Technical note | 001753

Clinical research

Streamlined workflow for absolute quantitation of therapeutic monoclonal antibodies using Promise Proteomics mAbXmise kits and a TSQ Altis Plus mass spectrometer

Authors

Yvonne E. Song[1], Dorothee Lebert[2], Jean-Valéry Guillaubez[2], Stephanie Samra[1], Edward Goucher[1], Bradley Hart[1]

[1]Thermo Fisher Scientific, San Jose, CA, United States

[2]Promise Proteomics, Grenoble, France

Keywords

TSQ Altis Plus mass spectrometer, Vanquish Flex UHPLC system, Hypersil GOLD C18 column, TraceFinder software, monoclonal antibodies, therapeutic drug monitoring, mAbs, TDM, Promise Proteomics, mAbXmise, OTDM, ITDM, LC-MS, mass spectrometry, adalimumab, infliximab, bevacizumab, cetuximab, ipilimumab, rituximab, nivolumab, pembrolizumab, trastuzumab

Application benefits

- Streamlined workflow and minimum up-front development efforts using the first commercial ready-to-use kits for quantitative analysis of mAbs, mAbXmise™ from Promise Proteomics, that provide all necessary reagents including stable isotope-labeled mAbs
- Ultimate quantitative performance achieved by a Thermo Scientific™ TSQ Altis™ Plus mass spectrometer, offering maximum sensitivity and productivity for routine protein quantitation
- Reliable and robust workflow for quantitative analysis of nine mAbs achieving excellent linearity with $R^2 > 0.997$ from 2 to 100 µg/mL, precision < 11% CV, and accuracy between 96 and 112% at LLOQ

Goal

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.

Introduction

Laboratories continuously seek improved productivity and efficacy for clinical research testing, ultimately impacting the turn-around time and sample throughput. Various efforts have been made to enable a laboratory to meet its business and profitability objectives, including rapid and high-throughput testing, automation, and high-end instrumentation. This also applies to therapeutic drug monitoring (TDM) during patient therapy. A TDM approach uses the quantitative measurement of the concentration of drugs in human blood, plasma, or serum to optimize dosing within the therapeutic range, such

thermo scientific

as therapeutic monoclonal antibodies (mAbs). In particular, mass spectrometry (MS) has gained significant popularity in clinical laboratories for quantitative analysis of mAbs due to its great versatility to measure highly complex biological proteins qualitatively and quantitatively.[1]

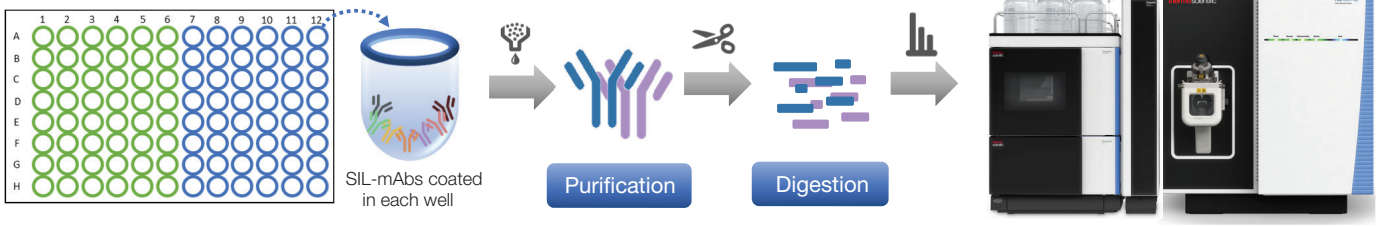
Automation or high-throughput assays have also emerged to provide a more user-friendly and streamlined workflow for MS-based quantitative analysis of mAbs such as the mAbXmise kits from Promise Proteomics (related patents numbers are US11543416, US11053303, US11630111, EP3165828, EP3165922, and EP3457139).[2,3] The mAbXmise kit is the first commercially available, ready-to-use kit that offers all the necessary reagents for the LC-MS/MS assay including the stable-isotope labeled (SIL) proteins of the target mAbs (SIL-mAbs). Often, the internal standards or SIL-mAbs are not available or do not meet the expected quality, which remained a noticeable challenge during assay development. The mAbXmise kits include the SIL-mAbs and come CE-IVD marked, meeting the quality and regulatory requirements of medical devices for *in vitro* diagnostics. The kits also provide written procedures and initial instrumental parameters including target peptide sequences so there is a minimal level of up-front method development effort needed by the end user.

Here we present a streamlined workflow for the absolute quantitation of therapeutic mAbs using the Promise Proteomics mAbXmise kits and the TSQ Altis Plus mass spectrometer. The kits used in this study include OTDM1 and ITDM1 kits used for oncology and inflammation disease treatment, respectively. The TSQ Altis Plus mass spectrometer is the latest generation of the TSQ MS series, offering superior acquisition speeds, sensitivity, selectivity, and robustness for routine and high-volume targeted protein quantitation.

Experimental

The workflow is described in Figure 1A, and more details are provided in the sections below. The experimental workflow followed the instructions for use (IFU) provided by Promise Proteomics with minor modifications (refer to instructions manual – https://customer.mabxmise.com). The kits used in this study include pre-coated plates with a full-length SIL-mAb corresponding to each target mAb sequence as internal standards with purity >95% and isotopic incorporation >98%. Thus, the ITDM1 kit contains two SIL-mAbs and the OTDM1 kit contains seven SIL-mAbs pre-coated in each plate. The kits also provide buffer stocks, reagents, six calibration standards including zero (CAL0 to CAL5), and two QC controls. Each kit provides 12 strips capable of preparing 96 samples. The calibrators and QC samples were added to the mAbXmise plate and subjected to immunocapture followed by trypsin digestion. The plate scheme is described in Figure 1B.

A



B

	Samples ITDM1						Samples OTDM1					
	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL 0	CAL 0	CAL 0	CAL 0	CAL 0	CAL 0	CAL 0	CAL 0	CAL 0	CAL 0	CAL 0	CAL 0
B	CAL 1	CAL 1	CAL 1	CAL 1	CAL 1	CAL 1	CAL 1	CAL 1	CAL 1	CAL 1	CAL 1	CAL 1
C	CAL 2	CAL 2	CAL 2	CAL 2	CAL 2	CAL 2	CAL 2	CAL 2	CAL 2	CAL 2	CAL 2	CAL 2
D	CAL 3	CAL 3	CAL 3	CAL 3	CAL 3	CAL 3	CAL 3	CAL 3	CAL 3	CAL 3	CAL 3	CAL 3
E	CAL 4	CAL 4	CAL 4	CAL 4	CAL 4	CAL 4	CAL 4	CAL 4	CAL 4	CAL 4	CAL 4	CAL 4
F	CAL 5	CAL5	CAL 5	CAL5	CAL 5	CAL 5	CAL5	CAL 5	CAL5	CAL 5	CAL5	CAL 5
G	QC1	QC1	QC1	QC1	QC1	QC1	QC1	QC1	QC1	QC1	QC1	QC1
H	QC2	QC2	QC2	QC2	QC2	QC2	QC2	QC2	QC2	QC2	QC2	QC2

Figure 1. Workflow and plate scheme

Reagent kits

- ITDM1: inflammation-TDM kit 1 for quantitation of two mAbs including adalimumab and infliximab (mAbXmise ITDM1, Promise Proteomics, France)
- OTDM1: oncology-TDM kit 1 for quantitation of seven mAbs including bevacizumab, cetuximab, ipilimumab, nivolumab, pembrolizumab, rituximab, and trastuzumab (mAbXmise OTDM1, Promise Proteomics, France)

Sample preparation

Buffers A and B were prepared according to the protocol when the kits were opened for the first time. An aliquot of 80 µL of Buffer A was added to each well of the mAbXmise plate followed by the addition of 20 µL of six calibrators from CAL0 to CAL5 and two QC samples. The concentration of the calibrators and QC samples are listed in Table 1 for each kit. The plate was sealed with an adhesive aluminum foil before incubation for 1 hour at room temperature with agitation at 450 rpm.

