A Bioanalytical Method for Quantitation of Monoclonal Antibody Therapeutics in Animal Biofluids by Liquid Chromatography and Tandem Mass Spectrometry

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PURPOSE

The recent decade has seen an exponential growth in the discovery and development of monoclonal antibody (mAb) therapeutics owing to their advantages over traditional small molecule drugs. More than 20 mAbs have been approved as drugs by the FDA, and nearly 300 mAbs are currently under development.[1]

Accurate bioanalytical methods are critical for successful quantitation of mAbs in biological matrices during preclinical and clinical development. Since a majority of these drugs are based on IgG1 isotype, a bioanalytical method that is applicable for quantitation of all IgG1 derived mAbs is highly desirable.

Currently, mass spectrometry based approaches are receiving attention as an attractive alternative to ligand binding assays (e.g. ELISA) that suffer from several limitations. Here we describe the development of an LC-MS/MS method for quantitation of IgG1 derived mAb therapeutics in animal matrices using rituximab (chimeric mAb) as a model drug.

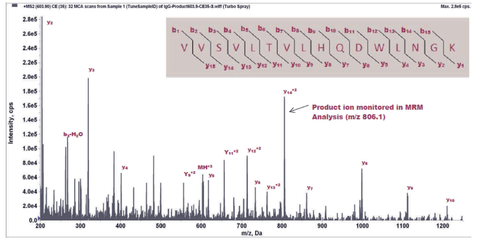
METHODS

Development of an LC-MS/MS method to quantitate mAb drugs in biological matrices required the selection of a surrogate peptide whose sequence satisfies selection rules.[2] Literature review and in silico analysis of the mAb sequence suggested that the VVSVLTVLHQDWLNGK sequence from the CH2 domain of the heavy chain could be a potential surrogate peptide.[3] A synthetic stable isotope ($^{13}C_5$ ^{15}N serine and $^{13}C_5$ ^{15}N threonine) labeled version of the surrogate peptide was then chosen as the internal standard (IS).

The assay method developed in this study involved extraction of the mAb from the matrix by protein precipitation followed by digestion and subsequent quantitation of the surrogate peptide by LC-MS/MS. Rat plasma was spiked with rituximab (model mAb) at various concentrations between 1 and 500 µg/mL. To 0.0250 mL of spiked rat plasma, 0.200 mL of MeOH was added and the resulting pellet was separated from the supernatant. The pellet was then reduced with dithiothreitol (DTT) and alkylated with iodoacetamide prior to trypsin digestion at 37 °C. The digested samples were analyzed using a Waters Acquity UPLC system equipped with an AB SCIEX API 5000 triple quadrupole mass spectrometer using an ESI source. Peptide ions were monitored in multiple reaction monitoring (MRM) mode. Surrogate peptide and IS ion transitions were 603.7→806.1 and 607.6→811.7 *m/z*, respectively.

RESULTS

Figure 1. MS/MS product ion spectrum of the surrogate peptide (precursor ion $[M+3H]^{3+}$; *m/z* 603.7)



MS/MS analysis confirmed the identification of the surrogate peptide generated by digestion.

Selectivity
Figure 2. Extracted ion chromatograms of (A) blank matrix and (B) rituximab plasma standard (1 µg/mL).

Linearity of Rituximab Plasma Standards

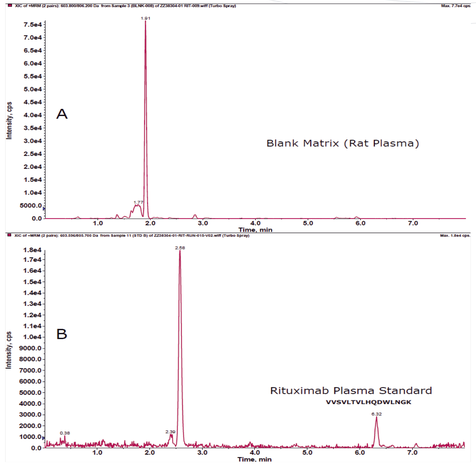
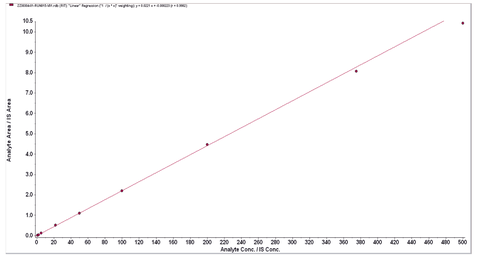


Table 1. Rituximab calibration standards from 1.00 – 500 µg/mL

Rituximab Nominal Concentration	Interpolated Concentration	% Nominal	Peak Area Ratio	Response Factor
1.00	1.02	102.0	0.0222	0.0222
2.00	1.84	92.2	0.0405	0.0202
5.00	5.52	110.0	0.122	0.0243
22.0	23.0	105.0	0.508	0.0231
50.0	49.4	98.9	1.09	0.0218
100	99.3	99.3	2.19	0.0219
200	202	101.0	4.46	0.0223
375	365	97.4	8.06	0.0215
500	472	94.4	10.4	0.0208

Standards were linear from 1.00-500 µg/mL

Figure 3. Calibration curve of rituximab plasma standards.



Precision & Accuracy
Precision and accuracy results at LLOQ, QC A, QC B and QC C concentrations met acceptance criteria

Table 2. Precision and accuracy results for rituximab quality control samples in rat plasma.

	LLOQ	QC A	QC B	QC C
	1.00	3.00	22.0	375
	1.02	3.10	23.3	349
	0.98	2.83	22.4	366
	1.08	2.88	22.2	385
	1.00	3.13	23.6	377
	0.86	3.90	21.1	380
	1.25	3.13	21.6	378
Mean	1.03	3.16	22.4	373
% CV	12.6	12.2	4.3	3.5
% Theoretical	103.0	105.3	101.8	99.5
n	6	6	6	6

Matrix Effect
Matrix effect assessed by quantitative accuracy in 6 lots of rat plasma (EDTA) fortified with rituximab at the LLOQ (1.00 ug/mL) and the high QC (385 ug/mL) met acceptance criteria.

Table 3. Matrix effect results for rituximab quality control samples in 6 lots of rat plasma.

		LLOQ		High	
	Lot#	1.00 ng/mL	% Dev.	375 ng/mL	% Dev.
	1	1.10	+10.0	397	+5.9
	2	0.978	-2.2	380	+1.3
	3	0.972	-2.8	389	+3.7
	4	0.987	-1.3	388	+3.5
	5	1.01	+1.0	379	+1.1
	6	0.997	-0.3	433	+15.5
Mean		1.01		394	
% CV		4.7		5.1	
% Theoretical		101.0		105.1	
n		6		6	

CONCLUSIONS & FUTURE WORK

- An LC-MS/MS assay method for quantitation of IgG1 mAb therapeutics was developed successfully
- Selectivity, precision and accuracy, and matrix effects results met acceptance criteria
- The LC-MS/MS method developed in the current study will be compared with an immunoassay method for quantitation of IgG1 mAb therapeutics
- The method will be evaluated in other animal matrices that are commonly used in pre-clinical studies

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3. Halquist, M. S. and Karnes, H. T. Biomed Chromatogr. 2011, 25, 47-58

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