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Applikationsbericht

LC-MS/MS Analysis of mAbs Using a Monoclonal Antibodies Quantification Kit – Spotlight on Infliximab

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Disclaimer

The data presented in this technical note combine the use of a kit dedicated to the preparation of samples and the use of liquid chromatography and mass spectrometry instrumentation to perform the quantitative analyses.

The mAbXmise Kit described has not been cleared by any regulatory entity for diagnostic purposes outside of Europe. The end user is responsible for completion of the method development and validation. Promise Proteomics mAbXmise Kits are not available for sale in all countries. For information on availability, please contact your local sales representative.'

Dies ist ein Applikationsbericht, der keinen detaillierten Abschnitt zu Versuchen enthält.

Abstract

Therapeutic monoclonal antibodies (t-mAbs) have been a revolution in the therapeutic tools available to clinicians for treating a variety of conditions. In the era of personalized medicine, there is increasing awareness of the need to measure mAbs for the purposes of dose optimization and cost management. The use of Ligand

Binding Assay (LBA) based techniques for measuring mAbs is well established but has some limitations, including poor performance, lack of standardization, a high cost when processing a limited number of samples, limited dynamic range, and the potential for cross-reactivity. Moreover, commercial kits are available for a limited number of mAbs. Mass spectrometry, a technology widely used in clinical laboratories for monitoring small molecules, is an interesting alternative to overcome these limitations. In this application brief, we demonstrate that Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) associated with the ready-to-use commercial mAbXmise kit is a simple way to implement mAbs measurement, providing high analytical performance, ease of use, and high flexibility for laboratory personnel. The use of this approach to perform Infliximab (IFX) measurement is highlighted in this application brief.

Introduction

t-mAbs are an important class of drugs used to treat a variety of conditions ranging from inflammatory bowel diseases to a variety of cancers. More than 100 antibody therapeutics are now approved by FDA and during the last five years, a mean of ten antibody therapeutics have been granted first approval in the EU and US each year. These molecules have revolutionized patient management and outcomes and are now a major part of therapeutic tools used by clinicians. A relationship between t-mAb concentration and efficacy has been reported, as well as loss of efficacy due to immunogenicity. Due to these observations and because of their high cost, there is increasing awareness of the need to measure t-mAbs.[1] Moreover, biosimilars are increasingly popular following the patent expiry of some of the pioneering mAbs on the market. In particular, IFX now has over five biosimilars on the market.

Ligand-binding assays were the first approaches available to measure mAb concentrations and soon became the go-to technology for their measurement in clinical laboratories. However, LBA can suffer drawbacks such as lack of reproducibility due to reagent lot-to-lot variability and poor harmonization among commercial methods.[2] To overcome these limitations, alternative technologies have been developed, such as methods based on LC-MS, which is a technology already used in laboratories for analysis of small molecules. The development of LC-MS/MS surrogate peptide approaches for protein-level analysis is well established in the field of proteomics but is yet to be widely adopted for routine quantification of t-mAbs in a clinical setting. This is mainly due to the lack of easy to implement commercial solutions available for the measurement of proteins using LC-MS/MS.

Use of LC-MS/MS and mAbXmise Kit for IFX Measurement

IFX is a well-established t-mAb used for treatment of inflammatory bowel conditions. Development of LC-MS/MS based methods for measuring IFX have been described in the literature.[3,4,5,6] Methods usually rely on a concentration measurement by LC-MS/MS through mass detection of signature tryptic peptides, which provide good levels of selectivity and therefore precision and accuracy for IFX analysis over a wide dynamic range. The recent availability of ready-to-use LC-MS kits to prepare plasma/serum samples prior to LC-MS analysis is certainly one important step to enable wider adoption of LC-MS approaches for mAbs measurement. The day-to-day reproducibility and usability of the LC-MS/MS LDT method could be optimized through the adoption of commercially available LC-MS kits such as the Promise Proteomics mAbXmise kits, which uses full-length isotopically labelled mAbs (SIL-mAbs) as internal standards, allowing for reproducible, robust, and accurate quantification of mAbs.[4] Furthermore, a kit format provides lot-to-lot reproducible standards, Quality Controls (QCs), reagents, and consumables to help with method harmonization across LC-MS platforms and facilitates adoption across different laboratories.