Table 1. Concentrations of the calibration and QC samples for OTDM1 and ITDM1 kits

Name	Concentration (µg/mL)	
	OTDM1	ITDM1
CAL0	0	0
CAL1	2	2
CAL2	10	5
CAL3	25	10
CAL4	50	20
CAL5	100	100
QC1	15	4
QC2	75	25

Sample purification

The PuriXmise plate was washed with 200 µL of Buffer A prior to the sample loading. The bottom of the PuriXmise plate was sealed with an adhesive aluminum foil and the samples were added to the plate followed by incubation for 1 hour at room temperature with agitation at 450 rpm. The plate was then washed with 200 µL of Buffer A three times. After the collection plate was placed on the bottom of the PuriXmise plate, 100 µL of Buffer B was added to each well. The plate was sealed with an adhesive aluminum foil and incubated for 10 minutes at room temperature with agitation at 450 rpm. The eluates were collected in the collection plate and this elution step was repeated one more time. The eluates were dried before digestion.

Sample digestion

The samples were resuspended by adding 50 µL of Buffer A. (If the color changes to yellow at this step, the NeutralX buffer should be added to adjust pH for trypsin digestion.) An aliquot of 200 µL of CutX buffer was added to a vial of CutXmise for reconstitution. An aliquot of 5 µL of CutXmise was added to the samples and the plate was sealed with an adhesive aluminum foil for overnight incubation at 37 ºC with agitation at 450 rpm. The plate was centrifuged shortly before removing the adhesive aluminum foil. The digestion was quenched by adding 5 µL of CutX Stop solution. (The sample color should turn yellow.) The final volume was adjusted to 80 µL using mobile phase A. Each well was analyzed once to evaluate the full-process replicates and confirm the complete sample preparation in each well. 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 for the assessment of LC-MS analytical performance.

Liquid chromatography

LC separation was performed using a Thermo Scientific™ Vanquish™ Flex UHPLC system with a Thermo Scientific™ Hypersil™ GOLD C18 column (2.1 x 50 mm, 1.9 µm, P/N 25002-052130). The LC gradient and other separation conditions are described in Table 2.

Mass spectrometry

Analysis was performed on a TSQ Altis Plus mass spectrometer with settings described in Table 2. Final SRM transitions of the target peptides are listed in Table 3.

Data acquisition and processing

Data acquisition, processing, and reporting were performed using Thermo Scientific™ TraceFinder™ software, version 5.1. The evaluation criteria for LC-MS analytical performance are listed in Table 4.

Table 2. 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 replicate analysis)		
Global MS parameters			
Source type	Heated electrospray ionization (H-ESI)		
Polarity	Positive		
Spray voltage (V)	3,500		
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 (s)	0.35		
Q1 resolution (FWHM)	0.7		
Q3 resolution (FWHM)	0.7		
CID gas (mTorr)	1.5		
Source fragmentation (V)	0		
Chromatographic peak width (s)	6		
RF lens (V)	60		
Dwell time priority	3 (normal)		

Table 3. List of SRM transitions

A: Transition list of OTDM1 peptides

mAb	Peptide sequence	Retention time (min)	Precursor *m/z*		Product *m/z*		CE (V)
			Endogenous peptide	SIL peptide	Endogenous peptide	SIL peptide	
Bevacizumab	FTFSLDTSK	3.4	523.264	527.271	563.30	571.32	19
					650.34	658.35	
					797.40	805.42	
					898.45	906.47	
Cetuximab	YASESISGIPSR	2.4	633.820	638.824	616.34	626.35	22
					729.43	739.43	
					1032.53	1042.54	
Ipilimumab	GLEWVTFISYDGNNK	4.9	871.923	875.930	797.34	805.36	30
					910.43	918.44	
					1158.54	1166.56	
Nivolumab	ASGITFSNSGMHWVR	3.2	550.600	553.936	610.79	615.79	17
					661.31	666.31	
					746.36	751.37	
Pembrolizumab	DLPLTFGGGTK	3.4	553.298	557.305	439.24	443.25	20
					667.34	675.36	
					877.48	885.49	
Rituximab	FSGSGSGTSYSLTISR	2.6	803.889	808.893	926.49	936.50	28
					1084.56	1094.57	
					1171.60	1181.60	
Trastuzumab	DTYIHWVR	2.8	545.278	550.282	597.33	607.33	22
					710.41	720.42	
					873.47	883.48	
	FTISADTSK	2.4	485.248	489.255	608.29	616.30	18
					721.37	729.39	
					822.42	830.43	

B: Transition list of ITDM1 peptides

mAb	Peptide sequence	Retention time (min)	Precursor *m/z*		Product *m/z*		CE (V)
			Endogenous peptide	SIL peptide	Endogenous peptide	SIL peptide	
Adalimumab	APYTFGQGTK	2.3	535.269	539.276	637.33	645.35	22
					738.38	746.39	
					901.44	909.46	
					998.49	1006.51	
Infliximab	SINSATHYAESVK	2.2	469.568	472.239	546.77	607.33	20
					603.79	720.42	
					833.42	883.48	
					934.46	942.48	
	SAVYLQMTDLR	3.5	648.834	653.838	504.28	514.29	23
					763.38	773.39	
					876.46	886.47	
					1138.59	1148.60	
	DILLTQSPAILSVSPGER	4.4	632.686	636.022	644.34	654.34	28
					731.37	741.38	
					844.45	854.46	
					1028.57	1038.58	

Table 4. Evaluation criteria for LC-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) is at least 5 times higher than 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 µg/mL ~ 100 µg/mL

Results and discussion

LC-MS method optimization

LC and MS conditions including LC gradient and SRM transitions were optimized using the Starter Kit TDM (P/N STAKIT01) prior to the evaluation. The Starter Kit TDM offers a mix of digested mAbs that only needs reconstitution before LC-MS injection. The final SRM transitions should be confirmed and optimized further by analyzing the calibrators CAL0 and CAL1 containing human serum to avoid interferences from co-eluting peptides. For example, the DILLTQSPAILSVSPGER peptide from infliximab was measured at a precursor charge state of 3 since its precursor at a charge state of 2 showed a high interference from a co-eluting peptide with a similar precursor *m/z* value, which could cause one of the criteria for the CAL0 and CAL1 responses to fail. Re-optimization of targeted transitions improved selectivity by removing interferences (data not shown). The final LC-MS/MS method resulted in a separation of all the target peptides across the LC gradient as shown in Figure 2. The upper panel represents XICs of OTDM1 peptides while the lower panel represents XICs of ITDM1 peptides.

