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Technical Report

LCMS Bioanalysis of Antibody Drugs Using Fab-selective Proteolysis "nSMOL Method"
— Selection of Signature Peptide —

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Abstract:

nSMOL (nano-surface and molecular-orientation limited proteolysis) is Shimadzu's completely new, proprietary, and innovative technique for selective proteolysis of Fab region of monoclonal antibodies. nSMOL allows analytical method development of antibody drugs independent of a variety of antibody drugs. Fab-derived peptide fragments produced by nSMOL can be precisely quantified by multiple reaction monitoring (MRM) using the Shimadzu LCMS-8050/8060 triple quadrupole liquid chromatograph mass spectrometer (TQ-LCMS). This report describes a selection protocol of signature peptides suitable for pharmacokinetic studies.

Keywords: nano-surface and molecular-orientation limited proteolysis, antibody drug, bioanalysis, LC/MS/MS

1. Introduction

Pharmacokinetic information provides some of the most fundamental indicators. The effective drug discovery is supported by the overall pharmacokinetic profile such as for drug efficacy and toxicity.

The current method used for measuring drug concentration in blood is enzyme-linked immunosorbent assay (ELISA). However, there are critical issues with ELISA, including influences from cross-reaction and inhibitory materials. In contrast, by MS, analysis is performed based on the structural information; thus, the aforementioned issues can potentially be resolved.

The LCMS analysis of high-molecular-weight proteins, such as antibodies, is normally performed after fragmentation of the protein into smaller peptides using a protease, such as trypsin or lysyl endopeptidase. However, this process also generates a large number of peptides including the signature peptides. These peptides increase the background noise and ionization suppression, and become a major cause of instability in the LCMS system. nSMOL can decrease these issues by selective proteolysis on the analytical target region of the antibody Fab. Therefore, the use of this approach can improve the reproducibility and robustness of the analytical system with maintaining antibody specificity.

A peptide for quantitation (signature peptide) is selected from the tryptic peptides containing a complementarity-determining region (CDR), which defines the specificity of the antibody. However, it is not possible that the CDR-containing peptide does not have the same amino acid sequence as that in the endogenous IgGs. At this point, it must be confirmed that there is no competition with the signature peptide in the biological matrix.

2. Structure and Specificity of Antibodies

Antibodies are large biological molecules with a heterotetrameric protein containing two heavy and light chains. Antibody structures are extraordinarily well conserved, and antibodies have a flexible hinge region between the rigid Fc and the variable Fab. A variable region, Fv, is positioned at the top of the Fab. The antibody specificity in this region is defined as amino acid substitution by somatic mutation. Portions of the Fv with a particularly high frequency mutation are called CDRs, which play a major role in antigen binding (see Fig. 1).

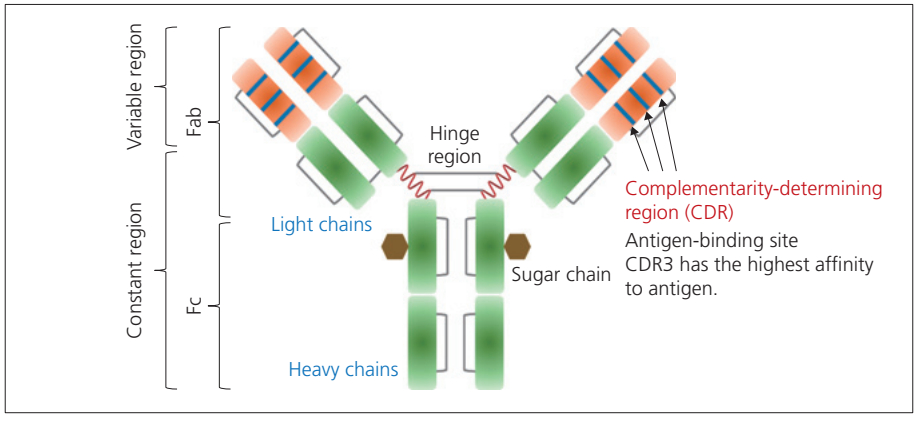

Fig. 1 Basic Antibody Structure

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3. Frequency of Amino Acid Substitution in the Fv

The Fv contains three regions of high-frequency amino acid substitution, namely CDR1, CDR2, and CDR3 (see Fig. 2). Almost all amino acid residues in these regions can undergo substitution; therefore, an extremely large number of possible sequences can be generated. Consequently, predicting amino acid sequences is difficult. Therefore, nSMOL bioanalysis requires the setting and selection of signature peptides based on actual analysis data.