Utilizing the surrogate peptide methodology and the Promise Proteomics mAbXmise sample preparation consumables, IFX can be measured using signature tryptic peptides, with selectivity of the method achieved through sample preparation, chromatographic separation, and Multiple Reaction Monitoring (MRM) mass detection.

Sample Preparation with Promise Proteomics mAbXmise Kit

The mAbXmise Instructions For Use (IFU) were followed to prepare the samples for LC-MS/MS analysis. The workflow procedure for the quantification of infliximab is highlighted in Figure 1. The workflow steps include:

1. Sample preparation: 20µL plasma samples, including calibrator, and quality controls, are dispensed into the mAbXmise plates containing lyophilized stable labelled infliximab.

2. Sample purification: Samples are transferred to the PuriXmise plate to perform the immunocapture of infliximab and allows washing of the sample to reduce matrix interferences. Samples are eluted into a collection plate and evaporated to dryness.

3. Sample digestion: Samples are resuspended, and the protease (CutXmise) is added to the sample to digest infliximab overnight. The digestion is quenched, with the samples immediately ready for analysis.

4. LC-MS analysis: Samples are injected and analysed on the LC-MS instrument to detect labelled, and nonlabelled peptides. Peptide transitions for infliximab and its SIL standard are shown in Table 1.

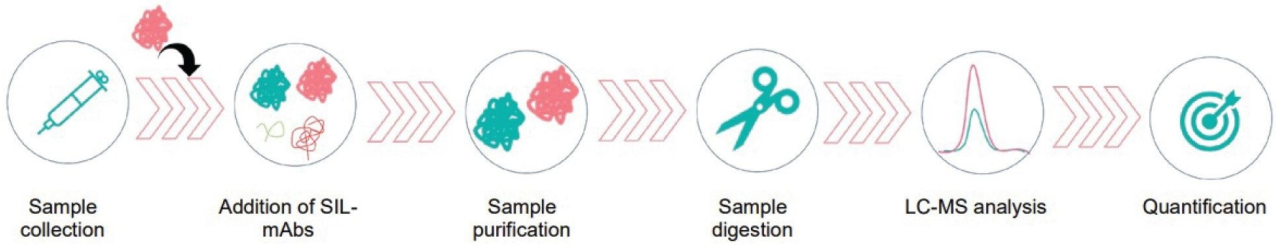

Figure 1. The LC-MS/MS workflow for the analysis of infliximab using the Promise Proteomics mAbXmise Kit (https://www.mabxmise.com/).

The calibrators, QCs and internal standard provided in the mAbXmise kit were used to demonstrate proof of performance, with calibrators ranging from 2–100 µg/mL and QCs at 4 and 25 µg/mL. In addition, analysis of 29 IFX plasma samples enabled comparison between different IFX signature peptide concentrations from the analysis.

LC-MS/MS Analysis

Samples were injected on to an ACQUITY UPLC™ I-Class FL with separation on the XSelect™ Premier HSS T3, 2.1 mm x 50 mm, 2.5 µm Column using an acetonitrile/water/formic acid gradient with a 4.5 minute run time and detection performed using the Xevo™ TQ-XS Mass Spectrometer. Re-analysis of the samples was performed using the same setup with a Xevo TQ-S micro Mass Spectrometer. Signature peptide MRM transitions for the analysis are shown in Table 1.