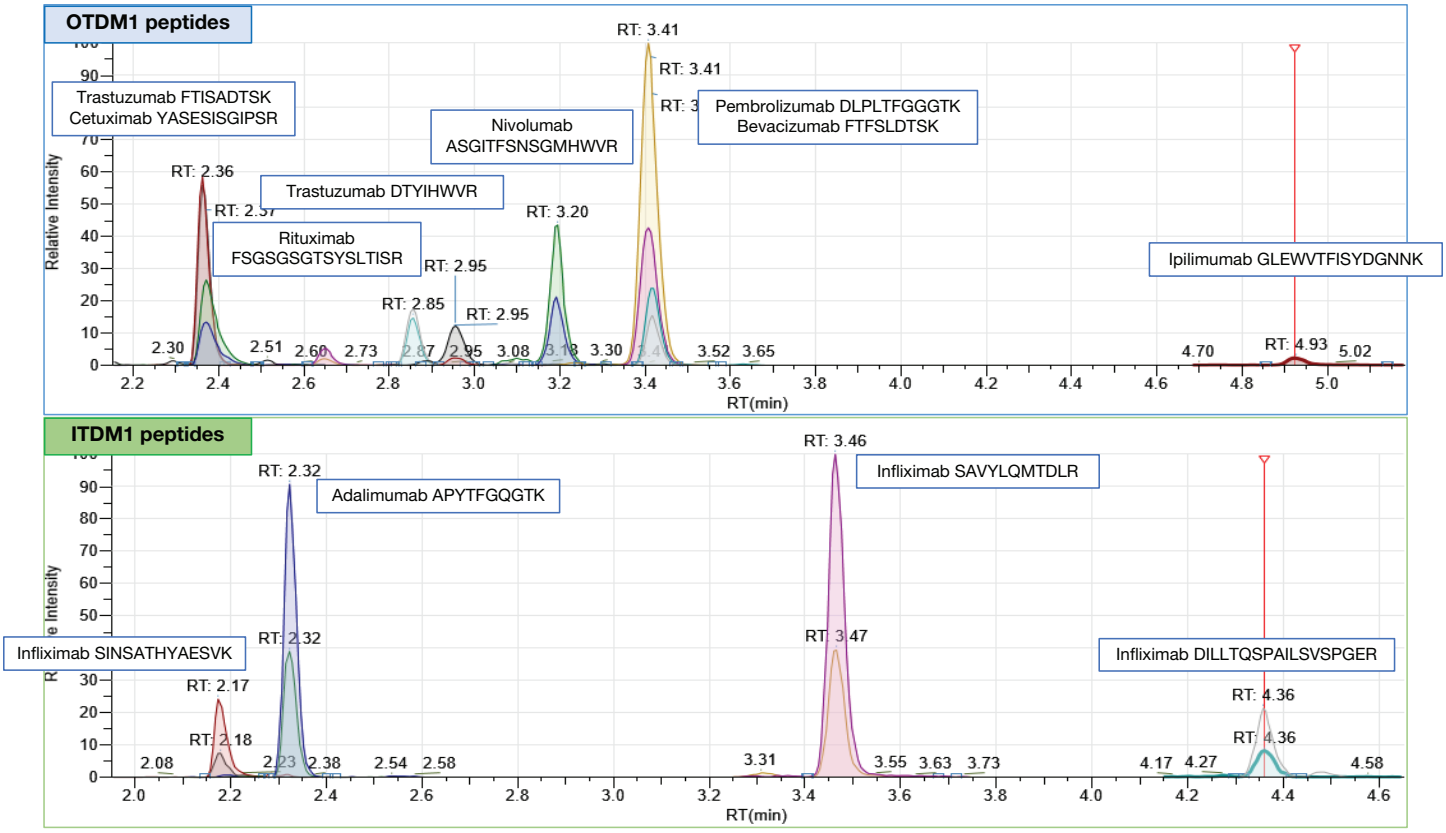


Figure 2. Representative XICs of OTDM1 and ITDM1 peptides

Evaluation of full-process replicates

The entire plate was successfully processed as shown in Figure 3. The highly comparable results were observed across all six replicates for both OTDM1 and ITDM1 kits. Figures 3A (OTDM1) and 3C (ITDM1) show reproducible area ratios and great linearity with $R^2 > 0.994$. Furthermore, the observed peak areas of all the SIL-mAb peptides support the reproducibility of the full plate sample preparation, with %RSD < 15 as shown in Figures 3B (OTDM1) and 3D (ITDM1). It should be noted that the injection volume was 5 µL, which was lower than recommended injection volumes, and was analyzed once for a quick check on sample preparation prior to the complete evaluation of LC-MS analytical performance. Nonetheless, the TSQ Altis Plus mass spectrometer generated very sensitive and reproducible detection of all the target peptides across the full plate.

