Regulated LC/MS/MS bioanalysis of therapeutic antibodies based on nano-surface and molecular-orientation limited (nSMOL) proteolysis method using a new reagent kit

1. Introduction

In preclinical and clinical trials of therapeutic monoclonal antibodies (mAbs), the construction of a measurement method for the drug concentration in blood or disease tissue is essential for its pharmacokinetic (PK) evaluation because overall PK pictures showing the correlation between antibody drug efficacy and clinical indicators is still insufficient. While binding assays were most used historically, alternative LC/MS/MS platforms have emerged. One such platform is LC/MS/MS quantitation based on nanosurface and molecular-orientation limited (nSMOL) proteolysis (Iwamoto N et al., Analyst, 2014). It is designed to keep antibody specificity with minimizing sample complexity and simply a sample preparation protocol, thus delivering robust quantitation with higher sensitivity (Figure 1). Immunglobulin (IgG) fractions containing therapeutic antibodies were collected from human plasma with IgG collection resin (diameter of pores: 100 nm). The IgGs immobilized on the inside of the pores are proteolyzed with trypsin-immobilized nanoparticles on the surface (diameter: 200 nm). Because IgC is immobilized at the pores, Fab oriented towards the solution is predominantly reacted with trypsin. Thus, nSMOL proteolysis minimizes the sample complexity by excluding both tryptic fragments other than those around the Fc region and trypsin enzyme, allowing a selective quantification of target mAb peptides with high efficiency and selectivity in combination with MRM measurement.

The practical validation dataset of several mAbs acquired by nSMOL method meets the validation guideline criteria issued by the U.S. FDA for Bioanalytical Method Validation of LCMS bioanalysis for low MW drug compounds (Figure 3, Reference II – VII). This fact demonstrates that the standard workflow for nSMOL improves robustness of analysis. It is dramatically lowered the matrix-derived background noise and increased the reproducibility of quantitative data with the standard sample preparation protocol which is significantly simplified from the state-of-the-art technologies in the field of quantitative proteomics.

In this presentation, we will introduce the analytical workflow using the new nSMOL reagent kit, and discuss for the application into the regulated LCMS bioanalysis of mAbs in human plasma.

2. Methods

nSMOL™ Antibody BA Kit is a ready-to-use providing almost all of reagents and the protocol required for nSMOL workflow. Five µL of plasma is treated according to standard protocol of nSMOL (Figure 2). The workflow requires just three steps: 1) Capturing the IgGs from the plasma sample, 2) nSMOL proteolysis, and 3) quantitation using LC-MS/MS in MRM mode. After the capturing steps, the nSMOL reaction solution or the enhanced nSMOL reaction solution were digested with internal standards and FG beads Trypsin DART™. Following nSMOL proteolysis, the reaction solution was centrifuged and the supernatant injected into a triple quadrupole mass spectrometer (LC/MS-8060/8050, Shimadzu Corporation, Japan), and monitored the signature peptides with the complementary-determining region (CDR) corresponding to each mAb.

3. Result

The data shown below are the results of practical bioanalysis of therapeutic antibodies in human plasma using nSMOL™ Antibody BA Kit (Figure 4, 5).

4. Conclusions

nSMOL proteolysis is our SHIMADZU original technology with a proven track record for the analysis of a variety of antibodies. nSMOL™ Antibody BA Kit provides a total strategy combining with a high-sensitivity triple quadrupole mass spectrometer LC/MS-8060/8050 which can surpass the conventional methods of monoclonal antibody analysis, including ligand binding assay and standard proteomics.

This original approach can maximize the use of LC/MS/MS in the development of antibody drugs or clinical studies. LC/MS/MS can also accelerate the speed of development for antibody pharmaceuticals just as it did in small molecule drug development. In addition, PK of therapeutic antibodies by nSMOL has the potential of becoming a standard approach for therapeutic drug monitoring (TDM).

5. Reference

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Disclaimer: nSMOL™ Antibody BA Kit and LC/MS-8060/8050 is intended for Research Use Only (RUO). Not for use in diagnostic procedures.