Signature peptide	Abbreviation	Target	MRM	Cone (V)	Collision (V)
SINSATHYAESVK	SIN	Quan (Qual)	469.8>603.8 (546.8)	40	10 (12)
		SIL	472.5>607.8	40	10
GLEWVAEIR	GLE	Quan (Qual)	536.8>488.3 (773.5)	40	14
		SIL	541.8>498.3	40	14
SAVYLQMTDLR	SAV	Quan (Qual)	648.8>763.4 (876.5)	40	18
		SIL	653.8>773.4	40	18
DILLTQSPAILSVSPGER	DIL	Quan (Qual)	632.9>731.4 (844.5)	40	16
		SIL	636.0>741.4	40	16
ASQFVGSSIHWYQQR	ASQ	Quan (Qual)	598.6>754.4 (818.4)	40	15
		SIL	602.0>685.8	40	15

Table 1. Infliximab signature peptide (quantifier and qualifier) and stabled labeled internal standard transitions.

Results and Discussion

Analytical Sensitivity and Calibration Linearity

Multiple signature tryptic peptides were measured for IFX and it was found that SINSATHYAESVK (SIN), followed by ASQFVGSSIHWYQQR (ASQ) provided the greatest level of analytical sensitivity at the 2 µg/mL calibrator standard for IFX with the highest peak response and Signal:Noise (S/N) using both the Xevo TQ-XS and Xevo TQ-S micro. All peptides provided a S/N (PtP) >10:1, indicating that these peptides are suitable for use as the LoQ for infliximab at 2 µg/mL. The calibration lines were found to be linear with r^2 >0.998 for each of the peptides on both systems.

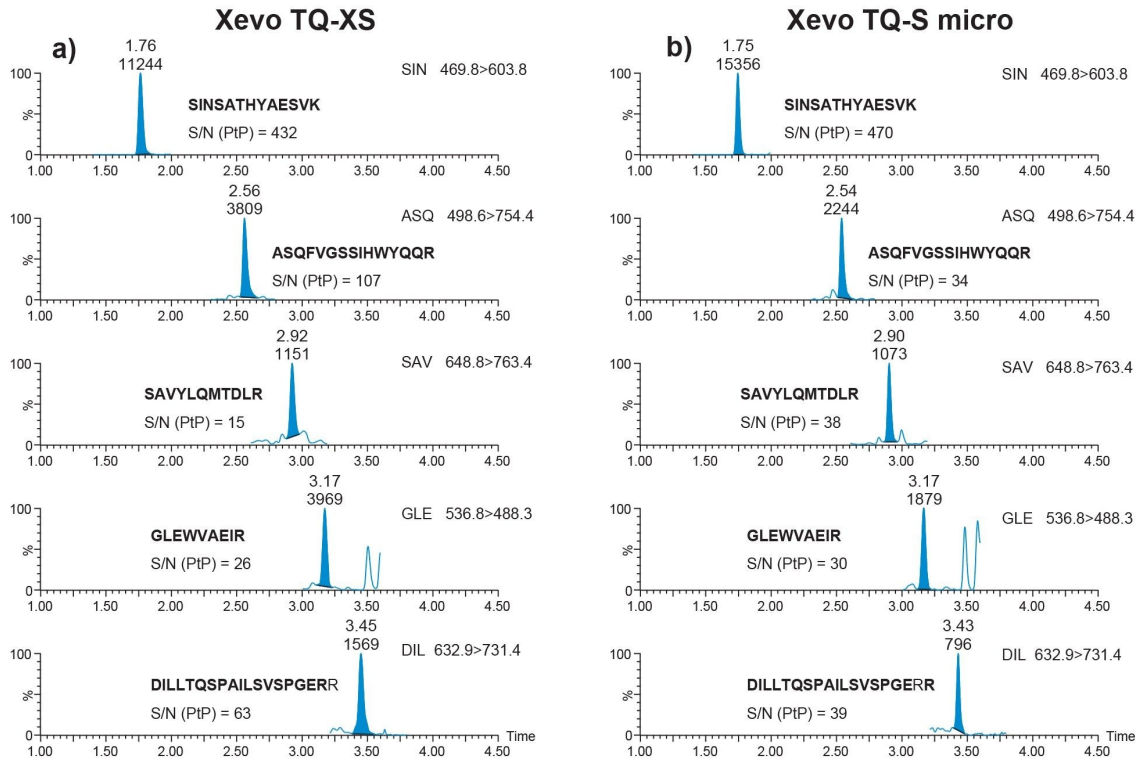

Figure 2. Analytical sensitivity of the method at 2 µg/mL for the five IFX signature peptides using the ACQUITY UPLC I-Class and Xevo TQ-XS (5 µL injection), and Xevo TQ-S micro (15 µL injection).

Precision

Precision on the Xevo TQ-XS and Xevo TQ-S micro was evaluated using the provided QC material over five replicates at 4 and 25 µg/mL. Total precision across the QC concentrations were ≤9.5% CV for the signature peptides of IFX with accuracies ranging from 95–108% compared to the nominal IFX concentrations (Table 2). The data demonstrates both the intra-day reproducibility of the Kit and the LC-MS/MS systems.

	QC 1 (4 µg/mL)				
	SIN	GLE	DIL	SAV	ASA
	Xevo TQ-XS				
Mean	4.1	4.2	4.1	4	4.2
SD	0.2	0.1	0.4	0.2	0.3
RSD	5.40%	1.20%	9.50%	4.80%	7.40%
Accuracy	104%	105%	102%	101%	105%
	Xevo TQ-S micro				
Mean	4	3.8	3.9	4.1	3.9
SD	0.1	0.3	0.2	0.3	0.4
RSD	3.70%	6.50%	6.20%	6.60%	10.00%
Accuracy	100%	96%	97%	102%	97%

	QC 2 (25 µg/mL)				
	SIN	GLE	DIL	SAV	ASA
	Xevo TQ-XS				
Mean	26	25.7	25.4	26.4	25.3
SD	0.7	0.6	0.9	1	1.3
RSD	2.70%	2.30%	3.40%	3.70%	5.00%
Accuracy	104%	103%	101%	105%	101%
	Xevo TQ-S micro				
Mean	26.3	25.5	27.1	26.8	23.7
SD	0.7	1.3	1.7	0.6	1.1
RSD	2.50%	5.10%	6.40%	2.20%	4.40%
Accuracy	105%	102%	108%	107%	95%

Table 2. Precision and accuracy of IFX signature peptides SIN, GLE, DIL, SAV, and ASA over five replicates at 4 and 25 µg/mL in serum using the Xevo TQ-XS and Xevo TQ-S micro.

Peptide Comparison

LC-MS/MS analysis of 29 IFX plasma samples was performed to enable comparison of different signature tryptic peptides of IFX. Figure 3 demonstrates the differences observed between the IFX signature peptides from the

Xevo TQ-XS analysis, with the SIN peptide observed at lower concentrations relative to the other peptides in the sample. The mean reproducibility for the five peptides across the 29 samples was much higher (CV 29%) compared to the mean reproducibility of the four peptides with SIN removed (CV 10%).

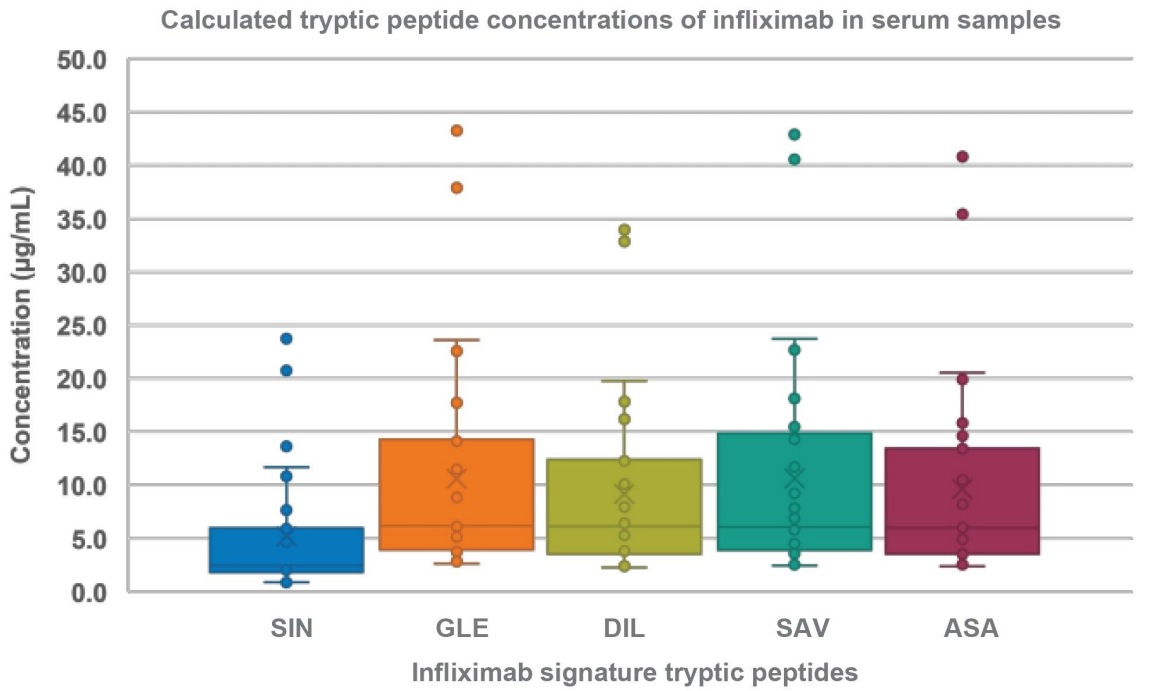

Figure 3. Comparison of the LC-MS/MS signature peptide concentrations for 29 IFX plasma samples.

The reduction in the IFX SIN peptide concentration could be explained by its susceptibility to deamidation, with an increase of 0.98 Da in its molecular mass resulting from deamidation at the asparagine-serine (N-S) motif in unadulterated plasma samples, causing a reduction in IFX SIN unlabeled peptide for the selected MRM transition, which isn't compensated by the calibrator and QC materials containing spiked materials.[7] Based on this data and supporting information, for the purposes of this method, it is recommended that laboratories evaluate and select peptides used to quantify infliximab.

Conclusion

Solutions for quantifying therapeutic mAbs using LC-MS/MS technology are now available for clinical studies. In this application brief, an example is given for quantifying Infliximab in plasma using a commercially available kit for sample preparation followed by LC-MS/MS analysis.

The use of the Promise Proteomics mAbXmise Kit makes the analysis accessible and easy to implement, while also being amenable to automation. The extended measuring range, between 2 µg/mL and 100 µg/mL, avoids the requirement for dilution sometimes observed with immunoassays, improving turnaround times for higher concentration samples. In addition, reduction in dilution and re-analysis improves utilization of the kit, which aids cost management. The Kit aids in the day-to-day reproducibility of the immunocapture and tryptic digestion of IFX for targeted LC-MS/MS analysis with the correct peptide selection. Compared to a direct digest surrogate peptide workflow, the process used in the kit reduces matrix interference, improves analytical sensitivity, and enables the use of lower sample volumes.

It has been demonstrated that the method can be run on both the Xevo TQ-XS and Xevo TQ-S micro Mass Spectrometers which provide the dynamic range to quantify IFX across the expected range, and the selectivity and analytical sensitivity to obtain low-level quantification of the IFX surrogate peptide in serum samples.

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Specific Mentions

This Application Brief is for Research Use Only, not for use in diagnostic procedures.

In Europe, mAbXmise kits are in vitro diagnostic medical devices for professional laboratory use. mAbXmise kits determine the plasma concentration of monoclonal antibodies. Analytical performance depends on the instrument characteristics and its settings. A validation of the analytical method shall be conducted according to internal practices. Consult the specific instructions for each for more information. PROMISE Proteomics products are distributed globally, so uses, applications, and availability of product in each country depend on local regulatory registration status. Products not registered and all other products are For Research Use Only. Manufacturer PROMISE PROTEOMICS SAS 7, parvis Louis Néel ▪ 38040 Grenoble France ▪ Phone +33 4 38 02 36 50 ▪ contact@promise-proteomics.com ▪ RCS Grenoble B 433 546 504

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