Quantification of Infliximab and Adalimumab and their anti-drug antibodies in human plasma by liquid chromatography-tandem mass spectrometry using ready-to-use kits

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Management of inflammatory bowel disease (IBD) has been performed and dominated by monoclonal antibodies (mAbs), specifically Infliximab (IFX) and Adalimumab (ADM), for almost 15 years. These biological molecules show a high interpatient variability and sometimes the formation of antidrug antibodies (ADAbs), leading to reduced efficacy. Therapeutic drug monitoring (TDM), measures the concentrations of drug and ADAbs in individuals to guide treatment decisions and has become a valuable tool to optimize therapy. Several assays have been previously developed to perform TDM, with the most common being enzyme-linked immunosorbent assays (ELISAs). However, in the last 5 years, LC-MS methods have been established and when combined with the use of full-length isotopically labelled mAbs (SIL-mAbs) can be powerful quantitative methods. Here, we describe a LC-MS based method using a ready-to-use kit for a rapid implementation of a multiplex quantitative assay for monitoring IFX and ADM and the ADAbs.

Global workflow including plasma sample preparation for quantification of mAbs

Plasma samples were prepared with the mAbXmise INF-TDM-01 kit developed and commercialized by PROMISE Proteomics, France. mAbXmise INF-TDM-01 is a ready to use kit dedicated to the quantification of Infliximab (IFX) and Adalimumab (ADM). The kit is a unique commercial solution combining stable-isotopically versions of therapeutic IFX and ADM, ready to use calibrators and QC, and all the reagents and consumables required for the plasma sample preparation prior to LC-MS injection.

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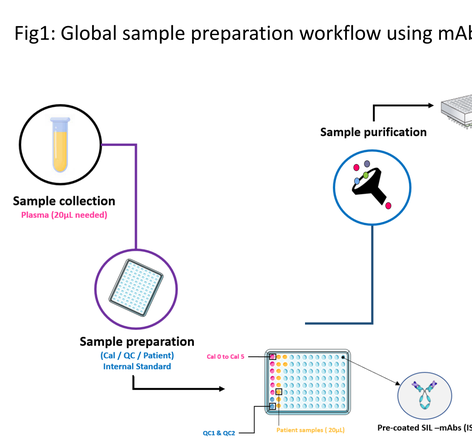
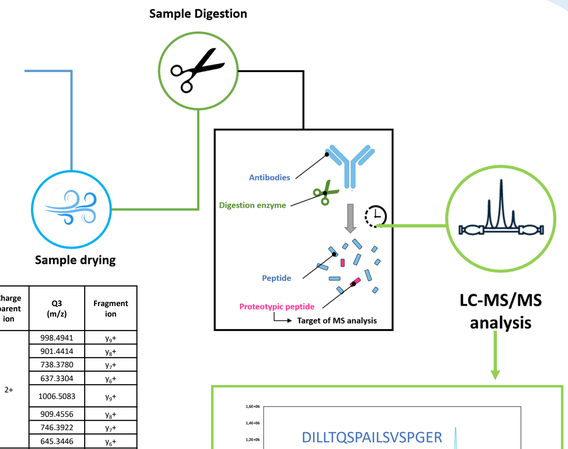
Fig1: Global sample preparation workflow using mAbXmise kit





Monoclonal antibody	Peptide sequence	Q1 (m/z)	Charge parent ion	Q3 (m/z)	Fragment ion
Adalimumab	APYTFGQGTK	535.26928	2+	998.4941	y_9^+
		535.26928		901.4414	y_8^+
		535.26928		738.3780	y_7^+
		535.26928		637.3304	y_6^+
Adalimumab	APYTFGQGT**[13C6,15N2]K**	539.27638		1006.5083	y_9^+
		539.27638		909.4556	y_8^+
		539.27638		746.3922	y_7^+
		539.27638		645.3446	y_6^+
Infliximab	DILLTQSPAILSVSPGER	948.52	2+	1212.6583	y_{13}^+
		948.52		1125.6262	y_{11}^+
		948.52		1028.5735	y_{10}^+
		948.52		957.5364	y_9^+
Infliximab	DILLTQSPAILSVSPGE**[13C6,15N4]R**	953.52		1222.6665	y_{12}^+
		953.52		1135.6345	y_{11}^+
		953.52		1038.5818	y_{10}^+
		953.52		967.5446	y_9^+

List of peptides monitored

Fig2: SRM chromatogram

Analytical performances of the LC-MS/MS assay developed for quantification of mAbs

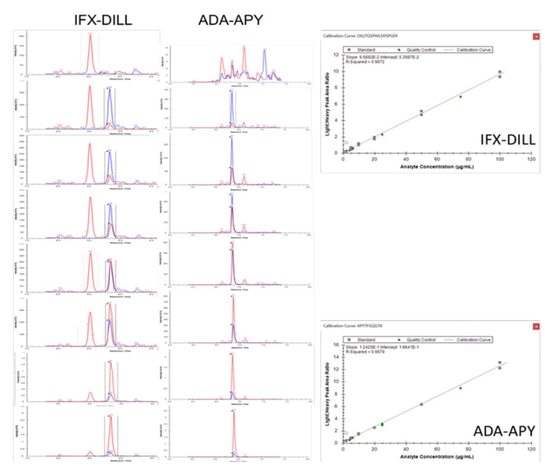
Chromatography: Chromatographic separation of peptides was achieved using a Phenomenex Biozen™ peptide XB-C18 column with a 2.6 µm particle size. Mobile phases consisting of water and acetonitrile with 0.1 % formic acid were used alongside a gradient with a final run time of 20 minutes.

