TANDEM QUADRUPOLE MS FOR THE QUANTIFICATION OF MONOCLONAL ANTIBODY SUBUNIT LIGHT CHAINS IN PLASMA

Caitlin M. Dunning, Mary E. Lame, and Mark D. Wrona
Waters Corporation, Milford, MA, USA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

INTRODUCTION

- As complexity of biotherapeutics and the desire to monitor these complexities *in vivo* increases, the need for both more sensitive and selective quantitative approaches rises.
- Although surrogate peptide methodology remains popular, more direct measurements of intact monoclonal antibodies (mAb) or subunits is useful.
- HRMS is preferred for initial characterization, monitoring of fine structure, and even discovery quantification. In many instances it may be appropriate and possible to transfer to tandems for longer term studies.
- We demonstrate here feasibility for the development and optimization of sample preparation and LC-MS/MS (tandem) methodology for the sensitive quantification of adalimumab subunit light chains.

METHODS

Sample Preparation

Adalimumab was immunopurified from rat plasma (10 µL) with biotinylated goat anti-human Fc Ab (15 µL of 0.5 mg/mL) coupled to streptavidin coated magnetic beads (25 µL of 20% slurry). The affinity purified eluates (50 µL) were neutralized to pH 8.0, then reduced with dithiothreitol to a mixture of light and heavy chains. Samples were then alkylated with iodoacetamide and finally acidified with formic acid (70 µL final volume) as seen in Figure 1.

LC System: **ACQUITY UPLC I-Class PLUS (Fixed Loop)**

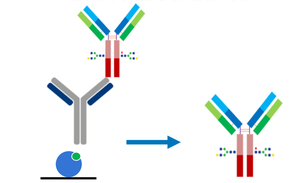
- Column: BioResolve RP mAb Polyphenyl[1], 450Å, 2.7 µm, 2.1 x 50 mm
- Column Temperature: 80°C
- Mobile Phases: A: 0.1% formic acid in water
  B: 0.1% formic acid in acetonitrile
- Cycle Time: 8.5 minutes
- Injection Volume: 10 µL

MS System: **Xevo TQ-XS Mass Spectrometer**

- Capillary Voltage: 2.4 kV
- Cone Voltage: 60 V
- Source Temperature: 150°C
- Desolvation Temperature: 600°C
- System Calibration: Low Resolution (1.0 Da FWHM)

WORKFLOW

A **AFFINITY CAPTURE OF BIOTHERAPEUTICS**



Capture with Anti-human Fc Ab

Release with acid

REDUCE TO SUBUNITS AND ALKYLATE FOR STABILITY

ProteinWorks Auto-eXpress Reduction/Alkylation Reagents

Mixture of Light and Heavy Chains

B **LC-MS/MS ANALYSIS**

Adalimumab Light Chain Sequence:
DIQMTQSPSSLSASVGDRVTITCRASQGIRNYLA
WYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGS
GTDFTLTISSLQPEDVATYYCQRYNRAPYTFGQG
TKVEIKRTVAAPSVFIF|PPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS
SPVTKSFNRGEC

FRAGMENT IDENTIFICATION			
mAb	Precursor (*m/z*)	Fragment (*m/z*)	Fragment Identity
Adalimumab	1236.02	1329.85	P119 – y96
Cetuximab (ISTD)	1236.81		

Figure 1. Sample preparation and LC-MS/MS workflow for the quantification of mAb subunit light chains[2]

- *(A) mAbs are immunopurified from rat plasma, reduced to light and heavy chain subunits, and alkylated for stability*
- *(B) A common MS/MS fragment (1329.85 m/z) was identified from the conserved region of the light chain and used for quantification of adalimumab and its ISTD cetuximab*

RESULTS

CHROMATOGRAPHY

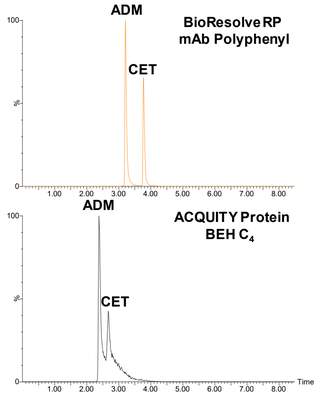


Figure 2. Chromatographic separation of adalimumab (ADM) and cetuximab (CET) via BioResolve and BEH C_4 columns