4. Amino Acid Sequence Alignment Using ClustalW

An example is shown the prediction of candidate signature peptides in four chimeric antibodies (Rituximab, Brentuximab vedotin, Cetuximab, and Infliximab) by ClustalW alignment (Fig. 3, black: common residues, gray: similar residues, white: unique residues).

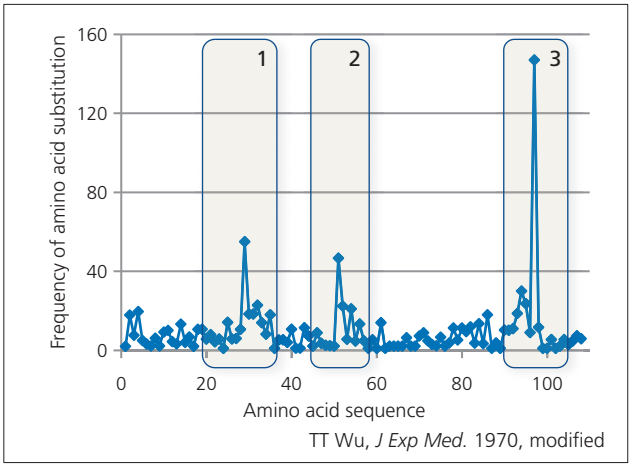

Fig. 2 Frequency of Amino Acid Substitution in Fv

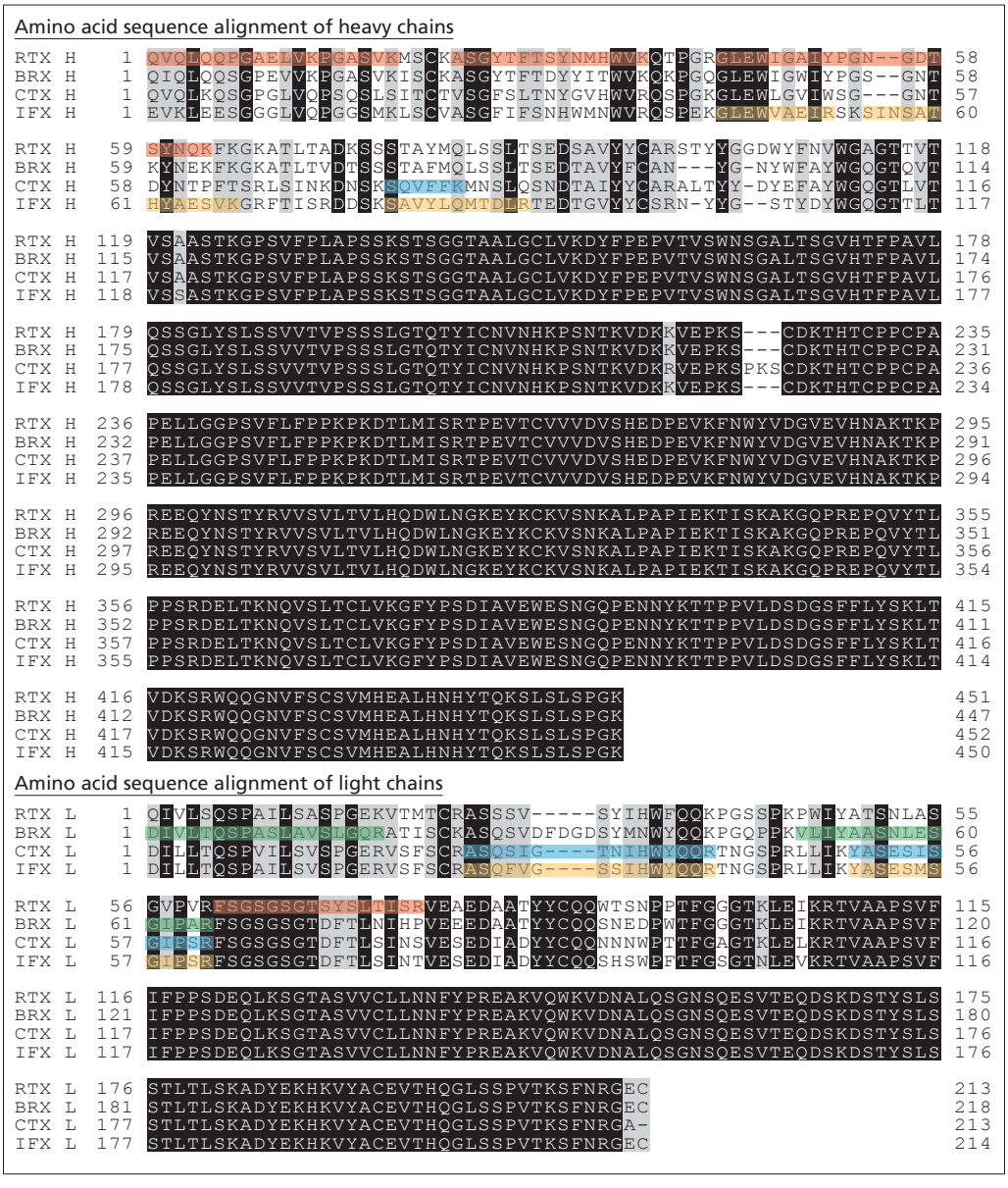

Fig. 3 Results of Amino Acid Sequence Alignment

5. Discussion of Alignment Analysis

From the sequence alignment analysis, it can be assumed that areas with a predominance of unique residues (white) are CDRs. The results also demonstrated the presence of unique residues throughout the Fv. The prediction of signature peptides is simplified by amino acid sequences of several representative antibodies according to antibody classification (chimeric, humanized, or fully human antibody).

During quantitative analysis, identified signature peptides are highlighted in for Rituximab, for Brentuximab vedotin, for Cetuximab, and for Infliximab. Some of the signature peptides include N-terminal sequences that may become heterogeneity during post-translational modification or may interfere with another biological matrix during preclinical studies. Therefore, N-terminal sequences are not used during an actual nSMOL bioanalysis.

6. Conclusions

A reliable selection of signature peptides is essential for clinical pharmacokinetic studies.

Fig. 4 shows a procedure from a structural analysis technique by mass spectrometry and a recent information-based approach. Identification of the peptide structure and its actual valence by high-resolution MS is the biggest advantage in the viewpoint of scientific evidence. The information-based approach configures MRM conditions based on sequence information. These two techniques should be used at different times depending on the circumstances.

Reference

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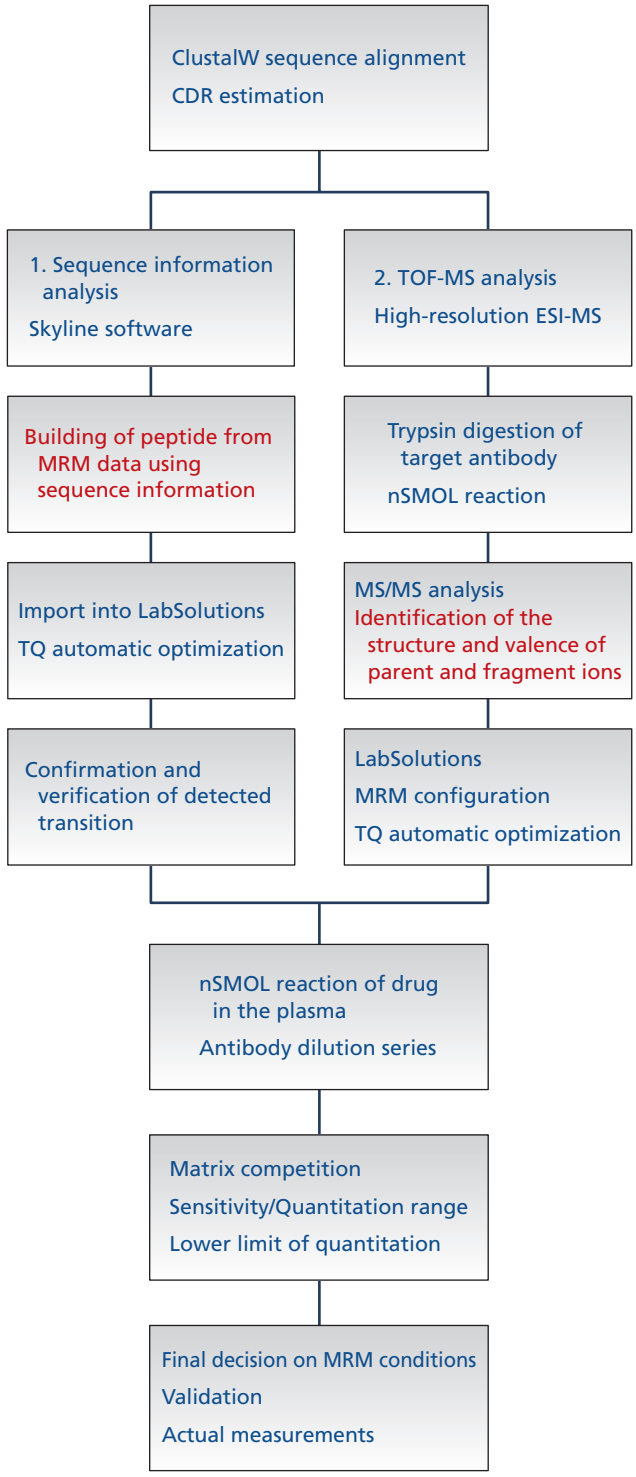


