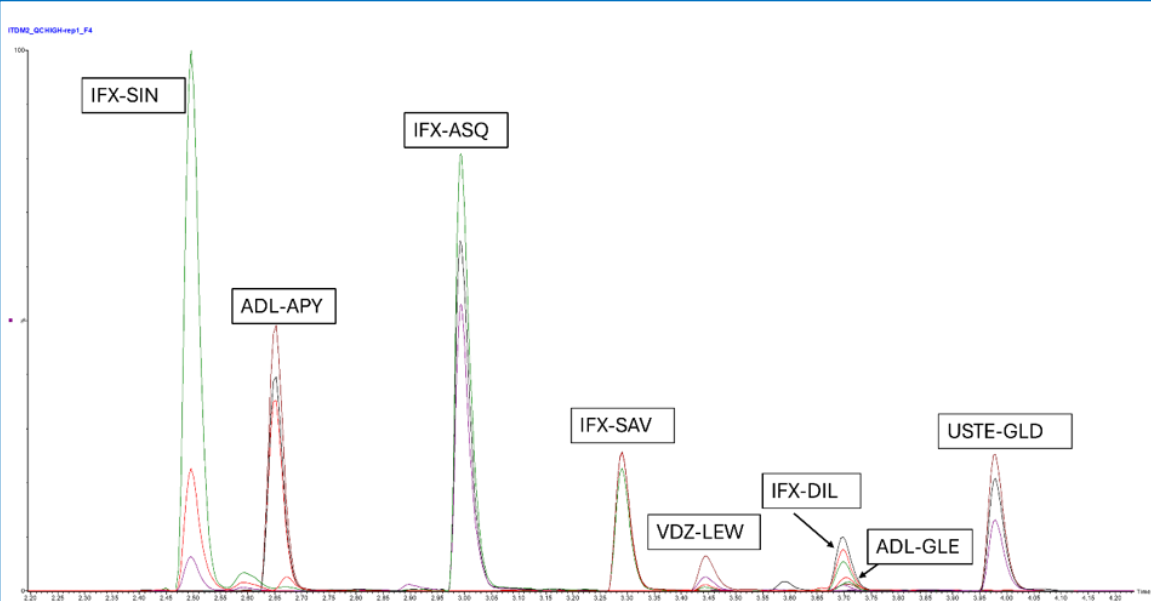


Figure 1: MRM chromatograms of IFX, ADL, USTE and VDZ peptides at 50 μg/mL.

	Concentrations measured in µg/mL								Reference immunoassay	
	mAbXmise + LC-MS/MS									
	IFX-DIL	IFX-SAV	IFX-ASQ	IFX-SIN	ADL-APY	ADL-GLE	Mean 4 peptides INFLIXIMAB	Mean 2 peptides ADALIMUMAB	INFLIXIMAB	ADALIMUMAB
Sample 1	25,3	26,7	26,3	19	<0,5µg/mL	<2µg/mL	24,3	<0,5µg/mL	28	no data
Sample 2	4,1	6,9	6,7	6,5	<0,5µg/mL	<2µg/mL	6,1	<0,5µg/mL	2,2	no data
Sample 3	<2µg/mL	1,2	1,1	1,4	<0,5µg/mL	<2µg/mL	1,2	<0,5µg/mL	<0,5µg/mL	no data
Sample 4	<2µg/mL	<0,5µg/mL	<0,5µg/mL	<0,5µg/mL	<0,5µg/mL	<2µg/mL	<0,5µg/mL	<0,5µg/mL	<0,5µg/mL	no data
Sample 5	<2µg/mL	<0,5µg/mL	<0,5µg/mL	<0,5µg/mL	15,2	13,5	<0,5µg/mL	14,4	no data	10,7
Sample 6	<2µg/mL	<0,5µg/mL	<0,5µg/mL	<0,5µg/mL	5,2	6,8	<0,5µg/mL	6,0	no data	3,6
Sample 7	<2µg/mL	<0,5µg/mL	<0,5µg/mL	<0,5µg/mL	interfered	<2µg/mL	<0,5µg/mL	<2µg/mL	no data	<0,5µg/mL

Table 2: concentration of Adalimumab and Infliximab in μg/mL found in patient samples estimated by mass spectrometry (ITDM2 kit – Promise Proteomics) and Immunoassay.