- *BioResolve: 450Å, 2.7 µm, 2.1 x 50 mm*
- *BEH C_4: 300Å, 1.7 µm, 2.1 x 50 mm*

MASS SPECTROMETRY

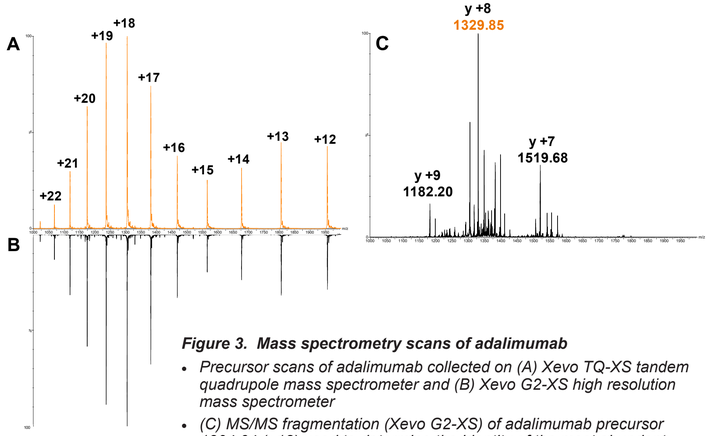


Figure 3. Mass spectrometry scans of adalimumab

- *Precursor scans of adalimumab collected on (A) Xevo TQ-XS tandem quadrupole mass spectrometer and (B) Xevo G2-XS high resolution mass spectrometer*
- *(C) MS/MS fragmentation (Xevo G2-XS) of adalimumab precursor 1304.64 (+18) used to determine the identity of the most abundant fragment ions*

QUANTITATIVE PERFORMANCE

A **CALIBRATION CURVE STATISTICS**

Curve (ng/mL)	Weighting	Linear Fit (R^2)	% Accuracy	LLOQ Amount on Column (pg)
25 – 100,000	$1/x^2$	0.993	87.0 – 108.9	35.7

B **QC STATISTICS**

QC Level	QC Concentration (ng/mL)	Mean (N=5) Calculated QC Concentration (ng/mL)	Mean (N=5) % Accuracy	% RSD
LLOQ	25	24.4	97.4	6.1
LQC	75	74.7	99.6	2.9
MQC	2,500	2798.3	111.9	2.5
HQC	80,000	71386.7	89.2	1.9

Table 1. Calibration and QC statistics for the quantification of adalimumab from rat plasma

- *(A) Calibration curves were linear (r^2 > 0.99) over the range of 25—100,000 ng/mL*
- *(B) QC sample statistics were excellent, achieving accuracies ranging 89—112% and single digit RSDs (< 6%)*

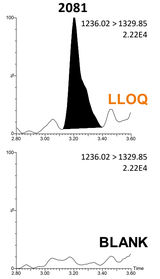


Figure 4. Representative blank and LLOQ QC chromatograms of adalimumab extracted from 10 µL of rat plasma

DISCUSSION

Chromatography

- The use of a **BioResolve** column vastly improved peak shape, tailing, and signal as compared to a **BEH C_4** column, enabling rapid separation of adalimumab and cetuximab light chain subunits *(Figure 2)*

Mass Spectrometry

- Comparable charge state envelopes were acquired for adalimumab on a **tandem quadrupole MS** *(Figure 3A)* and **high resolution MS** *(Figure 3B)* demonstrating the capability of TQ-MS systems to analyze larger molecules (> 10 kDa)
- Utilizing the accurate mass of our **HRMS system** during assay development, **common MS/MS fragments** were identified from the conserved region of human IgG light chains *(Figures 1B and 3C)*

Quantitative Performance

- Quantitative performance was excellent achieving a dynamic range of 25—100,000 ng/mL, and QC accuracies from 89—112% with RSDs < 6% *(Table 1)*
- Chromatographic performance at the **LLOQ** is illustrated in *Figure 4*, highlighting the sensitivity and specificity of the assay

CONCLUSIONS

A sensitive, fast, and widely applicable LC-MS/MS method for the accurate quantification of mAb subunit light chains via tandem quadrupole MS has been achieved and enabled by:

- Selective and specific sample preparation workflows
- Superior chromatography with the use of a BioResolve column
- Identification of generic and sensitive MS/MS fragments for quantification

REFERENCES

1. Nguyen, J.M.; Kizekai, L.; Walsh, D.; Cook, J.; Lauber, M.A.; A Novel Phenyl Bonded Phase for Improved Reversed-Phase Separations of Proteins. Waters Application Note 720006169EN, January 2018.
2. Dunning, C.M.; Lame, M.; Wrona, M.; Haynes, K.; Tackling Non-Specific Binding of Biotherapeutics Using LC-MS Compatible QuanRecovery Sample Plates with MaxPeak High Performance Surfaces. Waters Application Note 720006528en, March 2019.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS