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Application News

No.C130

Liquid Chromatography Mass Spectrometry

Development of MRM Methods for Monoclonal Antibodies Using Skyline

Monoclonal antibodies, or mAbs, have been used for over a decade in the treatment of a number of diseases but predominantly in the treatment of cancer and autoimmune diseases. Quantifying therapeutic mAbs in biological samples has been traditionally addressed by ligand binding assays (LBA's), however, there are major limitation in terms of extended method development times, reagent procurement, and matrix effects.

LC-MS/MS methods are emerging as an alternative approach to LBA's and this paper describes the application of MRM methods to the quantitation of peptide fragments containing β-amyloid antibody (6E10) CDR's (complementarity determining regions). CDR's are targeted by LC-MS/MS as antibodies share a conserved sequence and are only differentiated by the three loops (CDR1-3) which are diverse and contain antibody specific peptides. By selectivity detecting CDR1-3 by LC-MS/MS helps to characterize and quantify disease specific antibodies isolated from patient sera or plasma and can be used to identify differing therapeutic agents.

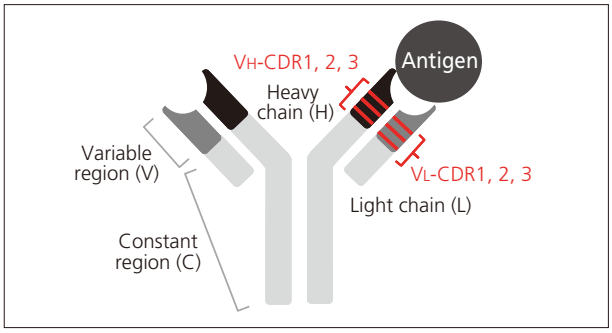


Fig. 1 Structure of Immunoglobulin and Complementarity Determining Regions (CDRs)

Development of MRM Method for Tryptic Fragment Containing CDR Using Skyline Software

Shimadzu LCMS-8050/8060 systems were used to identify CDR1-3 peptide fragments following a tryptic digest and Skyline software optimization[1]. The MRM method was set up to specifically detect peptide fragments containing β-amyloid antibody (6E10) CDRs.

Skyline was used to predict the peptide sequence for LC-MS/MS detection and also to optimize response for each precursor ion and product ion generated. The Skyline optimized MRM analysis method for CDR1-3 was then used to acquire highly specific and sensitive LC-MS/MS data.

1) Skyline is software developed by the MacCoss Lab of Biological Mass Spectrometry at the University of Washington.

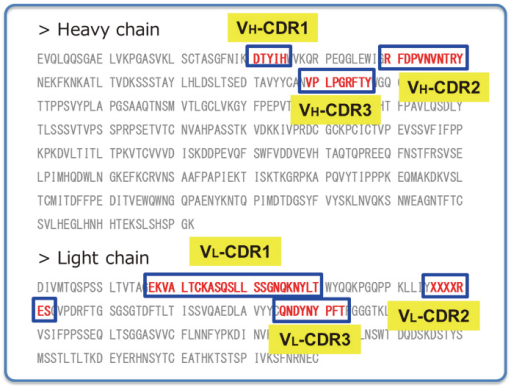


Fig. 2 Amino Acid Sequences and CDRs in β-Amyloid Antibodies (6E10)

