Clinical Research

Effortless and Streamlined Workflow for Absolute Quantitation of Therapeutic Monoclonal Antibodies using Promise Proteomics mAbXmise kits and TSQ Altis Plus Mass Spectrometer

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Abstract

Purpose: To simultaneously quantify the signature peptides of multiple therapeutic monoclonal antibodies in human serum in a streamlined and confident way using Promise Proteomics kits and the TSQ Altis™ Plus mass spectrometer.

Methods: Samples were purified and trypsin digested according to the instructions provided by Promise Proteomics with minor modifications (refer to instructions manual – https://customer.mabxmise.com). LC-MS/MS analysis was performed using Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to TSQ Altis™ Plus mass spectrometer.

Results: Successfully completed process replicate analysis and LC-MS/MS analytical performance evaluation and passed acceptance criteria according to the collaboration study plan.

Introduction

Laboratories continuously seek improved productivity and efficacy for clinical testing, ultimately impacting the turn-around time and sample throughput. Various efforts have been made including rapid and high-throughput testing, automation, and high-end instrumentation. In particular, mass spectrometry (MS) has gained significant popularity in clinical laboratories for TDM of mAbs due to its great versatility to measure such complex biological proteins qualitatively and quantitatively. Here we present the streamlined workflow for the absolute quantitation of therapeutic mAbs using the Promise Proteomics mAbXmise kits and the Thermo Scientific™ TSQ Altis™ Plus mass spectrometer.

Materials and methods

Sample Preparation

The workflow is described in Figure 1 including the plate scheme and the list of kits is below.

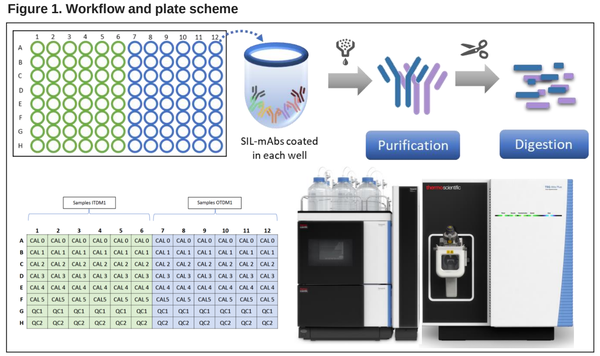
• ITDM1: inflammation-TDM kit 1 for quantitation of 2 mAbs including adalimumab and infliximab (mAbXmise® ITDM1, Promise Proteomics, France)

• OTDM1: oncology-TDM kit 1 for quantitation of 7 mAbs including bevacizumab, cetuximab, ipilimumab, nivolumab, pembrolizumab, rituximab, and trastuzumab (mAbXmise® OTDM1, Promise Proteomics, France)

Test Method(s)

LC-MS/MS analysis was performed using Thermo Scientific™ Vanquish™ Flex UHPLC system coupled to TSQ Altis™ Plus mass spectrometer. LC and MS conditions are listed in Table 1. Transitions can be found in Tech Note (TN001753). After sample preparation, each wall was injected once for process replicate analysis. The same calibrator and QCs were pooled and redistributed for analysis of LC-MS/MS analytical performance in triplicate.

Figure 1. Workflow and plate scheme



Data Analysis

Data acquisition, processing, and reporting were performed using Thermo Scientific™ TraceFinder™ 5.1 software and the evaluation criteria for LC-MS analytical performance are listed in Table 2.

Table 1. LC and MS conditions

LC gradient			
Time (min)	**% A**	**% B**	**Curve**
0.0	95	5	5
0.5	95	5	5
1.0	80	20	5
4.8	60	40	5
5.5	50	50	5
6.1	10	90	5
7.5	10	90	5
7.6	95	5	5
10	95	5	5
Separation conditions			
Mobile phase A	0.1 % formic acid in water		
Mobile phase B	0.1 % formic acid in 10: 10: 80 water: isopropanol: acetonitrile (v/v/v)		
Flow rate	0.25 mL/min		
Column temperature	40 °C (Still Air)		
Injection volume	10 µL (5 µL for full-process replate analysis)		
Global parameters			
Source type	Heated electrospray ionization (H-ESI)		
Polarity	Positive		
Spray voltage (V)	3500		
Sheath gas (Arb)	40		
Aux gas (Arb)	7		
Sweep gas (Arb)	1		
Ion transfer tube temp (°C)	325		
Vaporizer temp (°C)	275		
Divert valve A	0.0 min: position 1-6 (waste) 0.6 min: position 1-2 (MS) 6.2 min: position 1-6 (waste)		
Probe position (x-y-z)	Center - 1.5 - LM		
SRM scan parameters			
Cycle time (sec)	0.35		
Q1 resolution (FWHM)	0.7		
Q3 resolution (FWHM)	0.7		
CID gas (mTorr)	1.5		
Source fragmentation (V)	0		
Chromatographic peak width (sec)	6		
RF lens (V)	60		
Dwell time priority	3 (normal)		

Table 2. Evaluation criteria for LC-MS/MS analytical performance

Analytical characteristics	Acceptance criteria
QC accuracy	Mean concentration from 85 to 115 % for QC1 and QC2 samples
QC precision	CV ≤ 15 % for QC1 and QC2 samples
Lower limit of quantification (LLOQ)	• The analyte mean response at the LLOQ (CAL1) > 5 times the analyte response of the zero calibrator (CAL0 = blank sample).
	• LLOQ should be 2 µg/mL
	• Accuracy: mean concentration from 80 to 120 %
	• Precision: CV ≤ 20 %
Linear range	Linear fit: R^2 ≥ 0.99 on the expected linear range 2 ~ 100 µg/mL

