Poster

[P25-7] P25-7: Immunosuppressive drugs (2): Monoclonal antibody and genotyping

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[P25-7-4] A novel application for antibody quantitation method development and validation of infliximab in plasma by nSMOL and Skyline software

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Background

The quantitative and qualitative analysis for targeted protein is popular recently, such as biomarker detection, antibody analysis. Due to the high sensitivity and selectivity of ultra high performance liquid chromatographic/tandem mass spectrometry (UHPLC-MS/MS), it can be used for detection of targeted protein. However, the method development for proteins based on LC-MS/MS is very complex, and time consumable. Skyline aims to employ cutting-edge technologies for creating and iteratively refining targeted methods for large-scale quantitative mass spectrometry studies in life sciences. Therapeutic monoclonal antibody (mAb) bioanalysis using mass spectrometry is useful technology for mAbs measurement other than ELISA. A new method for sample pretreatment based on nano-surface and molecular-orientation limited (nSMOL) proteolysis has been promoted by shimadzu cooperation. This paper describes the application of MRM methods to the quantitation of peptide fragments in CDR’ s (complementarity determining regions). It is useful for LCMS bioanalysis of antibody drugs such as infliximab in human plasma.

Methods

A novel method using fab-selective proteolysis named nSMOL and Skyline software was used for method development. nSMOL is Fab-selective limited proteolysis which consists of the difference of protease nanoparticle diameter (200 nm) and antibody resin pore diameter (100 nm). For limited proteolysis of antibody, Protein A resin (pore: 100 nm) slurry was added to plasma including monoclonal antibody, and the antibody Fc region was immobilized to the resin at 25˚C for 10 min with gentle vortexing. Antibody-immobilized resin was washed with PBS, and limited proteolysis was performed with trypsin-conjugated FG beads (diameter: 200 nm). Limited proteolysis of Fab region on antibody was achieved by these two diameter difference. After nSMOL proteolysis, the generated peptides were collected by only simple filtration. Skyline is a open source Windows client application for building Selected Reaction Monitoring (SRM) / Multiple Reaction Monitoring (MRM) and analyzing the resulting mass spectrometer data. MRM method was built using Skyline 3.5.0.9319.

Results

Using nSMOL proteolysis method and Skyline software, there were only 7 peptides detected by LC-MS/MS for infliximab. Finally three peptides were selective for quantitative and qualitative analysis and peptide SINSATHYAESVK was selected for quantitation. The linear range for infliximab using this novel approach was 0.02 μg/mL~50 μg/mL with correlation coefficient 0.9960. LLOQ for this method was 0.02μg/mL and

RSD% of six replicates was 6.40%. The accuracy and precision for three levels QC samples fulfilled criteria of guideline on bioanalytical method validation.

Conclusions

Skyline was a powerful software for method development of typical peptides selection for infliximab. After nSMOL proteolysis, most of specific peptides from CDRs were released from infliximab. These results indicate that nSMOL is also significant method for precise quantification of infliximab in plasma.