3A

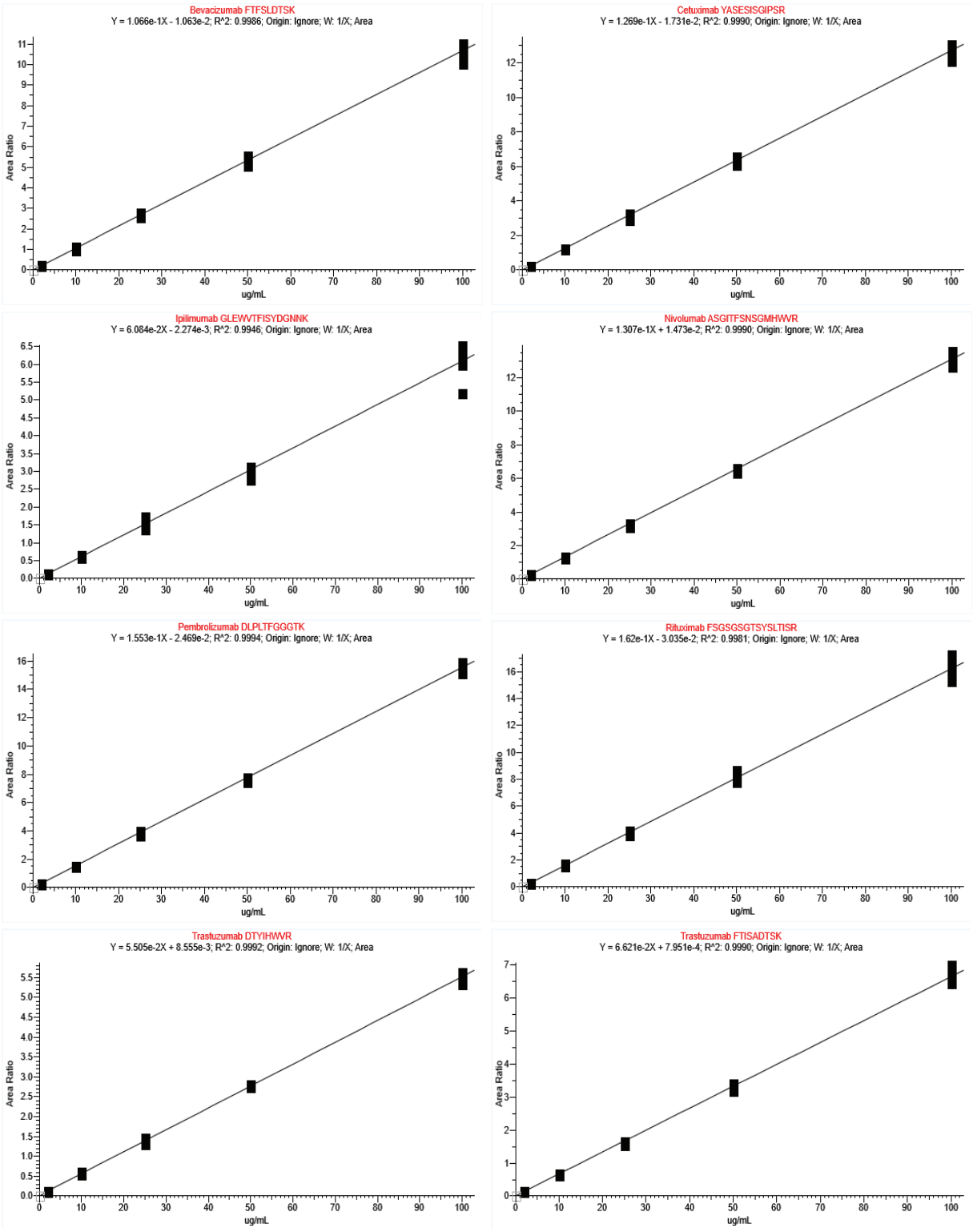


Figure 3. Results of measurement for full-process replicates including calibration curve for OTDM1 target peptides (A)

3B

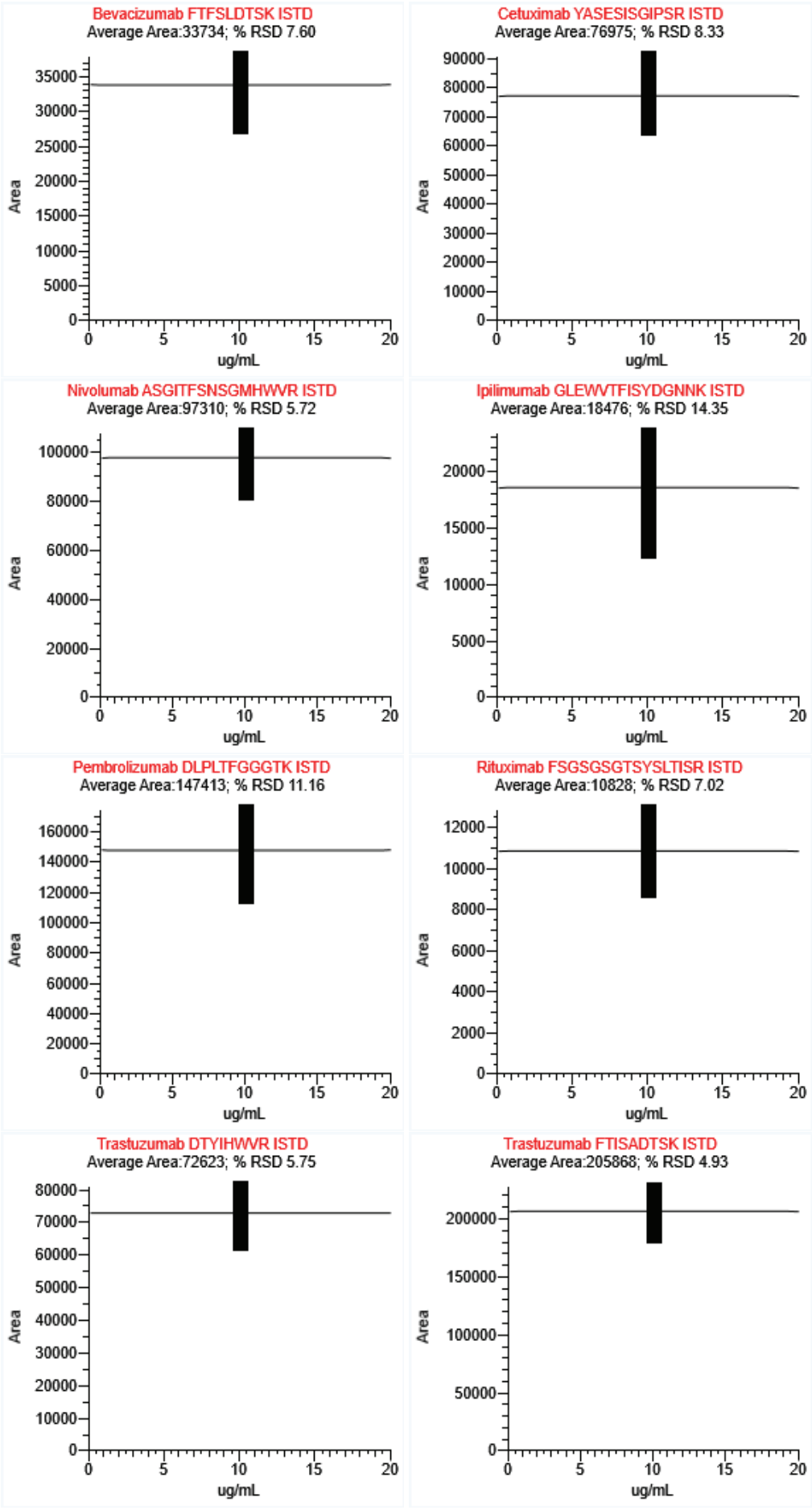
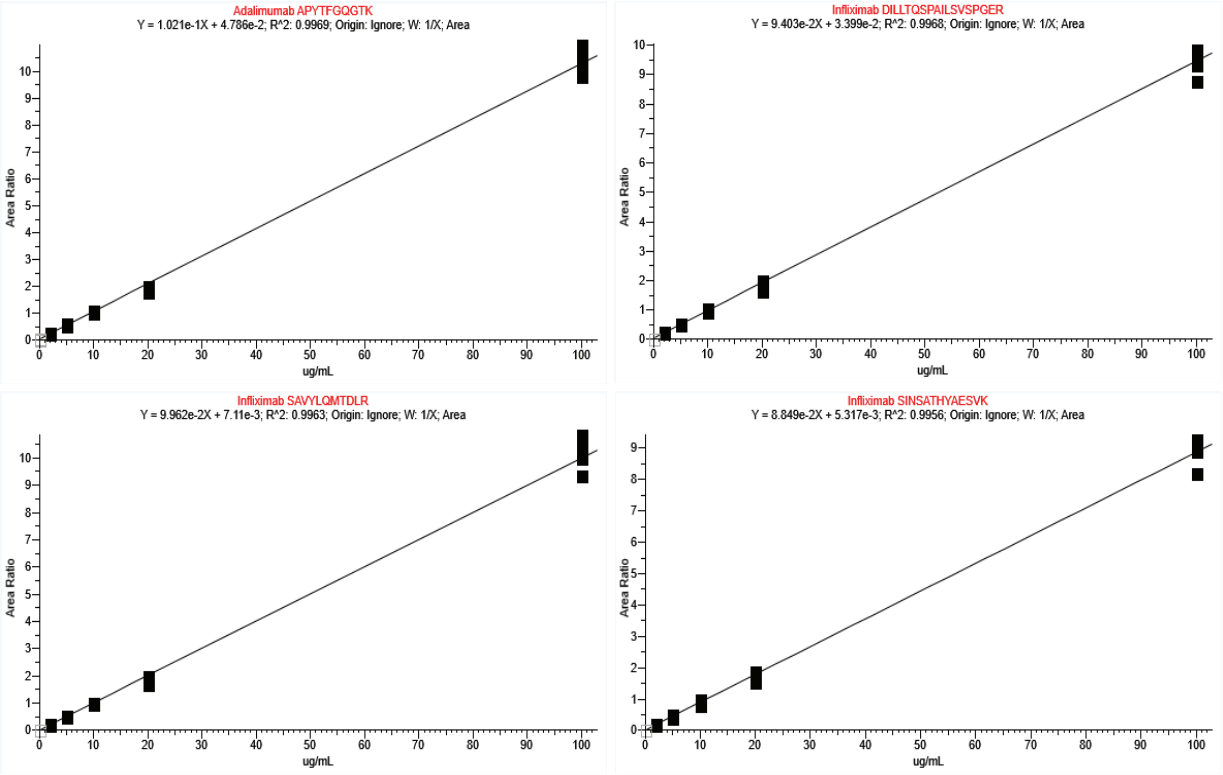