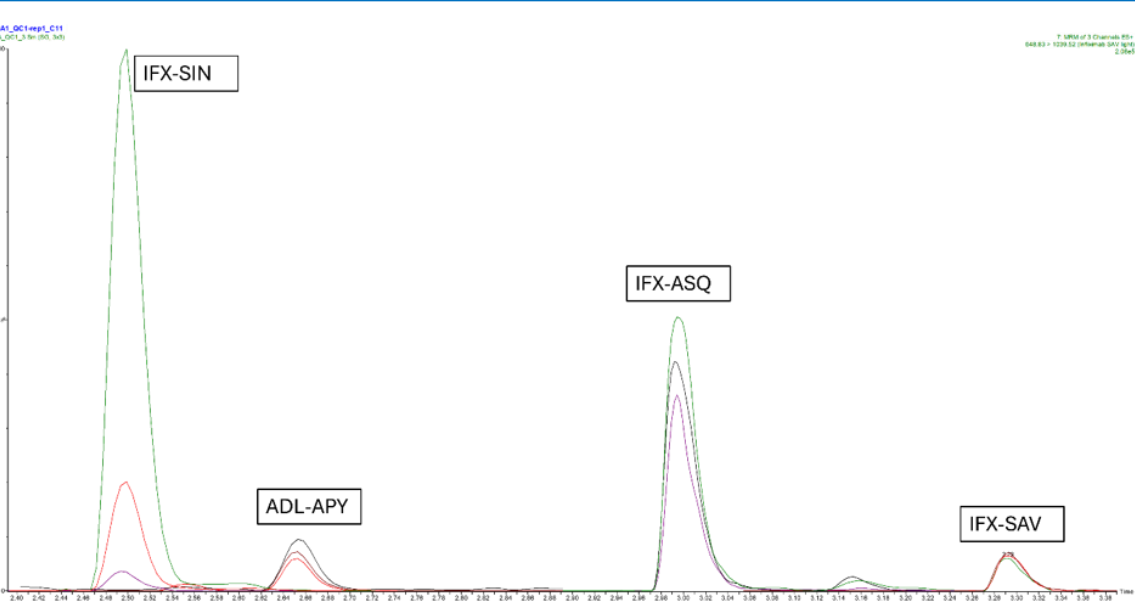


Figure 2: MRM chromatograms of IFX, ADL, USTE and VDZ peptides at 50 μg/mL.

	Concentrations measured in ng/mL							Reference immunoassay	
	mAbXmise + LC-MS/MS								
	Anti-INFLIXIMAB			Anti-ADALIMUMAB	Anti-INFLIXIMAB	Anti-ADALIMUMAB	Anti-INFLIXIMAB	Anti-ADALIMUMAB	
	IFX-SAV	IFX-ASQ	IFX-SIN	ADL-APY					
Sample 1	0	0	0	0	0	0	<2,5	no data	
Sample 2	0	0	0	0	0	0	<2,5	no data	
Sample 3	>400	>400	>400	0	> 400	0	>125	no data	
Sample 4	205,6	147,6	157,0	0	170,1	0	58,9	no data	
Sample 5	<LLOQ	0	0	0	0	0	no data	0	
Sample 6	<LLOQ	0	0	0	0	0	no data	0	
Sample 7	<LLOQ	0	0	> 200	0	> 200	no data	>125	

Table 3: concentration of anti-IFX and anti-ADL in μg/mL found in patient samples estimated by mass spectrometry (IADA1 kit – Promise Proteomics) and Immunoassay.

RESULTS

Analytical Performance

Parameter	ITDM2 (Drug mAbs)	IADA1 (Anti-drug Antibodies)
Analytes	IFX, ADL, UST, VDZ	Anti-IFX, Anti-ADL
Calibration range	0.5 – 100 μg/mL	10 – 400 ng/mL
Calibration model	Linear	4PL (sigmoidal)
Linearity / Curve fit	r ² > 0.99, within ±15%	4PL model (non-linear, sigmoidal response)
Precision (RSD)	< 10%	< 10%
Accuracy	94.2 – 114.2%	90.5 – 105.6%
LOQ	TDM range	Reported per peptide

Table 1: Analytical performance of drug mAbs and anti-drug antibodies methods.

