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應用手冊

A Generic Kit-Based Approach for Quantifying Monoclonal Antibody Drugs Through Direct Digestion of Discovery Study Samples

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Abstract

In this application note, we have used the ProteinWorks eXpress Direct Digest Kit to simplify and streamline the workflow process using the same universal protocol and reagents for all monoclonal antibody drugs tested.

The ProteinWorks eXpress Direct Digest Kit was successfully used to purify and simultaneously quantify infliximab, adalimumab, bevacizumab, and trastuzumab from a typical set of standard curve and QC samples in rat plasma. The standardized, kit-based approach enables inexperienced users to immediately obtain meaningful data in discovery studies in order to make time sensitive and critical project decisions.

Benefits

- Simple, standardized approach to protein quantification; broadly applicable optimized digest kit eliminates method development; samples are ready for LC-MS analysis in 4–6 hours

Introduction

Over the past 5–10 years, there has been a significant shift towards a greater % of biologics in pharmaceutical pipelines.[1] However, the industry finds itself in the middle of patent expiry for many of the critical monoclonal antibody and other protein-based drugs, with patent expiration dates ranging from ~2012–2020.[2] This has resulted in a focus on protein quantification in bioanalytical labs, innovator pharma and CRO's as well as biomarker research labs. While immunoassay (IA) methods are sensitive and simple to execute, poor reagent reproducibility, lack of standardization, cross-reactivity, limited linear dynamic range, and other short-comings have led the drive to convert to LC-MS. These LC-MS workflows however, encompass a multitude of sub-segments, each having many steps. Those that are common to most workflows may include affinity purification, denaturation, reduction, alkylation, digestion, and SPE clean-up (each requiring optimization). Such traditional protein quantification protocols often require as much as a day and half for completion. Furthermore, the margin and possibility of error is significant within each individual step. There is a strong need for simpler, more standardized workflows which enable scientists to complete sample preparation and start an analytical run by mid-day. At the same time, ideally using generic, kitted methods, assay sensitivity must be high enough to accurately and precisely quantify low enough levels of the target protein to make critical decisions in discovery. The typical workflow complexity as shown in Figure 1, often leads to errors and poor reproducibility or sensitivity. In this application note, we have used the ProteinWorks eXpress Direct Digest Kit to simplify and streamline the workflow process using the same universal protocol and reagents for all monoclonal antibody drugs tested. Infliximab, bevacizumab, trastuzumab, and adalimumab (Figures 2–5) in plasma were directly digested, and peptides extracted using SPE in under 4 hours total time. This enabled data to begin to be acquired the same day, with several 96-well plates being run by the following morning.

Experimental

Sample description

Infliximab, adalimumab, bevacizumab, or trastuzumab were spiked into human plasma. 35 µL plasma samples were then prepared for LC-MS analysis using the ProteinWorks eXpress Direct Digest Kit and Protocol. After digestion, signature peptides were cleaned-up using the ProteinWorks µElution SPE Clean-up Kit and Protocol.

Method conditions

LC System:	ACQUITY UPLC
Detection:	Xevo TQ-S Mass Spectrometer, ESI+
Column:	ACQUITY UPLC Peptide BEH C_{18}, 300 Å, 1.7 µm, 2.1 mm x 150 mm
Column temp.:	55 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Capillary (kv):	3
Cone (V):	30
Source offset (V):	50
Source temp. (°C):	150
Desolvation temp. (°C):	600
Cone gas flow (L/hr):	150
Desolvation gas flow (L/hr):	1000

Collision gas flow (mL/min):	0.15
Nebulizer gas flow (Bar):	7

Gradient

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0	0.3	100	0	6
1	0.3	100	0	6
7	0.3	50	50	6
8	0.3	10	90	6

Protein	Peptide	MRM transition	Cone voltage (V)	Collision energy (eV)
Infliximab	SINSATHYAESVK	469.60>603.80	40	10
Bevacizumab	FTFSLDTSK	523.30>797.48	16	14
Adalimumab	APYTFGQGTK	535.30>901.44	40	24
Trastuzumab	FTISADTSK	485.20>721.40	28	20
murine mAb	MNSLQTDDTAK (ISTD)	612.30>978.56	20	20

Table 1. MRM conditions for infliximab, adalimumab, trastuzumab, bevacizumab, and the murine mAb internal standard.