Figure 3. Results of measurement for full-process replicates including peak areas of OTDM1 SIL-mAb peptides (B)

3C



3D

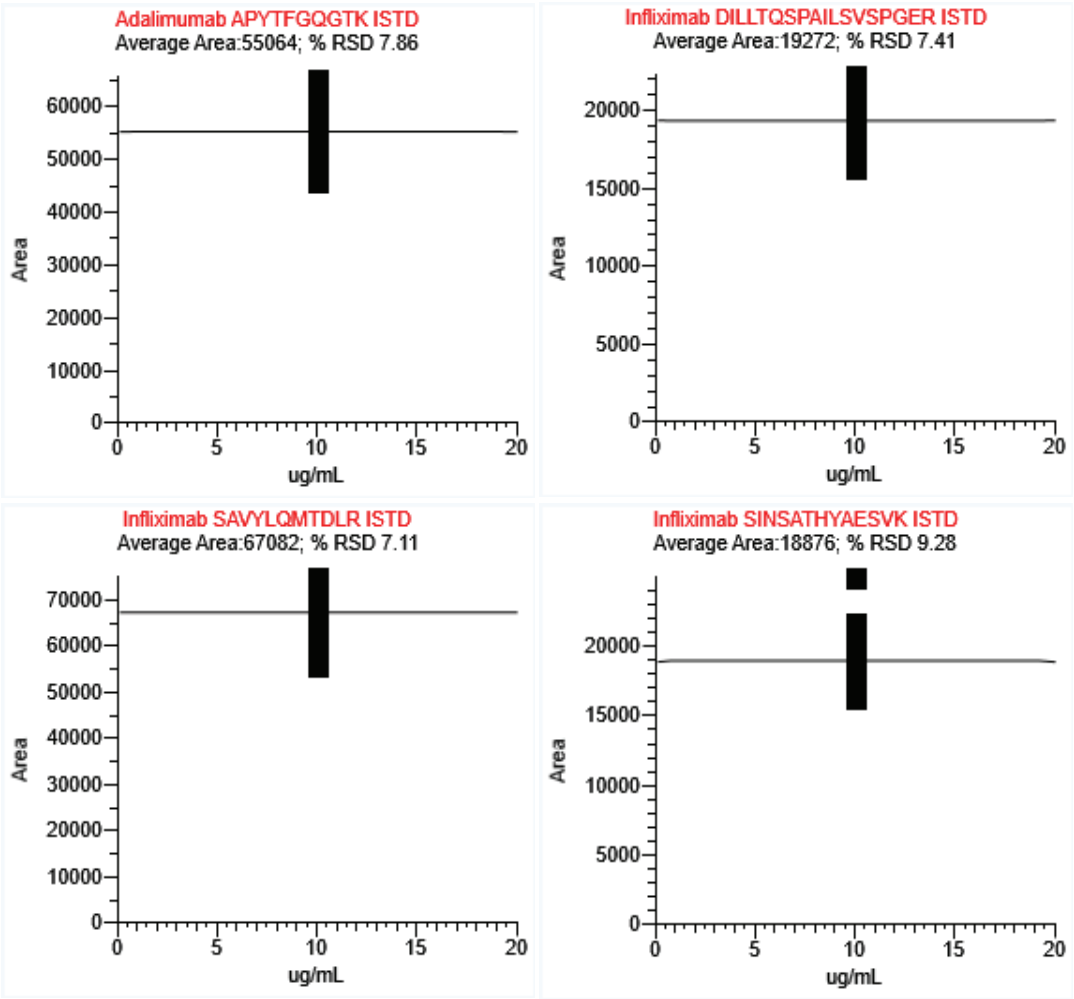


Figure 3. Results of measurement for full-process replicates including calibration curve for ITDM1 target peptides (C) and peak areas of ITDM1 SIL-mAb peptides (D)

Evaluation of LC-MS/MS analytical performance

The evaluation of LC-MS/MS analytical performance follows the criteria listed in Table 4. It should be noted that this study is not a full validation report but provides an overview of basic LC-MS analytical performance using the commercially available kits for quantitative analysis of mAbs. Table 4 describes the evaluation criteria for LC-MS/MS analytical performance including the accuracy and precision of two QC and LLOQ samples and the linear range of the calibration curve. As mentioned above, each well was analyzed in triplicate, generating 18 data points for each calibrator and QC sample. The results showed a successful evaluation of the LC-MS/MS analytical performance described in Table 5 and Figure 4.

Figures 4A and 4C show excellent linearity with $R^2 > 0.997$ over measured calibration concentration points for all the OTDM1 and ITDM1 target peptides. Figures 4B and 4D support the reproducible LC-MS measurement of the entire plate providing %RSD < 10. A summary of the assessed criteria can be found in Table 5 with actual data values. The accuracies of QC samples were determined to be between 94 and 103% for OTDM1 peptides with a precision of <7% CV and between 95 and 107% for ITDM1 peptides with a precision of <9% CV. The response of CAL1 (LLOQ) was 5 times higher than CAL0 for all the target peptides with accuracies between 96 and 112% and precision of <11% CV. Additionally, Figure 5 shows extremely reproducible retention times of all SIL-mAb peptides with RT difference ± 0.03 minutes during the evaluation.

