Quantification of Multiple Therapeutic mAbs in Serum
Using microLC-ESI-Q-TOF Mass Spectrometry

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Introduction

Therapeutic monoclonal immunoglobulins (mAbs) are an important class of drugs used to treat diseases ranging from autoimmune disorders to B cell lymphomas. We previously reported using microLC-ESI-Q-TOF mass spectrometry to quantify intact kappa light chains from the therapeutic mAb adalimumab (Humira, Abbvie Pharmaceuticals) spiked into normal human serum with a linear dynamic range of 10 – 5,000 ug/mL (1). Also, our group recently published a method for quantifying total IgG and all four IgG subclasses using proteotypic peptides and LC-MS/MS (2). Here we combine the two methodologies to describe an accurate and precise approach to quantifying multiple therapeutic mAbs in serum using a single assay. Our findings demonstrate that using accurate molecular mass of kappa light chains serves as a fast and efficient alternative to other non-mass spectrometry based methods for quantifying this class of drugs.

Therapeutic mAbs are proteins consisting of 2 identical heavy chains and 2 identical light chains. They are also referred to as homodimers since each half of the dimer contains a light chain linked to a heavy chain through a disulfide linkage. The molecule is made functional when the two identical dimers are linked to each other through multiple disulfide bonds between the heavy chains. Therapeutic mAbs usually have a heavy chain that belongs to the IgG isotype and a light chain that belong to the kappa isotype. The light chain portion of the molecule has a molecular mass ranging from 22,000 to 24,000 Da while the heavy chain has a molecular mass ranging from 50,000 to 60,000 Da. This is in contrast to typical therapeutic drugs which have molecular masses < 1,000 Da and often have structures that are different than endogenous metabolites. The rise in the use of therapeutic mAbs has opened up new challenges for clinical chemists interested in monitoring this class of drugs using mass spectrometry since they are larger and more complex than typical therapeutic drugs. In addition, they must be quantified in samples that

contain endogenous polyclonal immunoglobulins with identical structure. Fortunately, accurate molecular mass can be used to quantify therapeutic mAbs using mass spectrometry.

Methods

We have coupled two distinct approaches in order to quantify therapeutic mAb drugs using mass spectrometry. Therapeutic mAb do not currently have certified reference standards therefore we use an IgG specific proteotypic peptide measured by LC-MS/MS to determine the accurate amount of mAb in pharmaceutical preparations as previously described for measuring IgG in serum (2). Briefly, eculizumab was obtained from the manufacturer and used as provided. Infliximab, rituximab and vedolizumab were purchased from the pharmacy and reconstituted in water to 10 mg/mL. All therapeutic mAbs were reduced, alkylated and digested with trypsin. **Table 1** shows the total IgG concentration for the therapeutic mAbs infliximab, rituximab, and eculizumab determined using the LC-MS/MS assay and infliximab, rituximab, eculizumab, and vedolizumab using a nephelometric assay. The results show that the total IgG concentration determined using both LC-MS/MS and nephelometry is in good agreement with the known concentration of the pharmaceutical preparation listed on the packaging (shown under the name of the mAb name). The total IgG concentration calculated using the LC-MS/MS assay for infliximab, rituximab, and eculizumab, and the total IgG concentration calculated using the nephelometry assay for vedolizumab, was used for making a high concentration stock that contained all four therapeutic mAbs to be used as part of standard curve in commercial pooled serum. A standard curve was then made by diluting the high stock into pooled serum to give standard curves with a concentration range from 25 to 400 µg/mL. Immunoglobulins were purified from the serum standards using Melon Gel (Thermo Fischer), reduced with DTT, and then analyzed by microLC-ESI-Q-TOF mass spectrometry as previously described (1). Quantification was performed by first summing mass spectra over the retention time window for each mAb and then deconvoluting the mass spectrum using the Bioanalyst program within the Analyst TOF 1.6 software.

Results

Figure 1 shows a deconvoluted mass spectrum displaying the accurate molecular masses for the kappa light chains from each of the four therapeutic mAbs observed using microLC-ESI-Q-TOF mass spectrometry. Peak areas for each therapeutic mAb at each standard concentration were

determined by manually integrating the peak in the deconvoluted mass spectrum using the integration program in Analyst. **Figures 2** through **5** show the results for the linear regression analysis for each therapeutic mAb with the linear equation and R^2 values shown in the insert. In addition, 10 replicates were made in the same manner as the standards at a concentration of 200 ug/mL. **Table 2** shows the results from the replicates of all four therapeutic mAbs spiked together in normal pooled serum at a concentration of 200 ug/mL then analyzed using microLC-ESI-Q-TOF mass spectrometry. The concentrations of the replicates were determined for each mAb using the linear regression equation calculated from the standard curves found from standards containing all four therapeutic mAbs. The results demonstrate that the methodology has good accuracy (96% - 108%) and precision (%CV 14% - 15%).