Mass spectrometry: MS analysis was performed using the SCIEX QTRAP 6500 LC-MS/MS System with the IonDrive™ Turbo V Source using electrospray in the positive ionization mode. Both analytes and internal standards were optimized to determine compound dependent parameters (entrance potential (EP), declustering potential (DP), collision energy (CE), collision cell exit potential (CXP)).

Quantitative performances: The mAbXmise® kit provides optimal performance for the quantification of Infliximab and Adalimumab by the utilization of LC-MS and isotopically labelled internal standards. In performing method verification: sensitivity, specificity, precision, accuracy, linearity, carry-over, stability, recovery and matrix effects were assessed; passing the respective criteria set. Calibration curves were analysed by spiking known concentrations of analyte into plasma (0, 2, 5, 10, 20, 50, 100 mg/mL), with the LLOQ of the method for both analytes being established at 2 mg/L. Double blank samples were analyzed to assess specificity along with QC samples at four concentrations (2, 6, 25, 75 mg/L) to evaluate inter and intra assay precision and accuracy.

Table 1: Intra- and inter- assay accuracy and precision of adalimumab (ADL) and infliximab (IFX) measured in plasma with mAbXmise kit, Results obtained with originators drugs Remicade® and Humira®

Nominal concentration (µg/mL)	Mean Calculated concentration (µg/mL)	Intra-day accuracy (%, n = 8)	Intra-day precision (%, n = 8)	Mean Calculated concentration (µg/mL)	Inter-day accuracy (%, n = 16)	Inter-day precision (%, n = 16)
ADL - APYTFGQGTK						
2	1.9	-4.4	5.3	2.2	9.6	11.6
6	6.0	0.2	2.6	6.1	2.4	2.1
25	26.4	5.7	1.8	25.7	2.8	3.1
75	76.9	2.5	1.9	73.7	-1.7	4.9
IFX -DILLTQSPAILSVSPGER						
2	2.2	9.3	10.1	2.2	10.8	1.3
6	6.2	3.6	4.5	6.1	2.3	1.3
25	26.0	4.1	4.7	25.3	1.4	3.2
75	75.6	0.8	2.1	72.1	-3.9	4.4



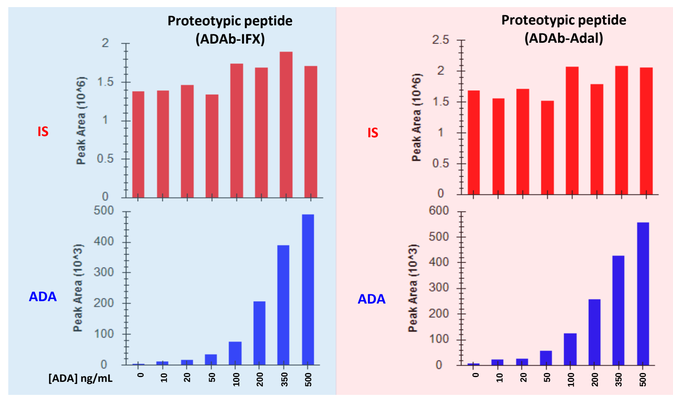
Analytical performances of the LC-MS/MS assay developed for quantification of ADAbs

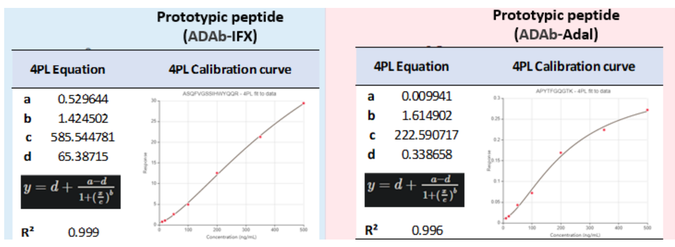
As any therapeutic proteins, drug mAbs can elicit an immune response with the resulting production of anti-drug antibodies (ADAbs). Promise Proteomics is developing a kit product to detect and quantify by LC-MS the ADAbs in plasma of patients treated with Infliximab or Adalimumab. This strategy, under patent, is used to develop a ready-to-use, multiplex and high throughput kit for *in vitro* diagnostic use.

Chromatography: Chromatographic separation of peptides was achieved using a Thermofisher Accucore Vanquish C18+ column with a 1.5 µm particle size. Mobile phases consisting of water and acetonitrile with 0.1 % formic acid were used alongside a gradient with a final run time of 15 minutes.

Mass spectrometry: MS analysis was performed using the SCIEX TQ 6500+ LC-MS/MS System with the IonDrive™ Turbo V Source using electrospray in the positive ionization mode. Both analytes and internal standards were optimized to determine compound dependent parameters (entrance potential (EP), declustering potential (DP), collision energy (CE), collision cell exit potential (CXP)).

Quantitative performances: The prototype kit provides optimal performance for the quantification of ADAbs of Infliximab and Adalimumab by the utilization of LC-MS. The range of quantification is 10-400 ng/mL with the LOD at 10ng/mL and the LLOQ at 10-20ng/mL. The curve is using 4PL regression with R^2 >0.99, an accuracy ≤ ± 20% and a precision RSD ≤ ± 20% for the back-calcutated concentrations of the calibrants and the quantification of QC.
This strategy provides advantage compare the ELISA assay: Multiplexing, Specificity and ability to point putative false results due to Rheumatoid factor.





Quantification of ADAbs using the kit patented by Promise Proteomics