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Application News

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nSMOL Antibody BA Kit

LCMS™ Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL – Part 7 - Tocilizumab Analysis -

Introduction - Immunological Mechanism of Tocilizumab -

Tocilizumab is a humanized monoclonal antibody against the human interleukin-6 (IL-6) receptor and may block the IL-6 signaling. IL-6 was identified in 1980's and recent studies have shown that it plays a role in inflammatory immune reactions and that it possesses a wide variety of physiological activities. In autoimmune inflammatory diseases, such as rheumatoid arthritis, Castleman's disease, and juvenile idiopathic arthritis, it is known that dysregulated production of IL-6 induces an out-of-control response leading to several immune disorders. Tocilizumab helps to control the rare diseases that have been designated as intractable by blocking and inhibiting inflammatory induction due to the dysregulated production of IL-6. Additionally, the recent evidence suggests that tocilizumab may be highly effective in the treatment of other diseases where IL-6 is involved in their pathogenesis. Therefore, this antibody is a promising option for treating cases of acute inflammation, such as the severe pneumonia associated with COVID-19 that is having a global impact.

nSMOL™ Antibody BA Kit Features

The nano-surface and molecular-orientation limited (nSMOL) proteolysis is a completely new, breakthrough technology from Shimadzu that enables selective proteolysis of the Fab of monoclonal antibodies. This technique facilitates the development of a method that is independent of a variety of therapeutic antibodies and achieves a paradigm shift in their bioanalysis. Furthermore, this is a method that meets the criteria contained in "Bioanalytical Method Validation Guideline for Industry" issued by Office of Communications, Division of Drug Information Center for Drug Evaluation and Research

Food and Drug Administration. Shimadzu has provided the validated LCMS method and protocol for each of these drugs.

This technique is validated using Shimadzu's LCMS™-8050 (hereinafter, LCMS-8050) and LCMS™-8060 (hereinafter, LCMS-8060), both of which are triple quadrupole mass spectrometers (Fig. 2).

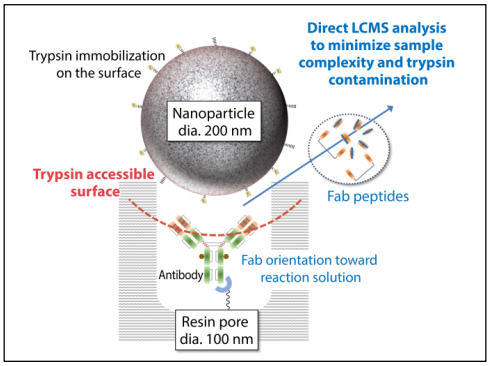

Fig. 1 Principle of nSMOL

Application of LC-MS to Analysis of Antibody Drugs

While the enzyme-linked immunosorbent assay (ELISA) has mainly been the assay technique to measure blood concentration of antibody drugs, there are methodological issues due to the effects of cross-reactivity and inhibitory substances. On the other hand, mass spectrometry may be able to solve these issues because of the structure-related analysis.

Additionally, recent advances in life science research has led the rapid development of antibody drugs and expanded indications for antibody drugs in the treatment of various diseases. While antibody drugs have significant clinical benefits in the treatment of various diseases previously considered as intractable, their continuous treatment reduces their therapeutic effects in some patients, among whom anti-drug antibodies have been identified.

Based on this background, the demand for the analysis of blood levels of antibody drugs in clinical and medical science studies is expected to increase in the future. An analysis method using LC-MS can make a real contribution to these studies.

Fig. 2 LCMS™-8050 (Left) and LCMS™ -8060 (Right)

Sample Pretreatment Protocol for Tocilizumab Using the nSMOL

With the nSMOL, the same assay platform can be applied to the pretreatment of any antibody drug. Some antibodies, however, are resistant to trypsin proteolysis, which may have a significant effect on the sensitivity of analyzed data. For even these antibody drugs, the acidified reduction process prior to the nSMOL reaction can improve the reaction efficiency and yield for a sufficient sensitivity.

[Sample Pretreatment Protocol]

First, IgG collection resin with 100 nm diameter pores is used to collect all IgGs in a serum/plasma sample within its pores. All proteins other than IgGs in the serum/plasma sample are washed out, and after 250 mM TCEP-HCl has been added, the mixture is incubated at room temperature for 30 minutes to reduce IgGs under an acidified condition. After washing the resin, nanoparticles (FG beads®, 200 nm in diameter) with immobilized trypsin are added to initiate proteolysis. After the reaction, reaction stop solution is added and then the resin and FG beads are removed with a spin filter. The resulting solution can be directly used for LC-MS analysis without the further treatment.

LCMS Analytical Conditions

The LC-MS analytical conditions are listed in Table 1 and the measurement parameters of peptides used for tocilizumab quantitation and the internal standard are listed in Table 2.