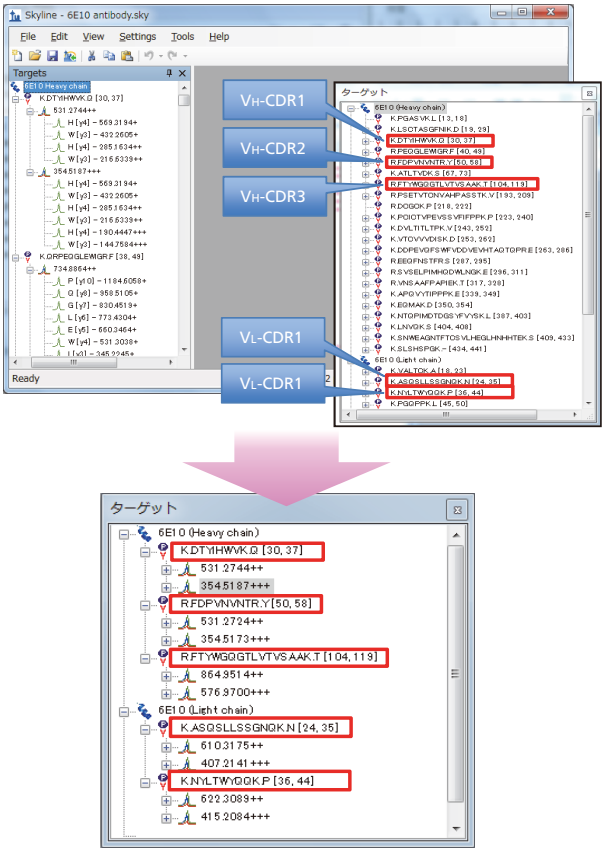


Fig. 3 Creating Analysis Methods Using Skyline (Selecting Trypsin Digestion Fragments Containing CDRs)

Amino acid sequence information of full-length monoclonal antibody (FASTA file) was imported to Skyline, and the peptide fragments produced by enzyme digestion were predicted. Further, peptide fragments containing CDRs were selected, and all the transitions and collision energies predicted were output as a LabSolutions LCMS analysis method.

Optimization of Analytical Method Using a Combination of the LCMS-8050/8060 and Skyline Software

Skyline targeted proteomics software (MacCoss et al., University of Washington, Seattle Washington) was used to predict precursor and product ions as well as collision energies and retention times using Shimadzu-specific models.

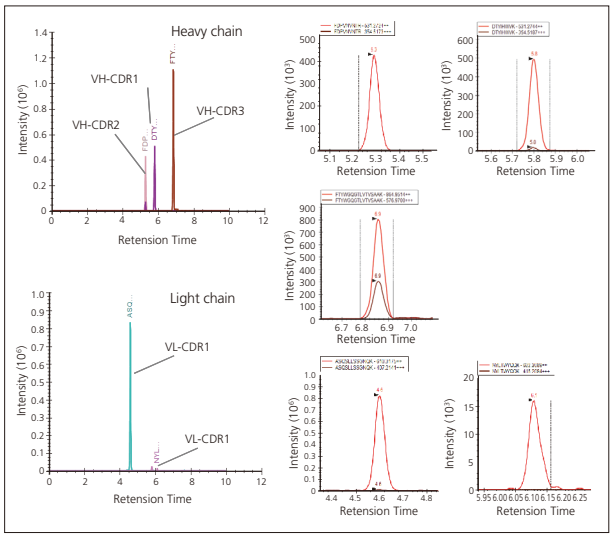


Fig. 4 Detection of Trypsin Fragments Containing CDR1-3 Using Standard Monoclonal Antibodies (6E10) Subjected to Trypsin Digestion (Data Analysis by Skyline)

Specific and sensitive tryptic peptide MRM's were selected and quantitative data was acquired using a calibration range from 6.67-6670 ng/mL.

