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Application News

No. C169

nSMOL™ Antibody BA Kit

LC-MS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL - Part 5 – Instrument comparison of precision and accuracy –

nSMOL™ Antibody BA Kit Features

nSMOL is Shimadzu's completely new and breakthrough LC-MS pretreatment technology 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 achieves a paradigm shift in the bioanalysis of antibody drugs.

Furthermore, nSMOL proteolysis 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 protocols for each antibody drug. nSMOL proteolysis is optimized for use with the Shimadzu LCMS-8050 and LCMS-8060 triple quadrupole mass spectrometers.

Comparison of the Shimadzu LCMS-8050 with Company A's LC-MS

In this study, we analyzed bevacizumab using the Shimadzu LCMS-8050 and vender products (LC-MS hybrid mass spectrometer) to compare accuracy and sensitivity. For both analyses, nSMOL proteolysis was used for pretreatment. Dwell time and cycle time of MRM methods are shown in Table 1.

Table 1 Dwell Time and Cycle Time of MRM Methods

Instrument	Dwell Time	Pause Time	Cycle Time (Max.)
LCMS-8050	10 msec	3 msec	182 msec
Vender products	15 msec	5 msec	280 msec

Analysis Conditions for Bevacizumab Using nSMOL

<Sample Processing Protocol>

With nSMOL proteolysis, the same sample processing protocol can be 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 (FG beads, 200 nm in diameter) with immobilized trypsin are added to cause proteolysis. 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 2 and the analytical conditions are listed in Table 3.

Table 2 Quantitation Peptides of Bevacizumab and the Internal Standard

Peptide	MRM Transition	Purpose
$P_{14}R$ (IS)	512.1>292.3 (b3+)	For quantitation
	512.1>389.3 (b4+)	For structure confirmation
	312.1>660.4 (b6+)	For structure confirmation
FTFSLDTSK	523.3>797.4 (y7+)	For quantitation
	523.3>898.5 (y8+)	For structure confirmation
	523.3>650.3 (y6+)	For structure confirmation

* Quantitation range in human plasma: 0.146 to 300 µg/mL
Averaged accuracy: 100.7 %

Table 3 Analytical Conditions

[LC] Nexera™ X2 System and vender products
- 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 : B conc. 1% (1.5 min) / 1-35 % (3.5 min) / 95 % (1 min) / 1 % (1 min)
- Flow rate : 0.4 mL/min (5 min)
- Injection volume : 10 µL

[MS] LCMS-8050 and vender products
- 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

Observations and Conclusions

nSMOL proteolysis enables the quantitation of bevacizumab in human plasma with high precision while also fulfilling the guideline criteria for small molecule drug compounds in terms of precision and accuracy in validation. Analyses of bevacizumab using the Shimadzu LCMS-8050 and vender products showed that analysis using the LCMS-8050 yielded higher accuracy and sensitivity and that measurements of the area value of the internal standard ($P_{14}R$) are stable (Table 4). In addition, analyses using the LCMS-8050 allowed acquisition of stable data for low concentrations as well. The type of collision gases and the structure of the mass spectrometers are different, suggesting that these differences affect the precision and accuracy.

Table 4 nSMOL-proteolyzed Bevacizumab Analysis Using the LCMS-8050 and Vender Products

Set Conc. (µg/mL)	LCMS-8050				Triple Quadrupole LC-MS (Company A)			
	Peak area Value	Conc. (µg/mL)	Accuracy (%)	$P_{14}R$ Area Value	Peak area Value	Conc. (µg/mL)	Accuracy (%)	$P_{14}R$ Area Value
0	445			640103	2185	< 0	N/A	382317
0.25	6436	0.257	102.8	610062	6502	0.217	84.7	416950
0.512	10358	0.485	94.8	623157	4166	0.537	104.9	150679
1.02	18712	1.04	101.8	597366	18452	1.00	98.2	408900
2.56	45959	2.63	102.9	623257	43997	2.66	103.7	409648
6.4	108651	6.57	102.6	609288	83687	6.85	107.1	315272
16	265218	15.6	97.3	634878	250600	16.6	103.7	396643
40	638375	38.7	96.8	617491	619589	38.9	97.3	420638
100	1662475	102	102.5	609322	1583550	105	105.2	399268
250	3927201	250	100.1	590061	3798713	253	101.3	398236
Average				615499				369855
%RSD				0.024				0.212

High Selectivity Specific to IgG and Easy Instrument Maintenance

nSMOL proteolysis selectively collects peptides for measurement and thereby reduces contamination of the analysis sample to a minimum. This can prevent the effects of matrices such as lower analysis precision, repeatability, and recovery rates, which are setbacks in bioanalyses using LC-MS, and thus contribute to the durability of an LC-MS for consecutive analyses.

In addition, a Shimadzu triple quadrupole LC-MS, such as the LCMS-8050, features easy maintenance. ESI capillary replacement is simple with no need for tools and the desolvation line can be replaced without breaking the vacuum, providing greater uptime. Furthermore, the cost of replacement parts is more affordable compared to other manufacturers.

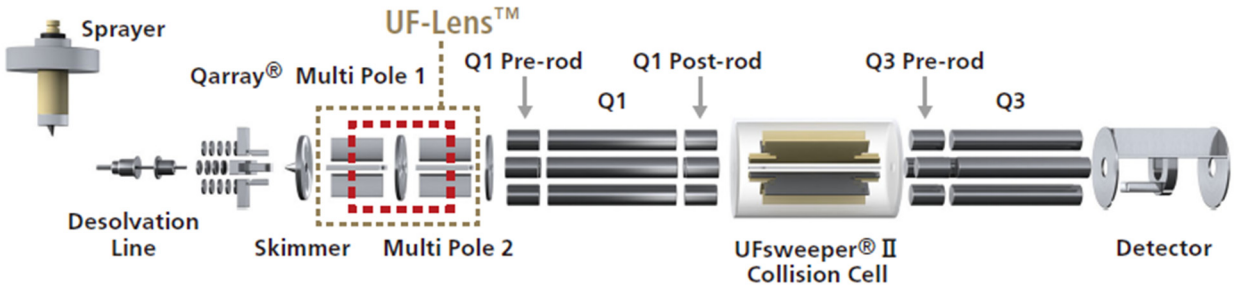

Fig. 1 Structure of the LCMS-8050 and the LCMS-8060

<References>
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