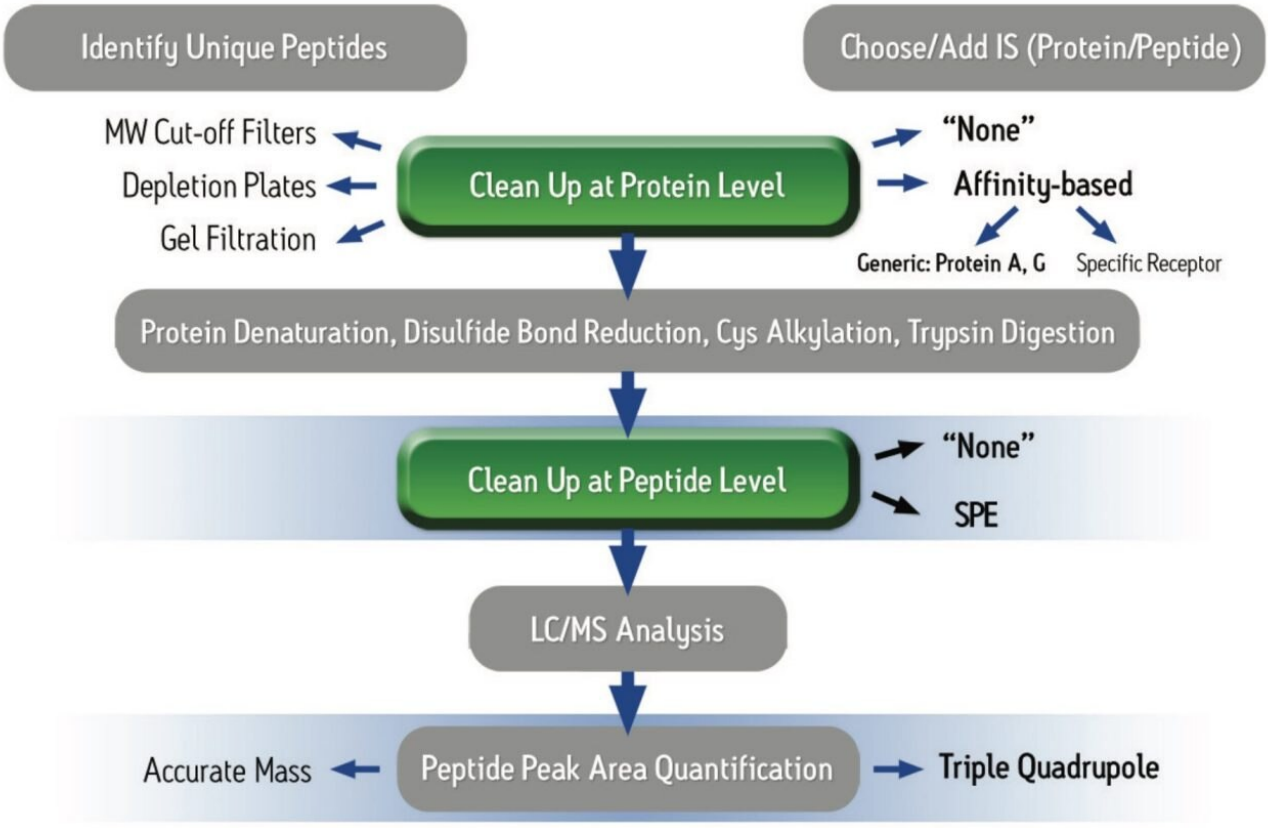

Figure 1. Typical protein bioanalysis workflow.