Table 1 LC and MS Analytical Conditions

[HPLC conditions] (Nexera™ X2)		
Column	:	Shim-pack™ GISS C18 (50 mm × 2.1 mm)
Column oven	:	50 °C
Solvent A	:	0.1% Formic acid / water
Solvent B	:	0.1% Formic acid / acetonitrile
Gradient	:	2 %B (0 - 1.5 min)/ 2-30 %B (1.5 - 4.5 min)/ 95 %B (4.5 - 5.5min)/ 2 %B (5.5 - 6.5 min)
Flow rate	:	0.4 mL/min
Injection volume	:	10 µL
[MS conditions] (LCMS-8050/8060)		
Ionization	:	ESI (Positive mode)
Mode	:	MRM
Nebulizing gas flow	:	3.0 L/min
Drying gas flow	:	10.0 L/min
Heating gas flow	:	10.0 L/min
DL temp.	:	250 °C
Block heater temp.	:	400 °C
Interface temp.	:	300 °C

Table 2 Peptides Used for Tocilizumab Quantitation and the Internal Standard

Peptide	MRM Transition	Purpose
VTMLR	310.2>520.2	For quantitation
	310.2>419.2	For structure confirmation
	310.2>201.0	For structure confirmation
P14R	512.1>292.3	For quantitation (IS)
	512.1>389.3	For structure confirmation
	512.1>660.4	For structure confirmation

* Quantitation range in human plasma (serum): 0.781 - 200 µg/mL
* P14R (internal standard) was added at the time of preparation of trypsin digestion reaction solution (the final concentration of approx.10 fmol/µL)

Validation Results for Tocilizumab

The validation results for accuracy and precision for tocilizumab in serum are shown in Table 3. The calibration curves are shown in Fig. 3. Representative chromatograms are shown in Fig. 4.

Table 3 Validation Data for Tocilizumab (Accuracy and Precision)

	Set concentration [µg/mL]	Data average [µg/mL]	Accuracy (%)	CV (%)
day1 (N=5)	2.34	2.39	102	5.87
	160	170	106	1.32
day2 (N=5)	2.34	2.35	100	3.40
	160	166	104	3.83
day3 (N=5)	2.34	2.59	111	3.32
	160	158	98.6	3.50
N=15	2.34	2.44	104	5.92
	160	165	103	4.31

Calibration Curve and MRM Chromatograms

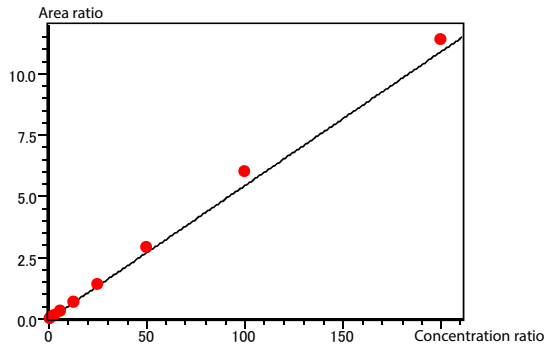

Fig. 3 Calibration Curve for Tocilizumab

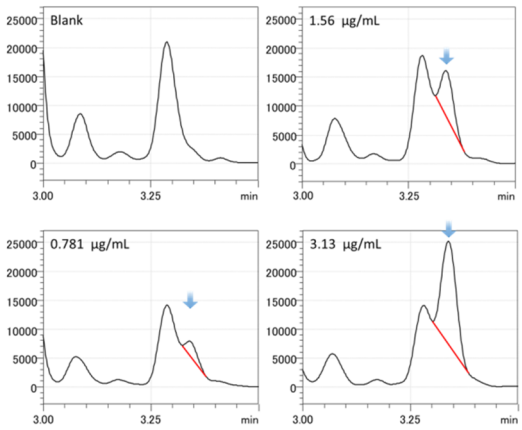

Fig. 4 Representative MRM Chromatograms (in human serum)

Discussion and Conclusions

The poor reaction efficiency of tocilizumab with trypsin using conventional nSMOL condition suggests that tocilizumab has a structure that causes a local resistance to trypsin. In this analysis, we were successful in developing a quantitative technique that meets the criteria contained in the validation guidance, within a range of 0.781 - 200 µg/mL, by incorporating an acidified reduction process.

These results demonstrate that nSMOL bioanalysis has sufficient accuracy and precision to monitor tocilizumab levels in circulation.

Other monoclonal antibodies have also been found to be resistant to trypsin and the user should choose the protocol depending on the monoclonal antibodies.

References

Iwamoto N et.al. Analyst, DOI:10.1039/c3an02104a
Iwamoto N et.al. J Pharm Biomed Anal, DOI: 10.1016/j.jpba.2018.11.019
Iwamoto N et al. J Immunol Methods, DOI: 10.1016/j.jim.2019.06.014
Hashizume M et. al. Folia Pharmacol Jpn, DOI: 10.1254/fpj.144.172

Note) The product described in this document has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination, treatment or related procedures.

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