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Application Note

High Sensitivity LC-MS/MS Quantification of Monoclonal Antibody Drugs in Rat Plasma Using a Standardized Sample Preparation Workflow

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This work demonstrates the highly sensitive, accurate and precise quantification of multiple mAb therapeutics in rat plasma using protein level affinity purification, ProteinWorks eXpress Digest Kits, and analysis on an ACQUITY UPLC and Xevo TQ-XS MS System, achieving limits of quantification of ≤5 ng/mL. Total sample preparation time, including affinity purification, was <8 hours. A standardized, kit-based approach to protein quantification enables inexperienced users to easily obtain accurate and robust quantification data to make time critical project decisions in support of drug discovery and development.

Benefits

Protein therapeutics, specifically monoclonal antibodies (mAbs), represent an increasing proportion of

commercialized drugs due to their target specificity, lower toxicity and higher potency. With many mAb drugs facing patent expiry, development of biosimilar and next generation mAbs has grown and has resulted in the increased demand for their bioanalytical quantification.

Introduction

As a large number of mAb biologics will lose or have already lost patent exclusivity, with patent expiries ranging from ~2012-2021, bioanalytical quantification in support of biosimilar[2-4] and next generation mAb therapeutics is increasing. Historically, this has been done using immunoassays. Although sensitive and easy to use, immunoassays can suffer from issues with specificity, standardization, limited multiplexing capabilities, and long method development times. In contrast, LC-MS with its many advantages (e.g., multiplexing, broad dynamic range, specificity, and faster method development) has emerged as a viable alternative for protein quantification. The surrogate peptide (bottom-up) approach, employing enzymatic digestion of the protein and quantification of resulting peptides using tandem quadrupole MS is commonly used, as sensitivity and detection limits required for pharmacokinetic, pharmacodynamic, and toxicokinetic studies can readily be achieved. While widely accepted across the industry, developing a bottom-up method requires extensive sample preparation optimization, making it difficult to quickly develop sensitive and robust methods. A strong demand exists for simplified, and standardized workflows which facilitate sensitive and accurate quantification for a diversity of mAbs.

Results and Discussion

Infliximab, adalimumab, trastuzumab, and SILu MAb (Sigma P/N MSQC3, used as internal standard) were spiked into rat plasma. The plasma samples (100 µL) were then immunopurified using goat-derived anti-human biotinylated IgG (Promega P/Ns V7820 and V7830) conjugated to a streptavidin bead slurry (25 µL). The post-affinity purified plasma sample (50 µL) was neutralized (pH 8) and digested using the ProteinWorks eXpress Digest Kit and 5-step digestion protocol, which includes reduction and alkylation. ProteinWorks eXpress Digest Kits are flexible, broadly applicable sample preparation kits, which contain pre-measured reagents and a

universal protocol designed to simplify and streamline the bottom-up, protein bioanalysis workflow. Following sample digestion, LC-MS/MS quantification of resulting peptides was performed using a Xevo TQ-XS tandem quadrupole MS. Chromatographic separation was achieved using an ACQUITY UPLC System and an ACQUITY UPLC Peptide BEH C_{18}, 300Å, 1.7 µm, 2.1 x 150 mm Column, using a linear gradient with 0.1% formic acid in water and acetonitrile (flow rate 0.3 mL/min) and a sample injection volume of 15 µL. Signature tryptic peptides and MS conditions used for mAb quantification are summarized in Table 1.

Protein	Tryptic peptide	Precursor charge state	MRM transition	Collision energy (eV)	Product ion identification
Infliximab	SINSATHYAESVK	[M+3H]3+	469.57 > 603.79	13	[2H+]2/y11
	LEESGGGLVQPGGSMK	[M+3H]3+	515.92 > 576.28	13	[1H+]1/y6
	ASQFVGSSIHWYQQR	[M+3H]3+	598.63 > 631.31	17	[2H+]2/y10
	DILLTQSPAILSVSPGER	[M+3H]3+	632.69 > 545.27	16	[1H+]1/y5
Adalimumab	NYLAWYQQKPGK	[M+3H]3+	499.26 > 609.84	11	[2H+]2/y10
	GLEWVSAITWNSGHIDYADSVEGR	[M+3H]3+	888.09 > 1039.48	23	[2H+]2/y19
	APYTFGQGTK	[M+2H]2+	535.27 > 499.75	16	[2H+]2/y9
	LLIYAASTLQSGVPSR	[M+3H]3+	559.32 > 602.33	16	[1H+]1/y6
Trastuzumab	IYPTNGYTR	[M+2H]2+	542.77 > 404.70	15	[2H+]2/y7
	FTISADTSK	[M+2H]2+	485.25 > 721.37	15	[1H+]1/y7
	DTYIHWVR	[M+2H]2+	545.28 > 597.33	24	[1H+]1/y4
	LLIYSASFLYSGVPSR	[M+3H]3+	591.66 > 602.33	18	[1H+]1/y6
SILuMAb (Internal Standard)*	DTLMISR	[M+2H]2+	423.22 > 516.28	14	[1H+]1/y4
	ALPAPIEK	[M+2H]2+	423.76 > 662.40	14	[1H+]1/y6
	GPSVFPLAPSSK	[M+2H]2+	597.83 > 426.24	21	[1H+]1/y4
	LMIYDATK	[M+2H]2+	481.76 > 718.39	17	[1H+]1/y6