Results and Discussion

In a pre-clinical setting, there is an emphasis on simple, broadly applicable, generic protocols as method development time and expertise are at a premium. Multiple signature peptides were used to quantify 4 different monoclonal antibody drugs in human plasma using direct digestion and the ProteinWorks eXpress Direct Digest Kit. For each protein, sensitivity, linearity, accuracy and precision data all met typical method validation requirements using the same broadly applicable ProteinWorks Kit. Through a direct digest of a 35 µL plasma sample, quantification limits ranged from 250 ng/mL–2.5 µg/mL for the 4 monoclonal antibody-based drugs. Standard curves were linear over 3.5–4 orders of magnitude with the average accuracies for standard curve points typically within 95–105%. Summary statistics from standard curves for infliximab, adalimumab,

trastuzumab, and bevacizumab are shown in Table 2 below.

Protein	Peptide	Std. curve range (µg/mL)	Weighting	Linear fit (r^2)	Mean % accuracy of all points
Infliximab	SINSATHYAESVK	0.25–250	1/X	0.996	101.74
Bevacizumab	FTFSLDTSK	0.50–500	1/X	0.999	100.00
Adalimumab	APYTFGQGTK	2.50–500	$1/X^2$	0.997	99.99
Trastuzumab	FTISADTSK	2.50–500	$1/X^2$	0.997	100.01

Table 2. Linear dynamic range, weighting, and average accuracy for standard curves for infliximab, adalimumab, trastuzumab, and bevacizumab in plasma digested and extracted using the ProteinWorks eXpress Direct Digest Kit.

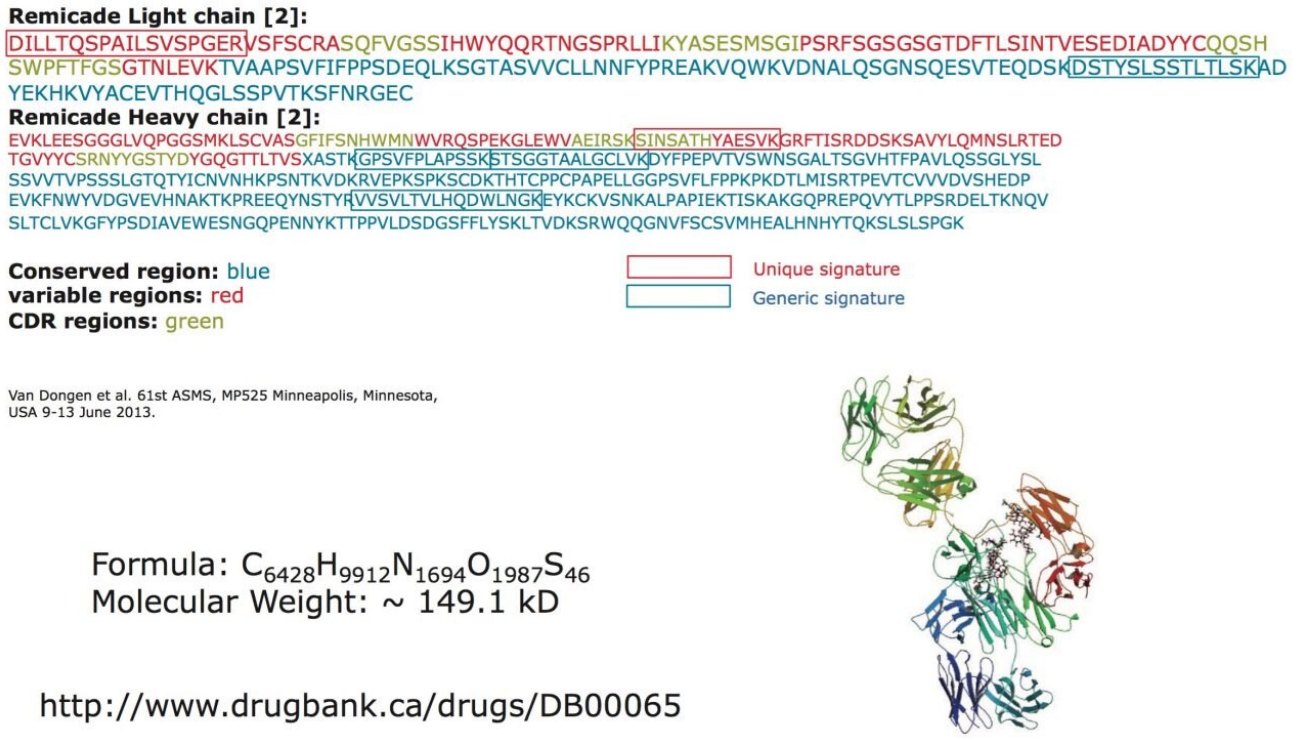

Figure 2. Structures of the monoclonal antibody-based drug infliximab (Remicade).