Conclusion

The data presented here demonstrate the ability of microLC-ESI-Q-TOF mass spectrometry to quantify multiple therapeutic mAbs in a single assay based on the known molecular mass of the light chain. This approach is similar to using an extracted ion chromatogram (EIC) to perform therapeutic drug monitoring on drugs with a smaller molecular mass. The innovative potential of this methodology is that it is not only capable of simultaneously quantifying therapeutic mAbs in the concentration range covering most target therapeutic concentrations, but also it is fast, inexpensive, and easy to perform.

Table 1

	IgG Subclass assay by LC-MS/MS (mg/mL)	
	IgG Total	% Total IgG calculated/known
Infliximab (10.0 mg/mL)	10.1	101%
Rituximab (10.0 mg/mL)	11.7	117%
Eculizumab (980 mg/mL)	11.6	118%
	IgG Subclass by nephelometry (mg/mL)	
	IgG Total	IgG Total calculated/known
Infliximab (10.0 mg/mL)	10.1	106%
Rituximab (10.0 mg/mL)	13.9	139%
Eculizumab (9.80 mg/mL)	9.05	92%
Vedolizumab (10.0 mg/mL)	12.8	128%

Figure 1

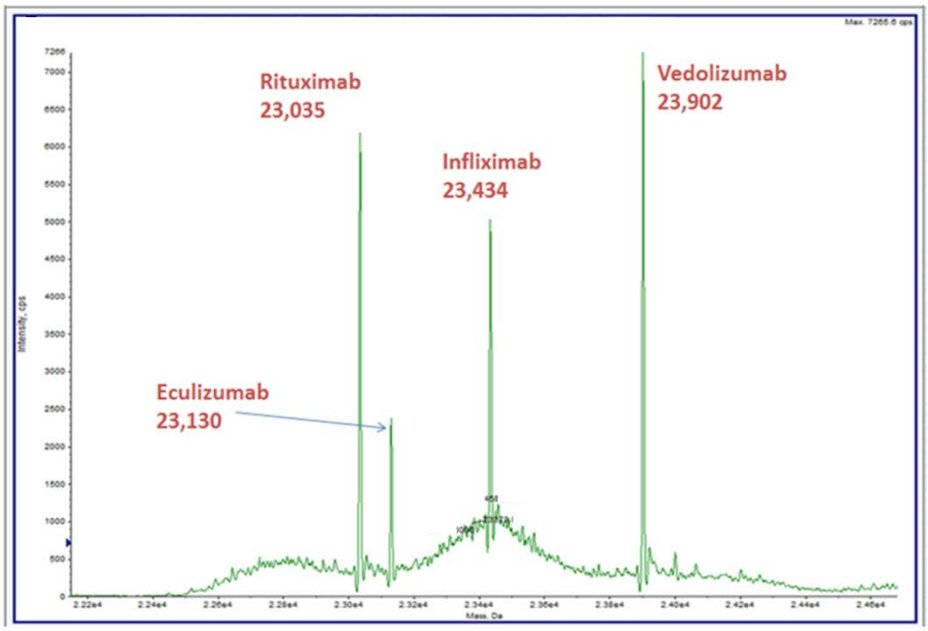

Figure 2

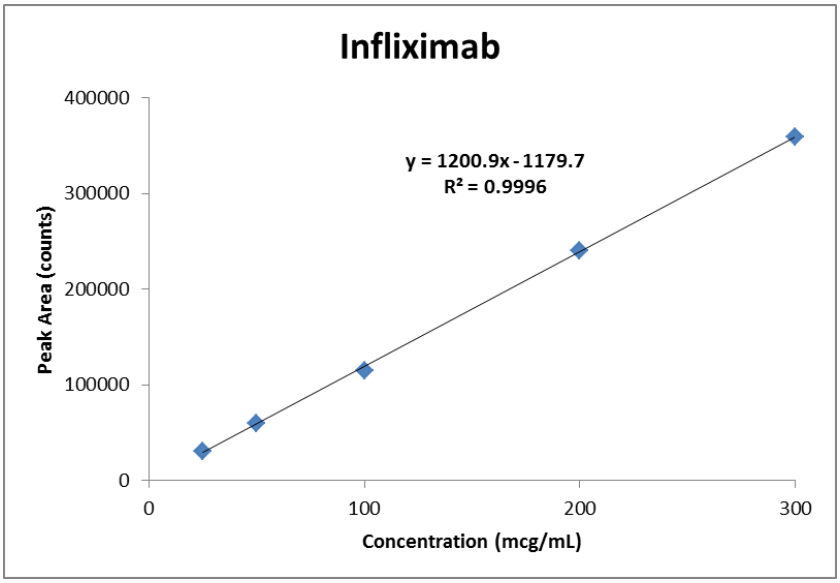

Figure 3

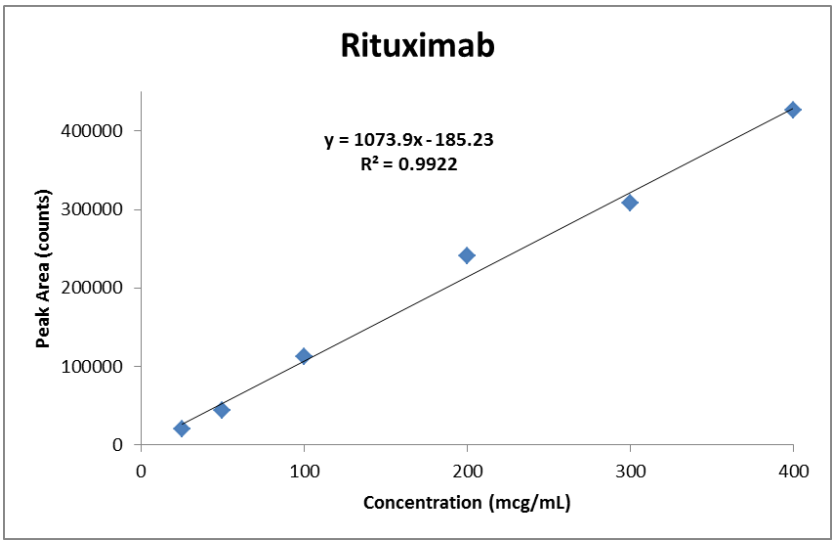

Figure 4

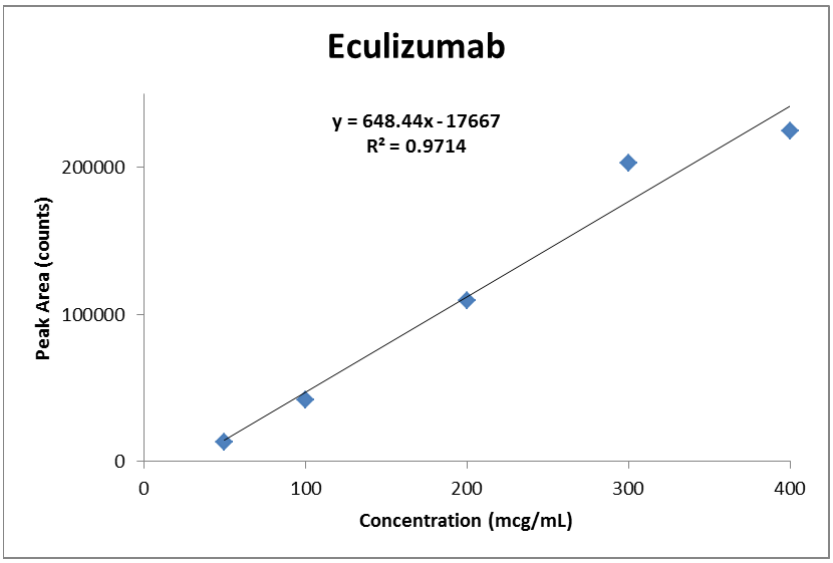

Figure 5

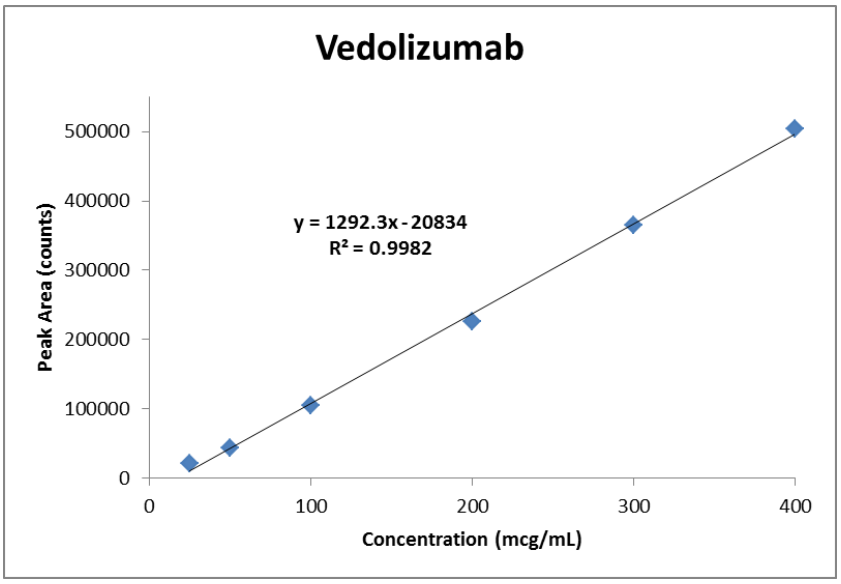

Table 2

Replicates N = 10 for all 4 mAbs spiked together in serum at 200 ug/mL

	Infliximab	Rituximab	Eculizumab	Vedolizumab
Mean (mg/mL)	192	217	208	194
SD	28.6	31.9	30.1	27.5
%CV	15%	15%	14%	14%
Accuracy	96%	108%	104%	97%

References

1. Barnidge DR, Tschumper RC, Theis JD, Snyder MR, Jelinek DF, Katzmann JA, Dispenzieri A, Murray DL. Monitoring M-proteins in patients with multiple myeloma using heavy-chain variable region clonotypic peptides and LC-MS/MS. J Proteome Res. 2014; 13(4):1905-10.
2. Ladwig PM, Barnidge DR, Snyder MR, Katzmann JA, Murray DL. Quantification of serum IgG subclasses by use of subclass-specific tryptic peptides and liquid chromatography—tandem mass spectrometry. Clin Chem 2014; 60(8): 1080-8.