Fig. 4 Signature Peptide Selection Procedure

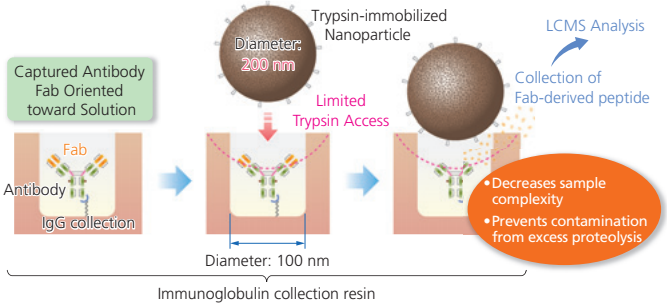
nSMOL Antibody BA Kit

LC/MS/MS Sample Prep Kit
for Quantitative Analysis of Monoclonal Antibodies

nSMOL Technology Increasing Confidence in Therapeutic Monoclonal Antibody Bioanalysis

nSMOL (nano-surface and molecular orientation limited proteolysis) is Shimadzu's proprietary, innovative technique that enables selective proteolysis of the Fab region of monoclonal antibodies. The nSMOL Antibody BA Kit is a ready-to-use reagent kit for collecting monoclonal antibodies from blood or other biological samples using immunoglobulin collection resin, and then performing selective proteolysis of the Fab region of these antibodies via trypsin-immobilized nanoparticles. Variable region-derived peptides produced by limited proteolysis can then be quantified via MRM measurements utilizing a high-performance LCMS-8050/8060 triple quadrupole liquid chromatograph mass spectrometer.

An unparalleled convenient and rapid workflow provided by the nSMOL Antibody BA Kit dramatically improves the productivity and robustness of LCMS mAb bioanalysis.



Kit Contents

The nSMOL Antibody BA Kit provides prepared reagents and protocols for sample prep via the nSMOL method.

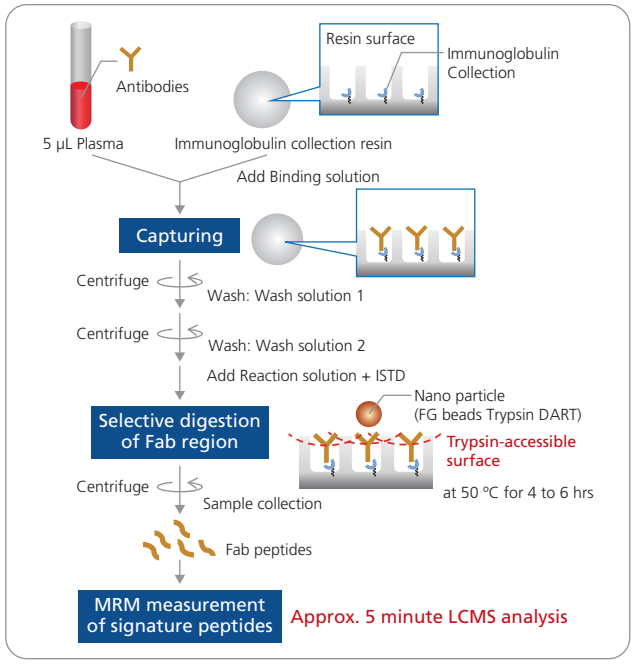
Reagent	Quantity	Capacity	Storage Temperature
Immunoglobulin collection resin	2	1.3 mL/each	4 °C
Wash solution 1 (Binding solution)	1	80 mL	4 °C
Wash solution 2	1	80 mL	4 °C
Reaction solution	1	10 mL	4 °C
Enhanced reaction solution	1	Freeze-dried	4 °C
Reaction stop solution	1	1 mL	4 °C
FG beads Trypsin DART	1	1.1 mL	–20 °C *1

*1: If it is being stored for a month or longer, store it at –80 °C.

Note: The reagent kit is transported at refrigeration temperatures (2 to 8 °C).

Simple Workflow

This kit enables highly reproducible data and avoids the troublesome steps of denaturing, reduction, and alkyation normally associated with protein digestion. There is also no need for solid phase extraction after reaction. After nSMOL preparation, samples can be injected directly onto the LCMS.



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