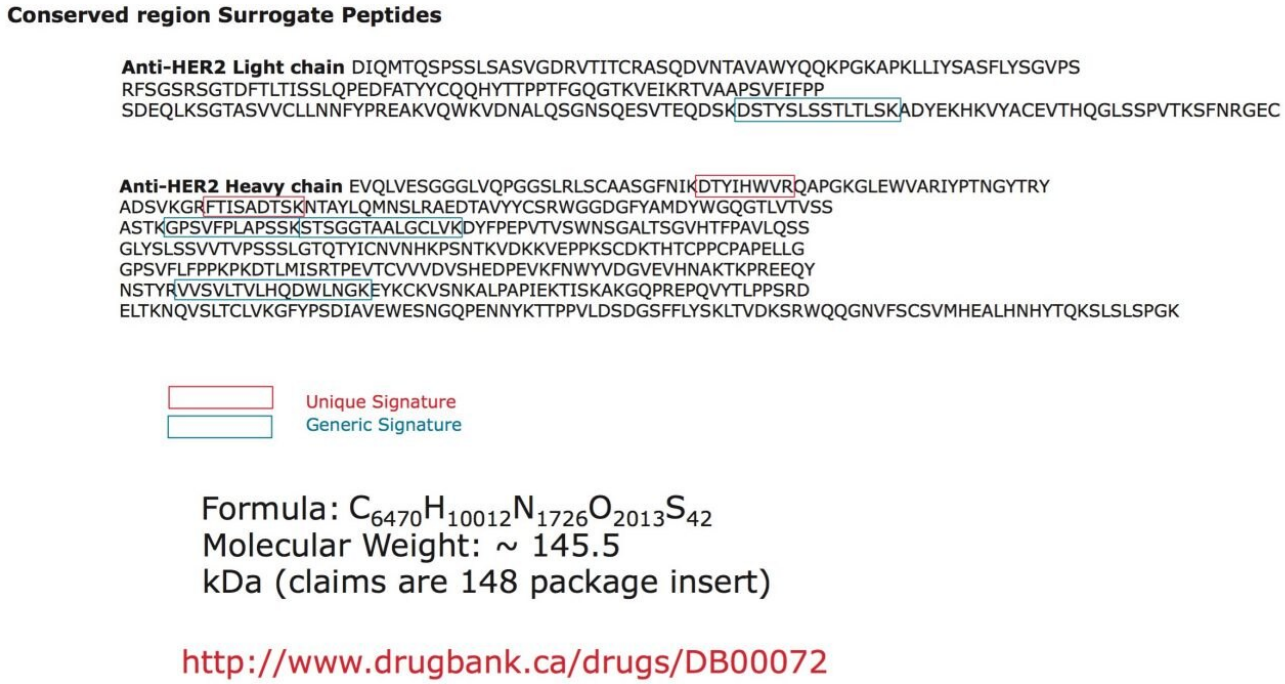

Figure 3. Structures of the monoclonal antibody-based drug trastuzumab (Herceptin).