*Table 1. MS conditions for infliximab, adalimumab, trastuzumab, and SILuMAb, including precursor and product ions. *$^{15}N^{13}C$ labeled mAb.*

Protein	Tryptic peptide	Curve range (ng/mL)	Weighting	Linear fit (R^2)	% Accuracy range	LOD (ng/mL)	LLOQ (ng/mL)
Infliximab	SINSATHYAESVK	1 - 10,000	$1/X^2$	0.997	90.0 - 105.6	0.5	1
	LEESGGGLVQPGGSMK	5 -10,000		0.991	95.1 - 107.7	1	5
	ASQFVGSSIHWYQQR	5 -10,000		0.992	90.4 - 108.8	5	5
	DILLTQSPAILSVSPGER	5 -10,000		0.993	94.0 - 105.0	5	5
Adalimumab	NYLAWYQQKPGK	5 - 10,000	$1/X^2$	0.995	95.3 - 107.7	1	5
	GLEWVSAITWNSGHIDYADSVEGR	10 - 10,000		0.991	92.8 - 107.7	5	10
	APYTFGQGTK	10 - 10,000		0.995	92.4 - 105.5	10	10
	LLIYAASTLQSGVPSR	50 - 10,000		0.988	91.4 - 110.3	50	50
Trastuzumab	IYPTNGYTR	5 - 50,000	$1/X^2$	0.990	88.3 - 112.3	1	5
	FTISADTSK	10 - 10,000		0.993	91.0 - 109.8	5	10
	DTYIHWVR	10 - 10,000		0.991	84.6 - 111.5	5	10
	LLIYSASFLYSGVPSR	10 - 10,000		0.993	93.2 - 106.4	5	10

Table 2. Linear dynamic range and standard curve statistics for the tryptic peptides used to quantify infliximab, adalimumab, and trastuzumab in plasma.

In this study, a kit-based sample preparation approach eliminated the need for discovery-stage method development, as was demonstrated with the accurate quantification of the 3 mAb drugs from rat plasma. Standard curves were linear over 3-4 orders of magnitude with average accuracies for standard curve points between 85-112%. Standard curve summary statistics for infliximab, adalimumab, and trastuzumab tryptic peptides used for quantification are shown in Table 2. For discovery studies, achieving detection limits ≤0.1 µg/mL (~1 nM) is typically required. Using protein-level affinity purification and subsequent sample digestion with the ProteinWorks Kit and universal protocol, limits of quantification between 1–5 ng/mL (10–50 pM) were readily achieved. Chromatographic performance and demonstration of sensitive quantification for the 3 mAbs is highlighted in Figure 1.