Table 5. Results of LC-MS/MS analytical performance associated with Figure 4

A. Results of OTDM1 kit evaluation

Analytical characteristics	QC accuracy 85 to 115% of nominal concentration		QC precision CV ≤ 15%		LLOQ				Linear range
Acceptance criteria	QC1	QC2	QC1	QC2	CAL0 response (5 x CAL0)	CAL1 response	Accuracy 80 ~ 120% of nominal concentration	Precision CV ≤ 20%	$R^2 \geq 0.99$ from 2 µg/mL to 100 µg/mL
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

B. Results of ITDM1 kit evaluation

Analytical characteristics	QC accuracy 85 to 115% of nominal concentration		QC precision CV ≤ 15%		LLOQ				Linear range
Acceptance criteria	QC1	QC2	QC1	QC2	CAL0 response (5 x CAL0)	CAL1 response	Accuracy 80 ~ 120% of nominal concentration	Precision CV ≤ 20%	$R^2 \geq 0.99$ from 2 µg/mL to 100 µg/mL
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

4A

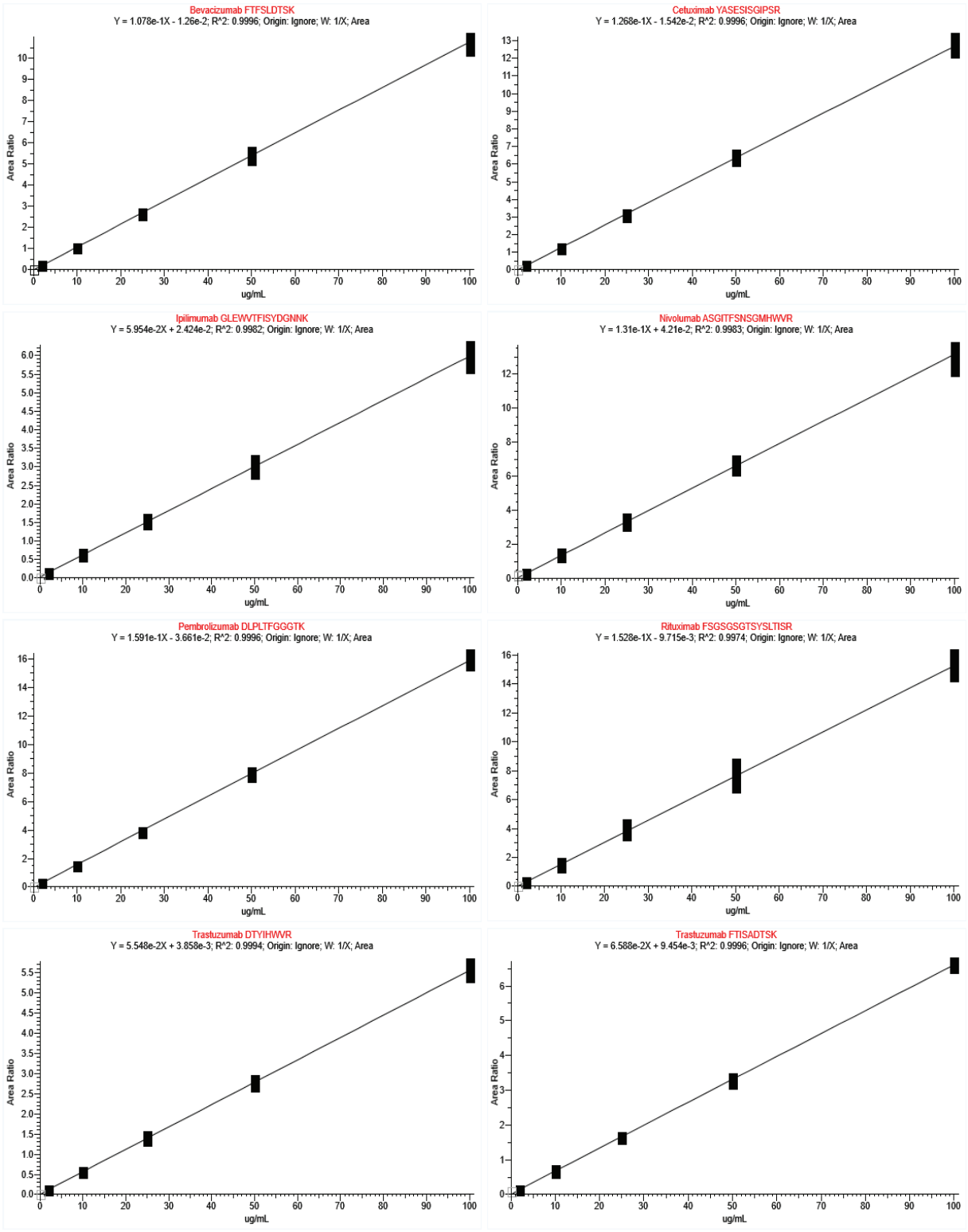


Figure 4. Results of measurement for LC-MS/MS analytical performance including calibration curve for OTDM1 target peptides (A)

