APPLYING A STANDARDIZED, KIT-BASED APPROACH TO ACHIEVE 10 NG/ML OF INFLIXIMAB FROM 35 µL OF PLASMA

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INTRODUCTION

Protein therapeutics, mAbs, and ADCs represent a growing class of drugs due to their target specificity, lower toxicity and higher potency. Historically, quantification of proteins has been done using immunoassays. However, due to the many benefits LC/MS affords (multiplexing, improved specificity, broader dynamic range and fast method development time), its use for the quantification of proteins is steadily gaining. However, LC/MS protein quantification still presents challenges. There is no single standardized workflow and the various workflow options can be complex and laborious, making it difficult for a scientist to achieve success.

This work describes the fast, sensitive quantification of infliximab using a flexible, kit-based approach and generic protocol, to achieve an LLOQ of 10 ng/mL from only 35 µL of plasma.

METHODS

Sample Preparation

Infliximab was immuno-purified from 35 µL of rat plasma using a generic affinity capture (Protein A). Samples were then prepared for LC/MS analysis using ProteinWorks eXpress Digest Kit and protocol. Signature tryptic peptides were purified using the ProteinWorks µElution SPE Clean-Up Kit and protocol.

LC/MS Conditions

LC-MS/MS analysis was performed on a Waters Xevo TQ-S triple quadrupole MS (ESI+). The tryptic peptides, DILLTQSPAILSVSPGER (633.1>731.8), SINSATHYAESVK (469.6>603.8), and DSTYSLSSTLTLSK (751.88>836.47) were selected for quantification. A murine mAb (Waters) was used as an internal standard. Chromatographic separation was achieved using an ACQUITY UPLC system with an ACQUITY UPLC Peptide BEH C18, 300Å, 1.7 µm, 2.1 mm x 150 mm column, at a flow rate of 0.3 mL/min using a linear gradient with 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Final injection volume was 10 µL.

Peptide	Std. Curve Range (µg/mL)	Weighting	Linear fit (r^2)	Mean % Accuracy of all points
DILLTQSPAILSVSPGER*	0.05-250	1/X	0.998	100.00
SINSATHYAESVK*	0.01-100	$1/X^2$	0.995	98.47
DSTYSLSSTLTLSK	0.10-500	$1/X^2$	0.997	99.34

Table 1.Linear dynamic range and standard curve statistics for signature peptides use to quantify infliximab in rat plasma.

RESULTS

Peptide	QC Conc (µg/mL)	Mean Cal. Conc (µg/mL)	Std. Dev.	%CV	Mean Accuracy
DILLTQSPAILSVSPGER*	0.035	-	-	-	-
	0.350	0.359	0.015	4.10	102.6
	3.500	3.210	0.026	0.81	91.7
	35.000	37.054	0.581	1.57	105.9
	350.000	327.304	13.672	4.18	93.5
Peptide	**QC Conc (µg/mL)**	**Mean Cal. Conc (µg/mL)**	**Std. Dev.**	**%CV**	**Mean Accuracy**
SINSATHYAESVK*	0.035	0.036	0.001	2.78	103.1
	0.350	0.331	0.003	0.80	94.5
	3.500	3.330	0.105	3.15	95.1
	35.000	38.287	1.168	3.05	109.4
	350.000	-	-	-	-
	QC Conc (µg/mL)	**Mean Cal. Conc (µg/mL)**	**Std. Dev.**	**%CV**	**Mean Accuracy**
DSTYSLSSTLTLSK	0.035	-	-	-	-
	0.350	0.333	0.010	2.85	95.3
	3.500	3.271	0.186	5.70	93.5
	35.000	36.256	1.999	5.51	103.6
	350.000	369.975	7.432	2.01	105.7

Table 2. Statistics for QC samples from all infliximab peptides used for quantification.