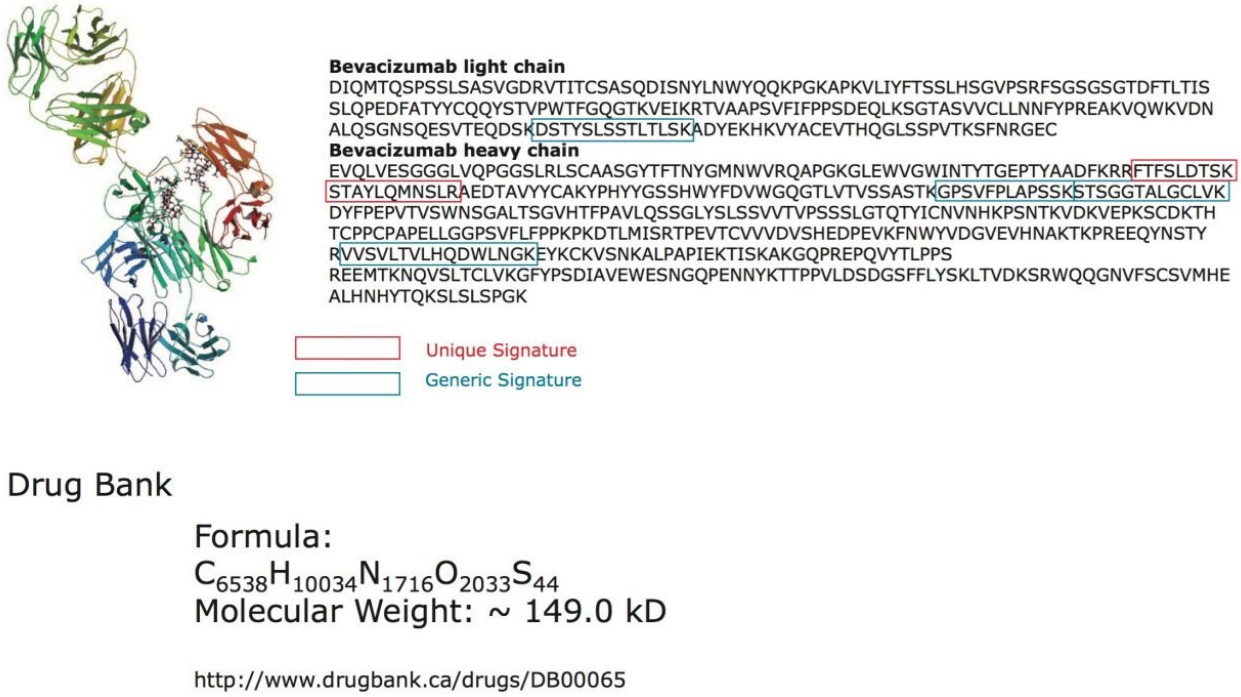

Figure 4. Structures of the monoclonal antibody-based drug bevacizumab (Avastin).