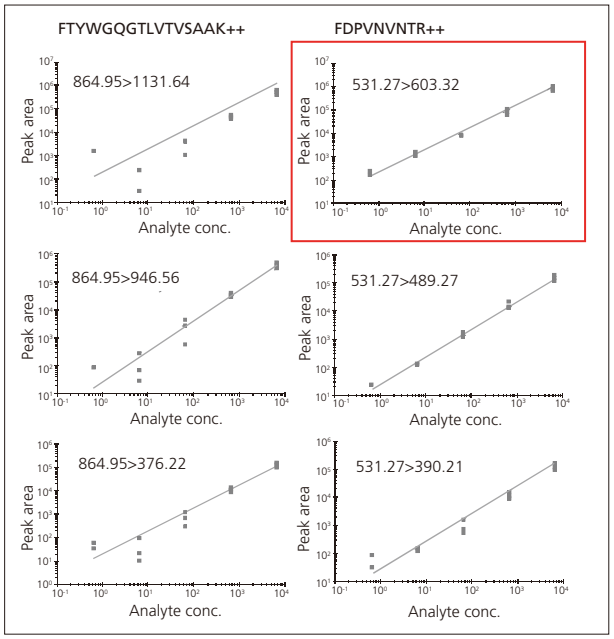


Fig. 5 Calibration Curve for Selected Peptides with Three Transitions.

The transition 531.27 > 603.32 was selected for quantitation as the dynamic range is 4 orders of magnitude. (The calibration curves are plotted on logarithmic scales).

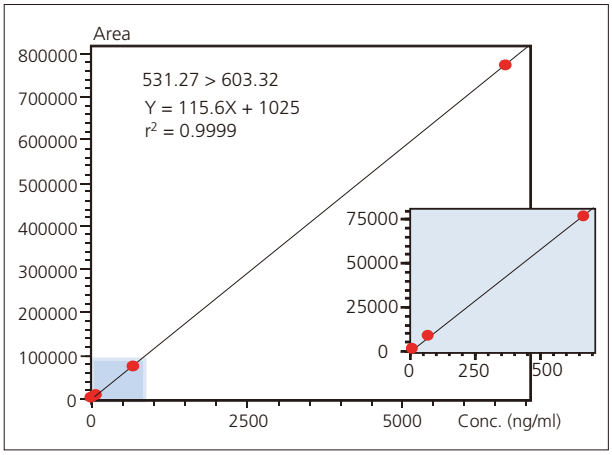
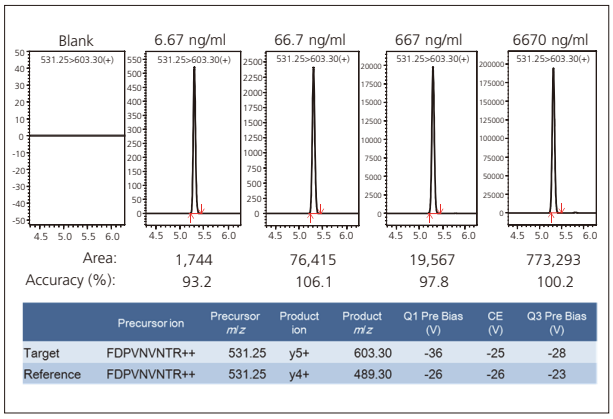


Fig. 6 Calibration Data for the Transition 531.27 > 603.32 on a Linear Scaling.

The LC-MS/MS method generates a linear response (r^2 = 0.999) for a calibration range over 4 orders of magnitude.



	Precursor ion	Precursor m/z	Product ion	Product m/z	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Target	FDPVNVNTR++	531.25	y5+	603.30	-36	-25	-28
Reference	FDPVNVNTR++	531.25	y4+	489.30	-26	-26	-23

Fig. 7 MRM Chromatograms for the FDPVNVNTR++ Peptide Transition 531.27 > 603.32.

The LC-MS/MS method acquired calibration data within an accuracy range of 93-106 % over the entire calibration range.

Conclusions

- The Shimadzu LCMS-8050/8060 system delivers an excellent solution for the quantitation of CDR peptide fragments.
- Wide dynamic range with a linear response of 4 orders of magnitude.
- High selectivity and sensitivity with high speed scanning capabilities reduces sample consumption even for very low amounts of therapeutic antibodies.
- Simple integration with Skyline software to help accelerate optimized method development and MRM analysis.

Notes

- The products mentioned in this article have not been approved/ certified as medical devices according to the Pharmaceutical and Medical Device Act in Japan.
- The analytical methods mentioned in this article cannot be used for diagnostic purposes.

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