4B

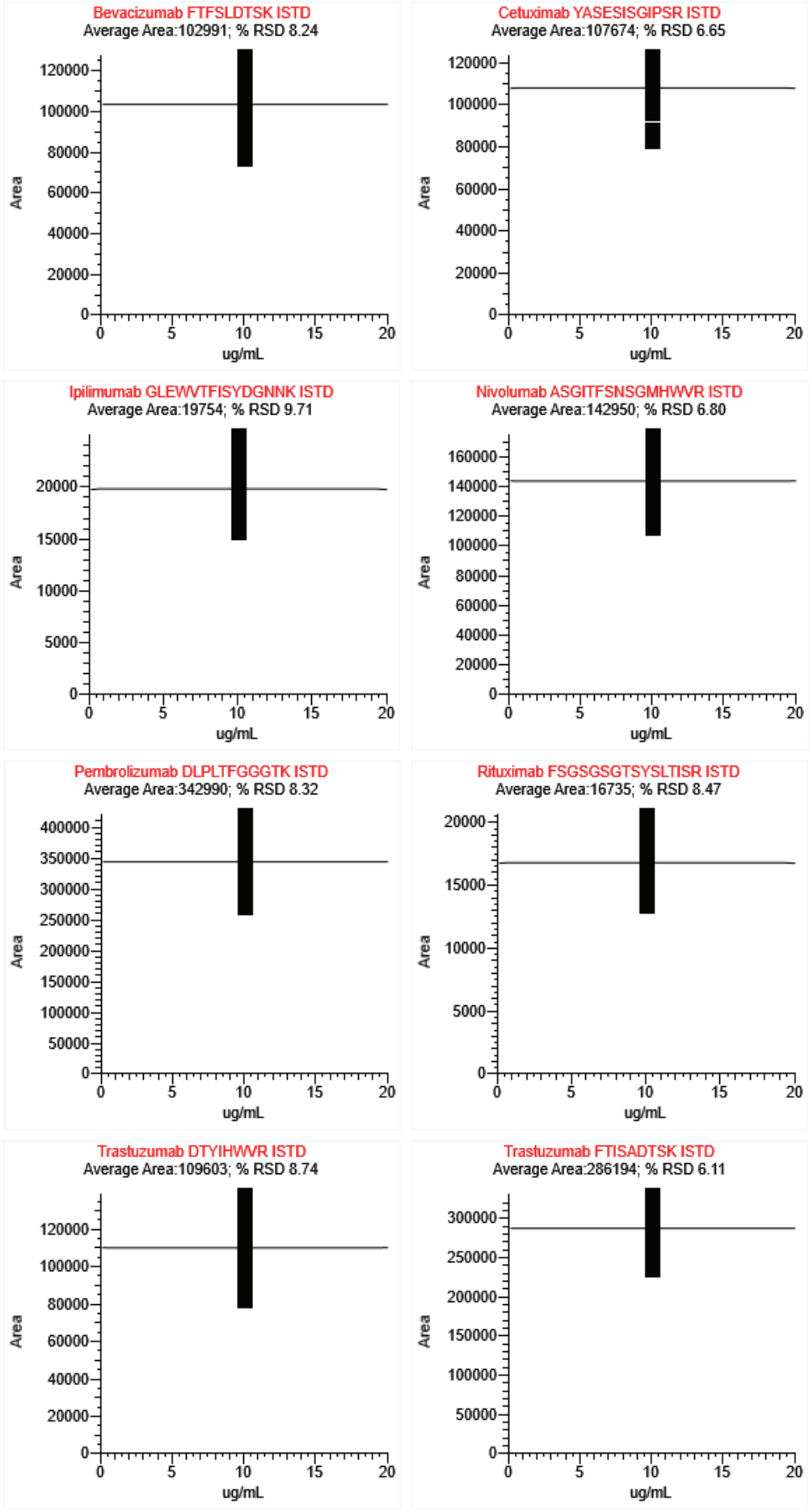
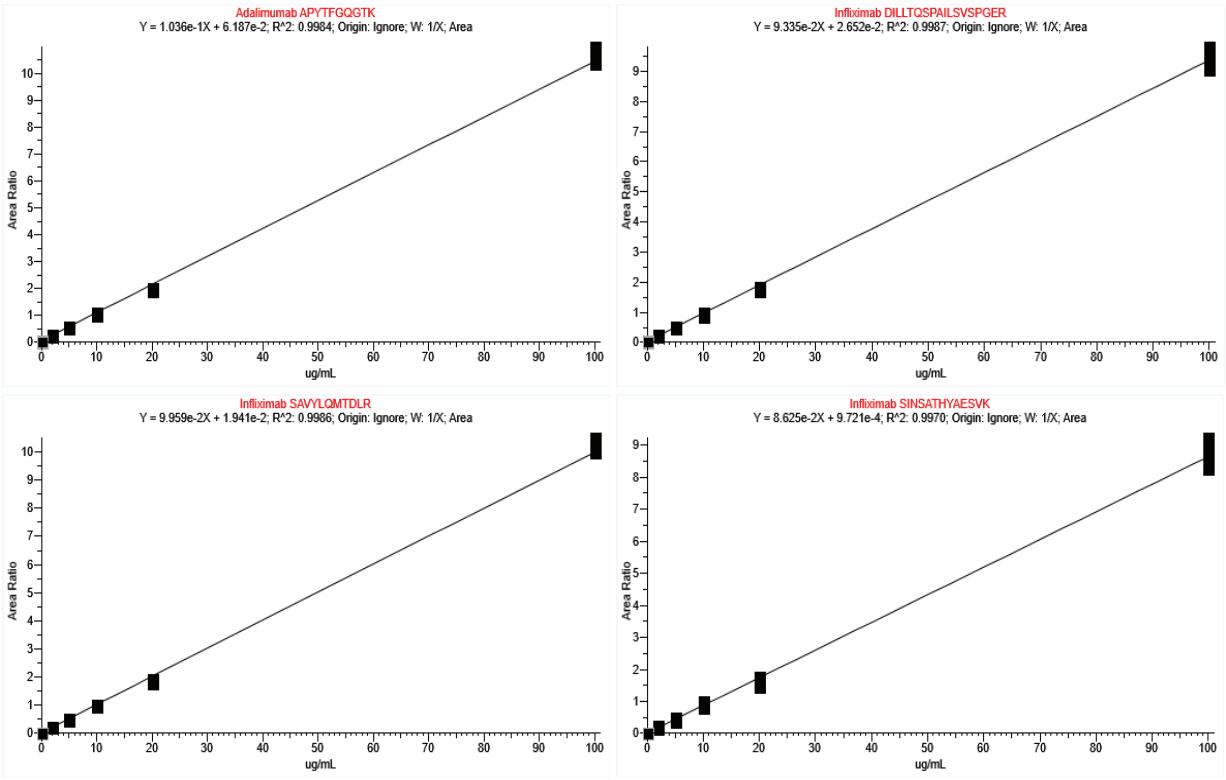


Figure 4. Results of measurement for LC-MS/MS analytical performance including peak areas of OTDM1 SIL-mAb peptides (B)

