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Application News

No. C174

nSMOL™ Antibody BA Kit

LCMS Bioanalysis of Antibody Drugs Using Fab-selective Proteolysis "nSMOL Method" - Part 6

- Improvement of Reaction Conditions for Automated Analyses -

nSMOL™ Antibody BA Kit Features

nSMOL is a new bioanalysis technology developed by Shimadzu Corporation that enables selective proteolysis of the Fab region of monoclonal antibodies. This technology facilitates method development independent of the variety of the antibody drug and is an innovative technology for the bioanalysis of antibody drugs.

Furthermore, nSMOL is the only method that has fulfilled the criteria of the "Guideline on Bioanalytical Method Validation in Pharmaceutical Development" (issued by the Japanese Ministry of Health, Labour and Welfare for small molecule drug compounds) with respect to multiple antibody drugs. Shimadzu also offers optimization methods and procedures for each antibody drug. nSMOL method is optimized for use with the Shimadzu LCMS-8050 (hereinafter, LCMS-8050) and LCMS-8060 (hereinafter, LCMS-8060) triple quadrupole mass spectrometers.

nSMOL reaction without shaking

Conventional nSMOL method involves trypsin digestion under constant shaking and therefore requires a shaker to be installed inside an incubator set to 50 °C. This study confirms that the proteolysis efficacy remains sufficient even when nSMOL proteolysis without constant shaking.

nSMOL Sample Processing Protocol

With nSMOL method, the same reagent and sample processing protocol is applied to all antibody drugs.

First, an immunoglobulin G (IgG) collection resin with pores of 100 nm in diameter is used to collect all IgG present in a plasma sample by fixing the collected IgG within its pores. All components other than IgG in the plasma are filtered and washed, and then nanoparticles with immobilized trypsin (FG beads, diameter 200 nm) are added. The proteolysis is carried out without constant shaking after shaking for 10 seconds. After the reaction, a reaction stop solution is added and then the resin and FG beads are removed with a spin filter. The resulting solution can be used for LC-MS analysis as is. The MRM conditions for the quantitation peptides are listed in Table 1 and the analytical conditions are listed in Table 2.

Table 1 Measurement Conditions

Antibody Drug	Peptide	MRM for Quantitation
Trastuzumab	IYPTNGYTR	542.80>404.70
Bevacizumab	FTFSLDTSK	523.30>797.40
Rituximab	ASGYTFTSYNMHWVK	598.10>817.50
Nivolumab	ASGITFSNSGMHWVR	550.80>661.50

Table 2 Analytical Conditions

[LC] Nexera™X2 System		
	Column	: Shim-pack™ GISS C18 (50 mm × 2.1 mm, 1.9 µm)
	Column temp.	: 50 °C
	Solvent A	: 0.1 % formic acid/water
	Solvent B	: 0.1 % formic acid/acetonitrile
	Gradient	: Trastuzumab·····B conc. 1 % (1.5 min)/1-25 % (3.5 min)/95 % (1 min)/1 % (1 min) Bevacizumab·····B conc. 1 % (1.5 min)/1-35 % (3.5 min)/95 % (1 min)/1 % (1 min)
	Flow rate	: 0.4 mL/min
	Injection Volume	: 10 µL
[MS] LCMS-8050		
	Ionization	: ESI Positive
	DL temp.	: 250 °C
	Block Heater temp.	: 400 °C
	Interface temp.	: 300 °C
	Nebulizer gas flow	: 3 L/min
	Drying gas flow	: 10 L/min
	Heating gas flow	: 10 L/min
	Probe position	: 2 mm

Differences in Reaction Between Constant Shaking and Static Condition

The enzymatic reaction step in nSMOL method was studied by comparing the analysis results from when the enzymatic reaction was performed with or without shaking. The antibodies Trastuzumab, Bevacizumab, Rituximab, and Nivolumab were used in the comparison. Fig. 1 compares the peak area values of the quantitation peptides obtained for a 50 µg/mL sample (N=3). When the recovery rate after constant shaking is considered to be 100 %, the reaction efficiency without constant shaking was 84.6 % for Trastuzumab and for the other three between 95.7 to 102 %. For all antibodies, the detected difference was not significant enough to affect the quantitation values. Furthermore, accuracy and sensitivity were not affected.

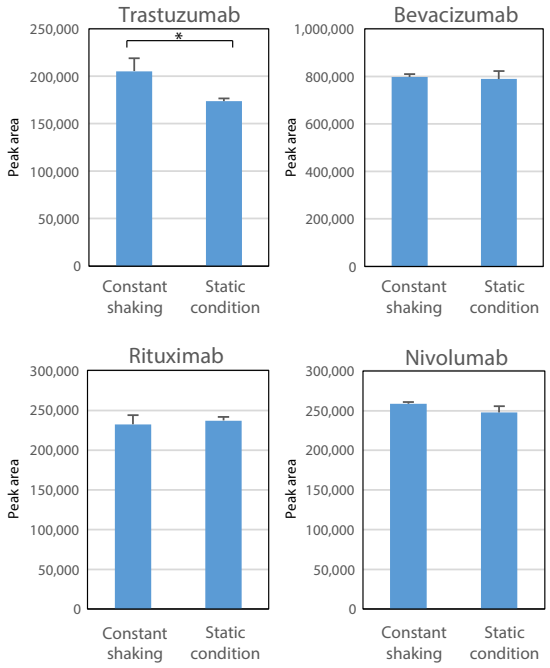

Fig. 1 Comparison of Peak Areas of Constant Shaking and Static Condition in Each Antibody Drugs (*: p<0.05)

Validation Results of Reaction Under the Static Condition for nSMOL Method

We performed validation analysis for multiple antibodies by implementing a test using the guideline criteria for small molecule drug compounds. This article introduces validation results regarding accuracy and precision for the following set concentrations with respect to Trastuzumab and Bevacizumab (Tables 3 and 4). The results indicate that both of accuracy and precision fulfilled the guideline criteria even when the reaction without constant shaking in nSMOL proteolysis.

Table 3 Analysis Results of Reaction Under the Static Condition for Trastuzumab

Set Concentration (µg/mL)	Data Average (N=7)	CV (%)	Accuracy (%)
0.244	0.254	8.72	104
200	207	4.58	103

Table 4 Analysis Results of Reaction Under the Static Condition for Bevacizumab

Set Concentration (µg/mL)	Data Average (N=7)	CV (%)	Accuracy (%)
0.439	0.417	14.3	95.0
240	229	2.83	95.5

Observations

Shimadzu's nSMOL technology enables the acquisition of CDR-peptides through the selective proteolysis of the Fab region. Until now, the nSMOL reaction was done while applying shaking agitation at 50 °C. However, as this article indicates, causing the reaction under static condition can sufficiently satisfy accuracy and sensitivity for the concentration range from approximately 0.1 to 300 ug/mL. This is because FG beads are nanoparticles and they can move homogeneously by Brownian motion. Since constant shaking is no longer necessary for reaction, a dedicated shaking instrument is also unnecessary, making sample processing much simpler.

The improved nSMOL protocol, which is carried out under static condition, is expected to be applied to the processing of multiple samples and automated assays, and also contribute to antibody pharmacokinetics for preclinical studies and clinical trials.

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

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