Chromatographic specificity

- Robust chromatographic separation enabled clear resolution of signature peptides for all targeted monoclonal antibodies and anti-drug antibodies enabling reliable integration and quantification as shown in Figures 1 and Figure 2 for ITDM2 Kit mAbs (IFX, ADL, USTE and VDZ) and IADA1 kit anti-drug antibodies (anti-IFX and anti-ADL).
- Peptide-level detection combined with MRM transitions ensured high analytical selectivity, minimizing interference from endogenous serum components. Simultaneous detection of multiple antibodies was achieved without cross-talk between transitions.

Calibration

Example calibration curves

- Infliximab (IFX, ITDM2) - Figure 3a
Demonstrated excellent linear response (r² > 0.99) across the calibration range (0.5–100 μg/mL) with all points within ±15%.

- Anti-Infliximab (Anti-IFX, IADA1) - Figure 3b
Calibration followed a 4-parameter logistic (4PL) curve (detection over 10–400 ng/mL), consistent with ligand-binding assay behaviour.

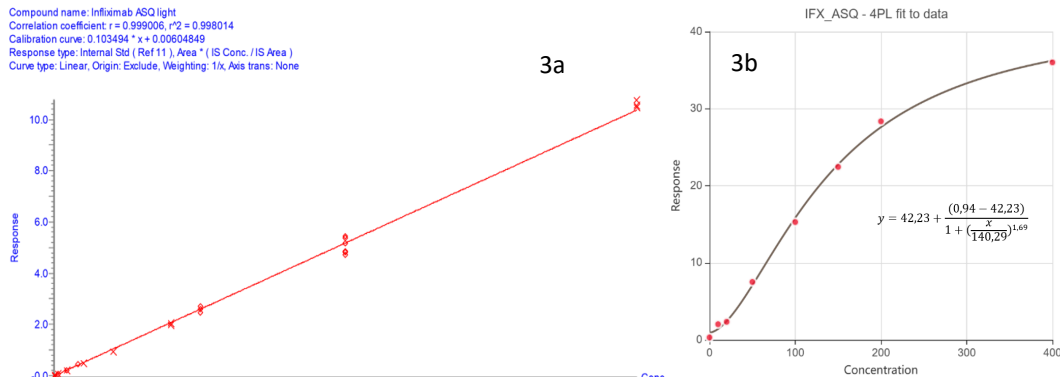


Figure 3: 3a is linear calibration for IFX-ASQ peptide (ITDM2) and 3b is 4PL curve for Anti-IFX-ASQ peptide.

Clinical sample analysis

- Seven patient samples (Infliximab or Adalimumab-treated) were analysed using the ITDM2 kit and compared with immunoassay results (Table 2). Good agreement was observed between mAbXmise + LC-MS/MS method and immunoassay, with expected method-dependent differences consistent with published literature.
- Parallel analysis with the IADA1 kit identified anti-drug antibodies in three patients (two IFX, one ADL), these results are consistent with ELISA results (Table 3).
- It should be noted that some of the LC-MS/MS concentrations exceeded the upper limit of quantification (ULOQ).
- The LC-MS/MS method performance showed good agreement with immunoassays for drug quantification and successfully detected anti-drug antibodies in patient samples.

CONCLUSION

Utilising the PROMISE Proteomics sample preparation kits and the robust, sensitive detection of the ACQUITY Premier System with Xevo TQ Absolute XR Mass Spectrometer

- Reliable quantification: The LC-MS/MS workflow demonstrated high sensitivity, precision (RSD <10%), and accuracy, supporting reliable multiplex quantification of mAbs and ADAs.
- Strong clinical agreement: Drug levels and ADA detection demonstrated good agreement with established immunoassays, confirming suitability for therapeutic drug monitoring.
- Efficient and scalable solution: The combination of standardised mAbXmise kits and LC-MS/MS enables rapid implementation, high specificity, and multiplex capability, offering a practical alternative to conventional assays.

For Research Use Only. Not for use in diagnostic procedures.

©2026 Waters Corporation

Waters, Xevo, ACQUITY and CSH are trademarks of Waters Corporation or its affiliates. mAbXmise is a trademark of Promise Proteomics. All other marks are the property of their respective owner.