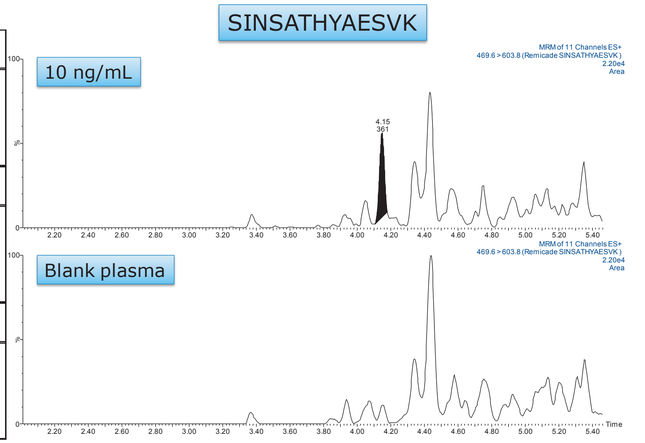

Figure 2. Chromatogram showing 10 ng/mL of infliximab in rat plasma, as compared to blank rat plasma. Infliximab is quantified using the unique peptide SINSATHYAESVK

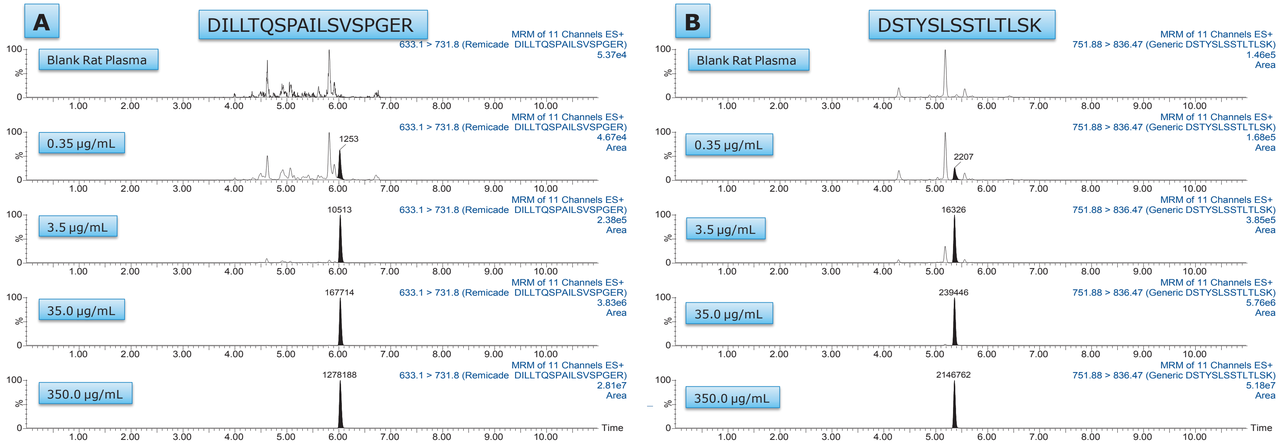

Figure 1. Representative QC chromatograms of Infliximab using the DILLTQSPAILSVSPGER (A) and DSTYSLSSTLTLSK (B) tryptic peptides.

DISCUSSION

With the infliximab US patent expiration date of 2017 drawing ever closer[1], the focus on this important drug in CRO's as well as biosimilar research labs has increased. In this work, we have used a commercially available digest kit to simplify and streamline the protein quantification workflow.

- Including affinity purification (Protein A), digestion with a commercially available kit, and peptide purification (SPE), total sample preparation time was <6 hours.
- Using several tryptic peptides, successful quantification of infliximab was achieved with standard curves linear between 3.5-4 orders of magnitude and mean accuracies >98% for all points (Table 1).
- QC samples (Table 2) at all levels easily met recommended FDA regulatory criteria[2] with mean accuracies ranging from 92-110% and %CVs <6%, indicating an accurate, precise, and reproducible method. Representative chromatograms are highlighted in Figure 1.
- Using the optimized protocol and reagents provided in the kit and only 35 µL of plasma, a limit of 10 ng/mL was achieved for the SINS peptide, as seen in Figure 2.[3]

CONCLUSION

A generic kit-based approach was successfully used to purify infliximab from a typical set of standard curves and QC samples in rat plasma. A limit of quantification of 10 ng/mL was readily achieved, while maintaining excellent linearity and single digit precision. The total sample prep time, including an affinity purification step, was under 6 hours. The total digest prep time was just over 2 hours. The universal, kit-based approach allows novice users to achieve ultra-low detection limits with a simple, step-wise protocol and a set of standardized, pre-measured reagents, ensuring both the sensitivity required and the transferability desired of such methods.

References

1. McKinsey and Company; Data Source: Evaluate Pharma, US Patent Expiration Dates.
2. FDA Guidance for Industry for Bioanalytical Method Validation, CDER
3. Application Note: 720005535EN, High Sensitivity Quantification of Infliximab in Rat Plasma Using a Fast, Standardized Kit-Based Approach

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