Results

The highly comparable results were observed across all six replicates for both OTDM1 and ITDM1 kits with great linearity with R^2 > 0.994 and % RSD <15, indicating the reproducibility of the full plate sample preparation. Then, the six replicates of each calibrator and QC from each kit were pooled together, distributed to another plate following the same plate scheme, and analyzed in triplicates, generating 18 data points for each calibrator and QC sample. We successfully evaluated the LC-MS/MS analytical performance, showing excellent linearity with R^2 > 0.997 and % RSD <10 (Figure 3 and Tables 3 and 4). Additionally, Figure 4 shows extremely reproducible retention times of all SIL-mAb peptides with RT difference ± 0.03 minutes during the evaluation.

Figure 2. Representative XICs of OTDM1 and ITDM1 peptides

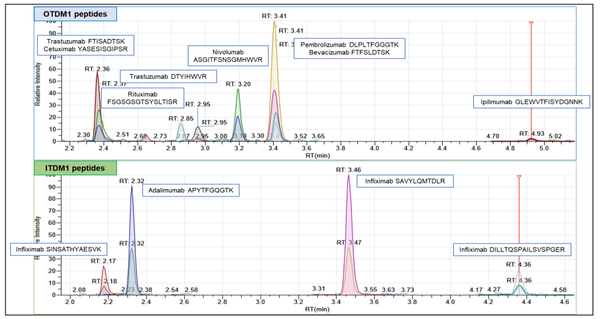


Figure 3. Calibration curves for OTDM1 (blue) and ITDM1 (green) peptides (#data points=18)

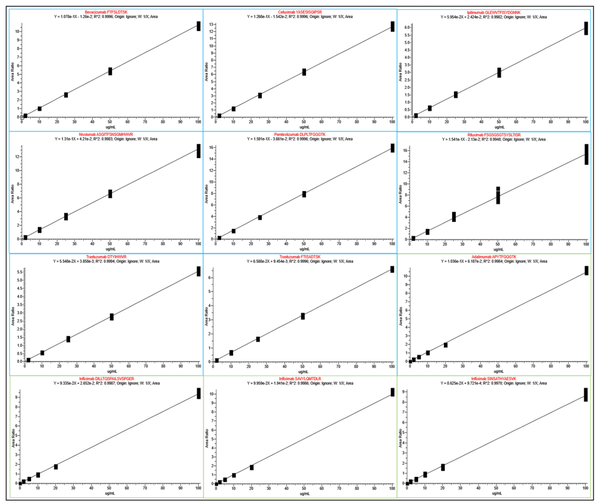


Table 3. Results of LC-MS/MS analytical performance of ITDM1 peptides

Analytical characteristics	QC accuracy		QC precision		LLOQ				Linear range
Peptide	QC1	QC2	QC1	QC2	CAL0 response (5 x CAL0)	CAL1 response	Accuracy	Precision	R^2 ≥ 0.99
Adalimumab APYTFGQGTK	107.0%	95.2%	3.1%	1.9%	0.050 (0.250)	0.267	99.0%	3.2%	0.9984
Infliximab SINSATHYAESVK	99.8%	100.4%	8.7%	3.7%	0.002 (0.010)	0.193	111.6%	8.4%	0.9970
Infliximab SAVYLQMTDLR	103.0%	98.7%	2.6%	1.9%	0.016 (0.080)	0.219	100.2%	2.1%	0.9986
Infliximab DILLTQSPAILSVSPGER	96.4%	96.2%	2.8%	2.6%	0.024 (0.120)	0.223	105.4%	6.0%	0.9987

Table 4. Results of LC-MS/MS analytical performance of OTDM1 peptides

Analytical characteristics	QC accuracy		QC precision		LLOQ				Linear range
Peptide	QC1	QC2	QC1	QC2	CAL0 response (5 x CAL0)	CAL1 response	Accuracy	Precision	R^2 ≥ 0.99
Bevacizumab FTFSLDTSK	101.1%	102.2%	1.9%	1.6%	0.009 (0.043)	0.138	98.3%	3.8%	0.9996
Cetuximab YASESISGIPSR	97.3%	96.6%	1.5%	1.8%	0.003 (0.015)	0.243	98.3%	3.6%	0.9996
Ipilimumab GLEWVTFISYDGNNK	95.0%	101.2%	6.1%	2.9%	0.009 (0.045)	0.136	106.3%	7.4%	0.9982
Nivolumab ASGITFSNSGMHWVR	95.2%	94.0%	3.3%	3.2%	0.040 (0.200)	0.297	102.6%	6.2%	0.9983
Pembrolizumab DLPLTFGGGTK	100.3%	100.0%	2.0%	1.4%	0.014 (0.070)	0.294	96.0%	1.8%	0.9996
Rituximab FSGSGSGTSYSLTISR	101.9%	105.3%	6.4%	5.9%	0.002 (0.010)	0.285	100.8%	10.6%	0.9971
Trastuzumab DTYIHWVR	101.2%	101.3%	3.1%	2.3%	0.010 (0.050)	0.116	99.1%	3.9%	0.9994
Trastuzumab FTISADTSK	100.2%	99.4%	1.8%	0.8%	0.028 (0.140)	0.177	102.6%	4.4%	0.9996

Figure 4. Variation in observed retention times of all SIL-mAb peptides during the evaluation of LC-MS analytical performance