4C



4D

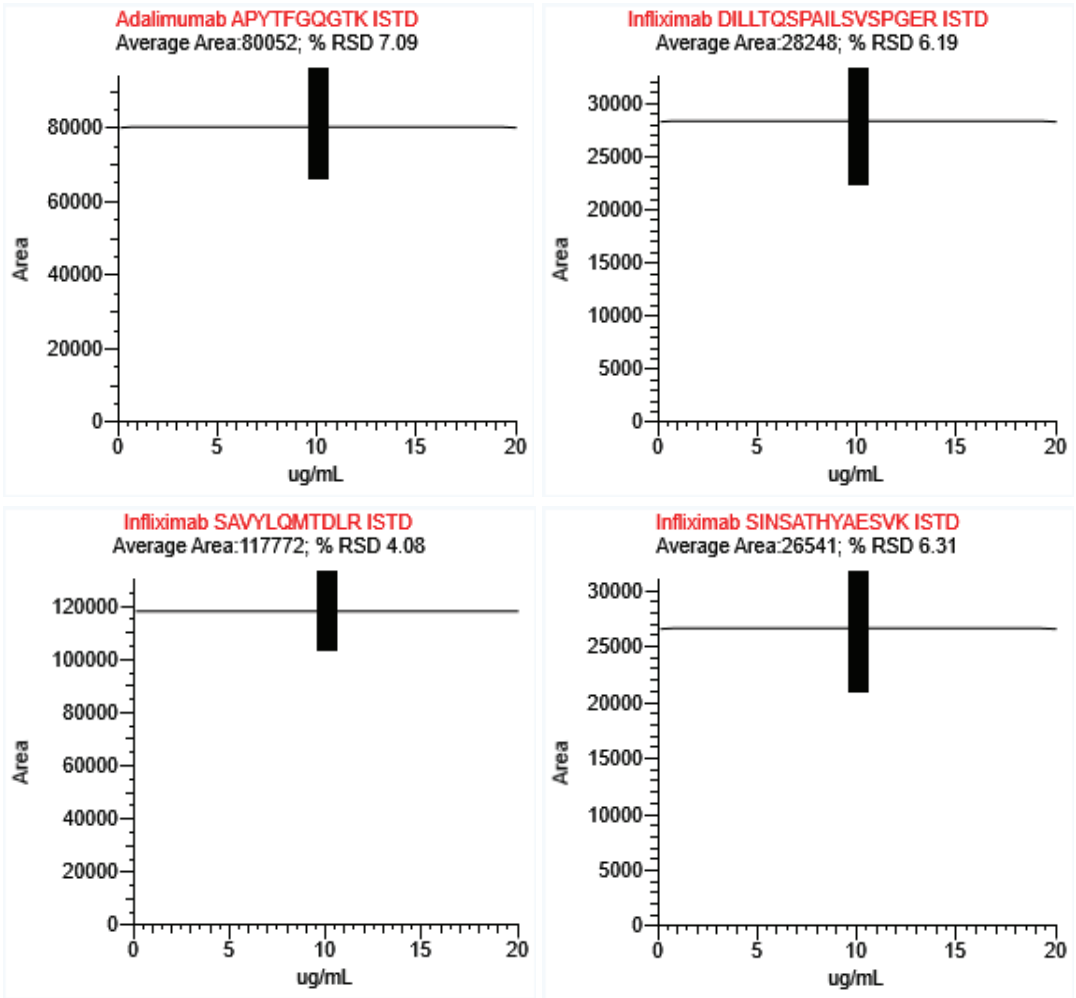


Figure 4. Results of measurement for LC-MS/MS analytical performance calibration curve for ITDM1 target peptides (C) and peak areas of ITDM1 SIL-mAb peptides (D)

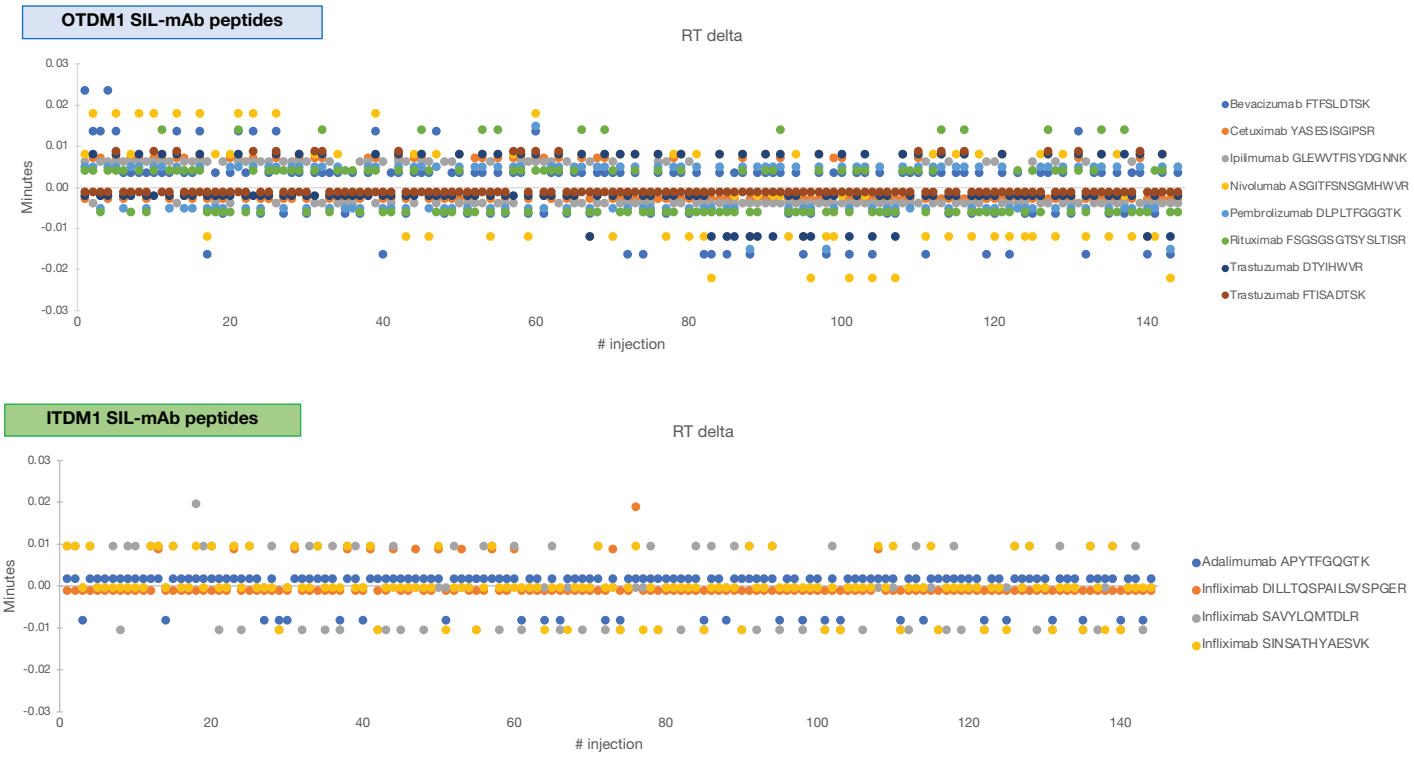


Figure 5. Variation in observed retention times of all SIL-mAb peptides during the evaluation of LC-MS/MS analytical performance

Conclusion

In this report, we have successfully evaluated the Promise Proteomics mAbXmise kits in the TSQ Altis Plus mass spectrometer for quantitative analysis of mAbs. The mAbXmise kits generated very reproducible sample sets across the full plate in a user-friendly and high-throughput way. The kits provided all necessary reagents and consumables, including aluminum foils and plates, so there was no need to purchase additional items for the sample preparation. Alternative items still can be used based on the user's preference, such as Thermo Scientific™ Nunc™ 96-Well Cap Mats (P/N 276011) to cover the collection plate for LC-MS injection.

With LC-MS/MS analysis using the TSQ Altis Plus mass spectrometer, we were able to quantify the lowest level of calibrators with high reproducibility and accuracy. The overall data support that the workflow tested in this report generated sensitive and robust data with high confidence, emphasizing the capability of MS-based assay of mAbs as routine testing. Furthermore, monitoring multiple mAbs in a single workflow promotes the productivity and efficiency of laboratories. This is because the workflow can be applied to other mAbs without developing mAb-specific reagents or buffers and further allows batching different mAbs into the same plate. Automation of the kits can significantly reduce hands-on time while further improving the productivity of users.

References

1. Ladwig, P. M., Barnidge, D. R., Willrich, M. A. V. Mass spectrometry approaches for identification and quantitation of therapeutic monoclonal antibodies in the clinical laboratory. Clinical and Vaccine Immunology 2017, 24(5). https://doi.org/10.1128/CVI.00545-16
2. Tron, C. et al. Quantification of infliximab and adalimumab in human plasma by a liquid chromatography tandem mass spectrometry kit and comparison with two ELISA methods. Bioanalysis 2022, 14, 831–844.
3. Marin, C. et al. Cross-validation of a multiplex LC-MS/MS method for assaying mAbs plasma levels in patients with cancer: A GPCO-UNICANCER study. Pharmaceuticals 2021, 14(8), 796.

Learn more at **thermofisher.com/ClinicalResearchApps**