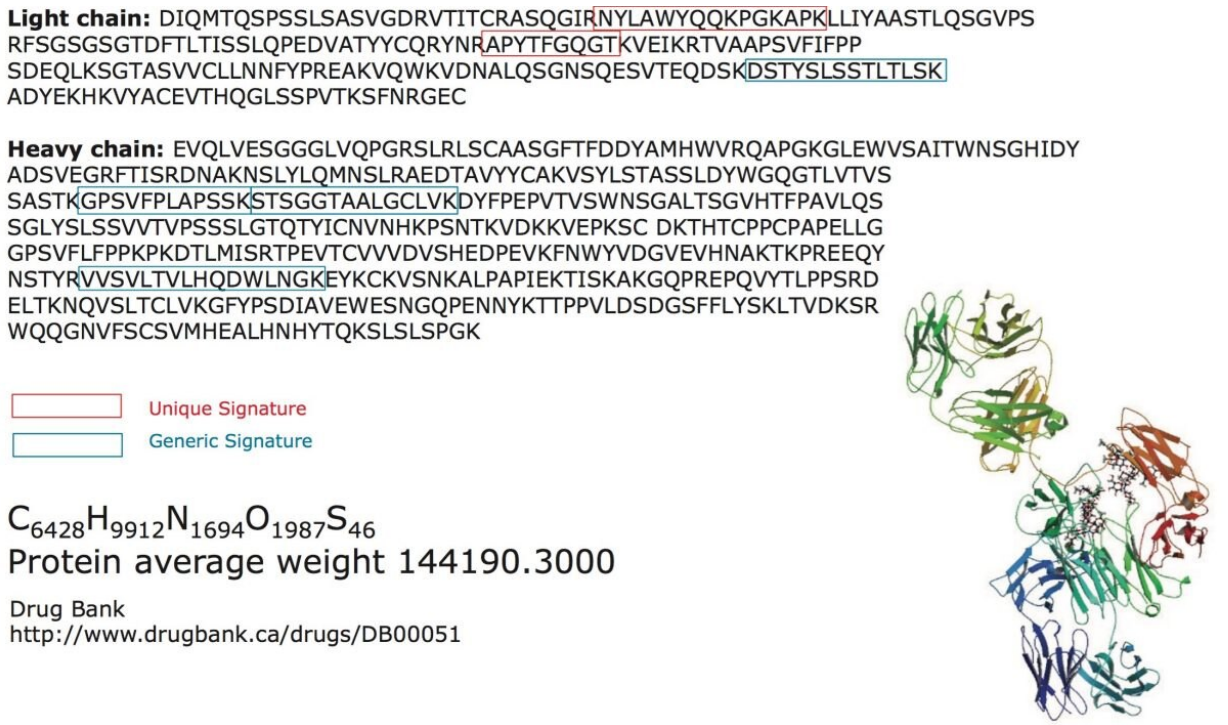

Figure 5. Structures of the monoclonal antibody-based drug adalimumab (Humira).

At the same time, QC statistics (summarized in Table 3 below) also easily met regulatory guidelines,[3] with average precision values well under 15%, and averaging in the single digits.

For a typical discovery study, detection limits ~1 µg/mL are common for monoclonal antibody type drugs. Using the ProteinWorks eXpress Direct Digest kit, these limits are easily obtained for all 4 drugs evaluated. Low QC chromatograms are shown in Figure 6 which demonstrate that adequate sensitivity is achieved with a single universal protocol and the kit.

In this study, the single universal digest protocol and SPE method designed for tryptic peptides eliminated the need for discovery-stage method development. The fact that the kit was able to accurately and precisely quantify 4 monoclonal antibody drugs in plasma without the need for optimization demonstrates its broad applicability and utility in an environment where time is critical and experience with protein bioanalysis may be limited. Furthermore, the application of a kit with lot-traceable, pre-measured reagents ensures that methods may be seamlessly transferred across sites and labs, or analysts.

Protein	Peptide	QC conc (µg/mL)	Mean cal. conc (µg/mL)	Std. dev.	%CV	Mean accuracy
Infliximab	SINSATHYAESVK	0.350	0.333	0.010	3.10	95.0
		3.500	3.816	0.098	2.56	109.0
		35.000	36.075	0.576	1.60	103.1
		350.000	359.301	19.892	5.54	102.6
		QC conc (µg/mL)	Mean cal. conc (µg/mL)	Std. dev.	%CV	Mean accuracy
Bevacizumab	FTFSLDTSK	0.350	0.356	0.004	1.08	101.7
		3.500	3.393	0.196	5.78	96.9
		35.000	38.461	1.282	3.33	109.9
		350.000	369.788	28.066	7.59	105.6
		QC conc (µg/mL)	Mean cal. conc (µg/mL)	Std. dev.	%CV	Mean accuracy
Adalimumab	APYTFGQGTK	0.350	–	–	–	–
		3.500	3.978	0.570	14.34	113.7
		35.000	36.567	1.023	2.80	104.5
		350.000	380.963	18.143	4.76	108.8
		QC conc (µg/mL)	Mean cal. conc (µg/mL)	Std. dev.	%CV	Mean accuracy
Trastuzumab	FTISADTSK	0.350	–	–	–	–
		3.500	3.663	0.067	1.82	104.7
		35.000	39.182	2.389	6.10	112.0
		350.000	374.080	14.010	3.75	106.9

Table 3. Statistics for QC samples of infliximab, adalimumab, trastuzumab, and bevacizumab in plasma digested and extracted using the ProteinWorks eXpress Direct Digest Kit.