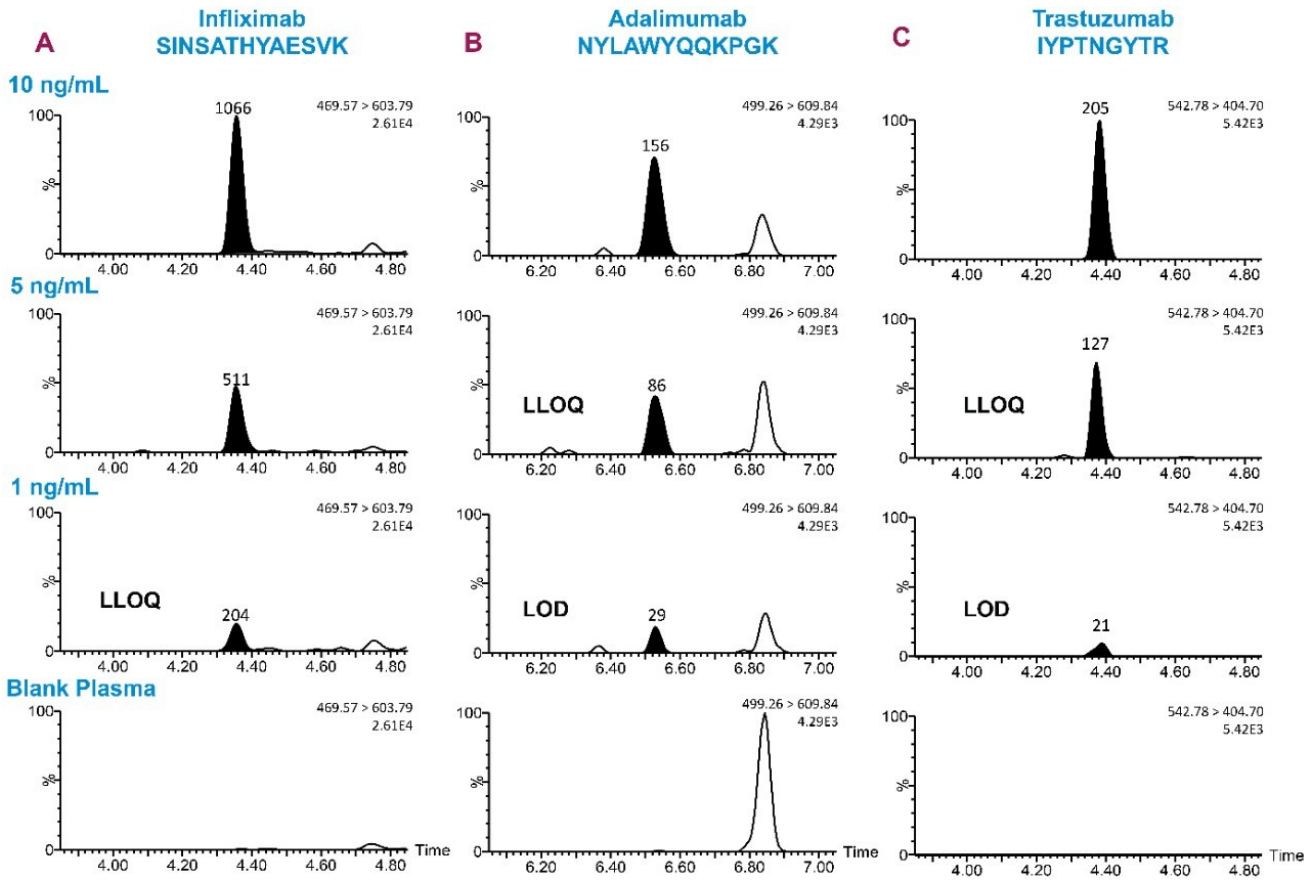

Figure 1. Representative chromatograms, demonstrating sensitivity (≤1 ng/mL), for the SINS (A), NYLA (B), and IYPT (C) signature peptides of infliximab, adalimumab, and trastuzumab, respectively. Plasma samples were immunopurified and subsequently digested using the ProteinWorks eXpress Digest Kit.

LC conditions

LC system:	Acquity UPLC
Column temp.:	55.0 °C
Injection vol. (µL):	15
Loop size (µL):	20

Mobile phase A:	0.1% Formic Acid in Water
Mobile phase B:	0.1% Formic Acid in Acetonitrile

MS conditions

MS system:	Xevo TQ-XS
Capillary (kV):	2.9
Cone (V):	30
Source offset (V):	30
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
Nebuliser gas flow (Bar):	7

LC Gradient Infliximab

Time (min)	Flow rate (mL/min)	% A	% B	Curve
Initial	0.300	98.0	2.0	Initial
1.00	0.300	98.0	2.0	6
7.00	0.300	60.0	40.0	6
8.00	0.300	10.0	90.0	6
8.80	0.300	10.0	90.0	6
9.00	0.300	98.0	2.0	6
10.00	0.300	98.0	2.0	6

LC Gradient Infliximab

LC Gradient Adalimumab

Time (min)	Flow rate (mL/min)	% A	% B	Curve
Initial	0.300	95.0	5.0	Initial
1.50	0.300	95.0	5.0	6
9.50	0.300	65.0	35.0	6
10.00	0.300	10.0	90.0	6
11.00	0.300	10.0	90.0	6
11.50	0.300	95.0	5.0	6
13.50	0.300	95.0	5.0	6

LC Gradient Adalimumab

LC Gradient Trastuzumab

Time (min)	Flow rate (mL/min)	% A	% B	Curve
Initial	0.300	95.0	5.0	Initial
1.50	0.300	95.0	5.0	6
9.50	0.300	50.0	50.0	6
10.00	0.300	10.0	90.0	6
11.00	0.300	10.0	90.0	6
11.50	0.300	95.0	5.0	6
13.50	0.300	95.0	5.0	6

LC Gradient Trastuzumab

Conclusion

This work demonstrates the highly sensitive, accurate and precise quantification of multiple mAb therapeutics in rat plasma using protein level affinity purification, ProteinWorks eXpress Digest Kits, and analysis on an ACQUITY UPLC and Xevo TQ-XS MS System, achieving limits of quantification of ≤5 ng/mL. Total sample preparation time, including affinity purification, was <8 hours. A standardized, kit-based approach to protein quantification enables inexperienced users to easily obtain accurate and robust quantification data to make time critical project decisions in support of drug discovery and development.

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