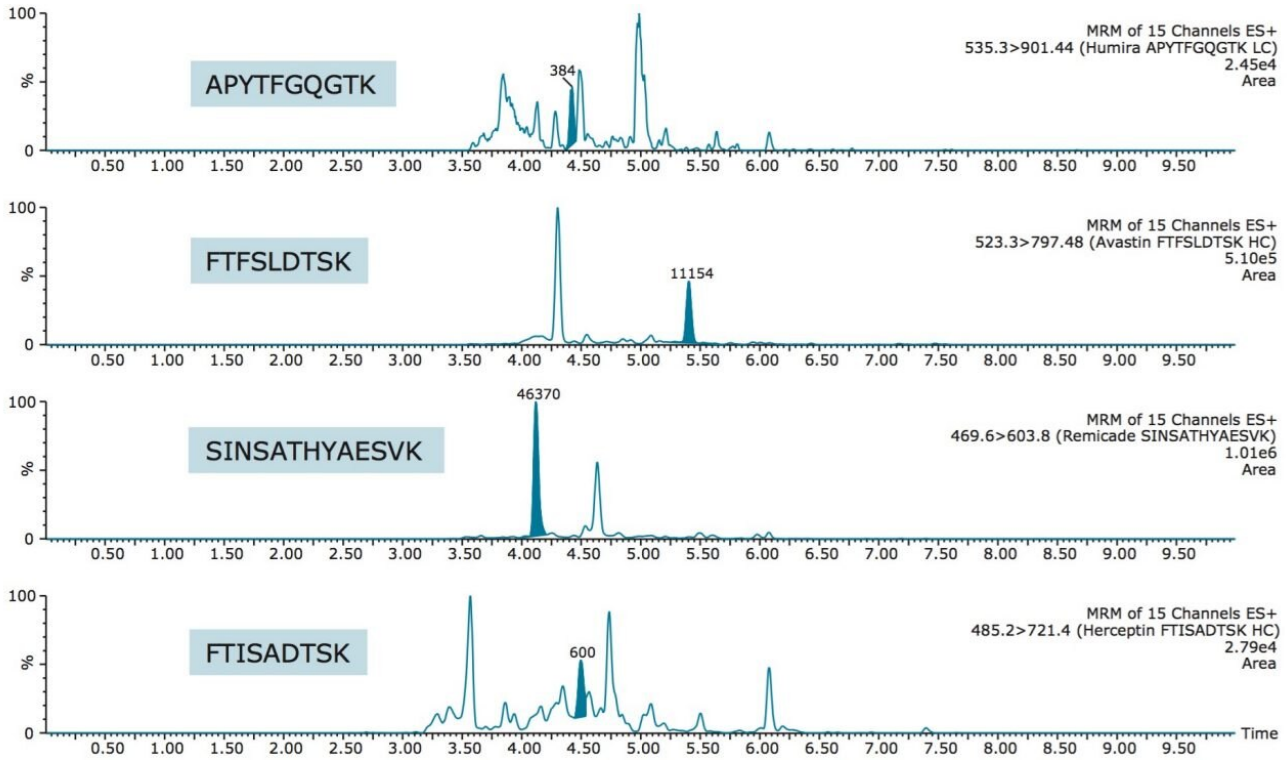

Figure 6. Low QC chromatograms (3.5 µg/mL) for bevacizumab, adalimumab, infliximab, and trastuzumab.

Conclusion

The ProteinWorks eXpress Direct Digest Kit was successfully used to purify and simultaneously quantify infliximab, adalimumab, bevacizumab, and trastuzumab from a typical set of standard curve and QC samples in rat plasma. Quantification limits of 250 ng/mL to 2.5 µg/mL for each antibody were readily achieved, while maintaining excellent linearity and precision. The total sample prep time including digestion and SPE was just over 3 hours. The standardized, kit-based approach enables inexperienced users to immediately obtain meaningful data in discovery studies in order to make time sensitive and critical project decisions.

References

1. Dalzell, Managed Care, October 2013.
2. McKinsey and Company; Data Source: Evaluate Pharma, US Patent Expiration Dates.
3. FDA Guidance for Industry for Bioanalytical Method Validation, CDER.

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ACQUITY UPLC Peptide BEH C18, 300Å, 1.7 µm, 2.1 mm X 150 mm Column <
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720005541, November 2015