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Application News

No. C146A

nSMOL™ Antibody BA Kit

LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL- Part 2 - Bevacizumab analysis -

nSMOL™ Antibody BA Kit Features

nSMOL is Shimadzu's completely new and breakthrough technology that enables selective proteolysis of the Fab region of monoclonal antibodies. This technique facilitates method development independent of a variety of antibody drugs and achieves a paradigm shift in the bioanalysis of antibody drugs.

Furthermore, this is the only method with respect to antibody drugs that has fulfilled the criteria of "Guideline on Bioanalytical Method Validation in Pharmaceutical Development" for low MW drug compounds issued by the Japanese Ministry of Health, Labour and Welfare. Shimadzu also offers optimization methods and protocols, and nSMOL can be applied to clinical research at various institutions.

Antibody Drug Classification and Selection of Quantitation Peptides

Monoclonal antibodies are produced from the hybridoma with mouse spleen lymphocyte and myeloma cells. While mice are predominantly used as hosts, in recent years a variety of hosts are now available to produce monoclonal antibodies.

Furthermore, production of the variable Fv by phage display technology and high-throughput screening of affinity sequence has become alternative standard procedure.

Antibody drugs are classified into four classes according to specific structure.

The complementarity-determining region (CDR) of antibody specificity against human IgGs becomes smaller according to the "mouse" → "chimeric" → "humanized" → "fully human" antibody. More precise selection of quantitation peptides becomes particularly important in the nSMOL, which is used to perform structure specificity-indicated analysis.

The nSMOL enables selective proteolysis in variable regions. This allows selection of quantitation peptides that reflect the structural characteristics of antibodies. Antibodies have three CDRs on each heavy and light chain, CDR2 is known as the region that makes first contact with an antigen. The signature peptide by nSMOL are mainly from CDR2 containing peptides.

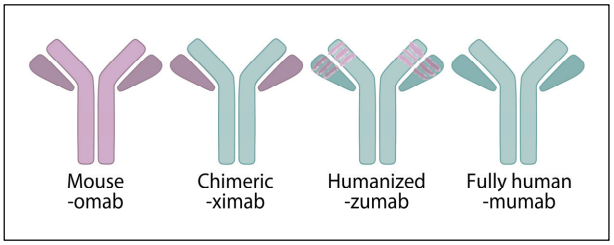

Fig. 1 Antibody Drug Classification

Analysis Conditions for Bevacizumab Using the nSMOL

<Sample Processing Protocol>

With the nSMOL technique, the same sample processing protocol can be applied to all antibody drugs. For details, refer to Shimadzu Application News (Trastuzumab analysis).

<LCMS Analysis Conditions>

[LC] NexeraX2 System

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	: 1 %B (1.5 min)/1-35 %B (3.5 min)/ 95 %B (1 min)/1 %B (1 min)
Flow rate	: 0.4 mL/min
Injection	: 10 µL

[MS] LCMS-8050, 8060

Ionization	: ESI Positive
DL	: 250 °C
Heat Block	: 400 °C
Interface	: 300 °C
Nebulizer gas	: 3 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min

Bevacizumab Quantitation Peptides

Peptide	MRM transition	Purpose
$P_{14}R$	512.1>292.3 (b3+)	For quantitation (IS)
	512.1>389.3 (b4+)	For structure confirmation
	512.1>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
STAYYLQMN SLR	642.3>748.4 (y6+)	For quantitation
	642.3>861.5 (y7+)	For structure confirmation
	642.3>620.3 (y5+)	For structure confirmation
VLIYFTSSLH SGVPSR	588.3>775.9 (y14++)	For quantitation
	588.3>602.3 (y6+)	For structure confirmation
	588.3>939.5 (y9+)	For structure confirmation

* Quantitation range in human plasma : 0.15 to 300 µg/ml
Averaged accuracy : 101.3 %

MRM Chromatograms

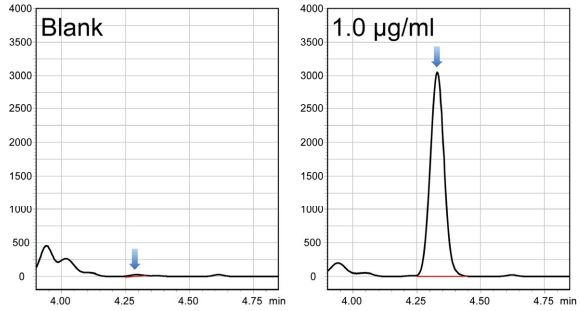

Fig. 2 MRM Chromatograms of FTFSLDTSK (in Human Plasma)

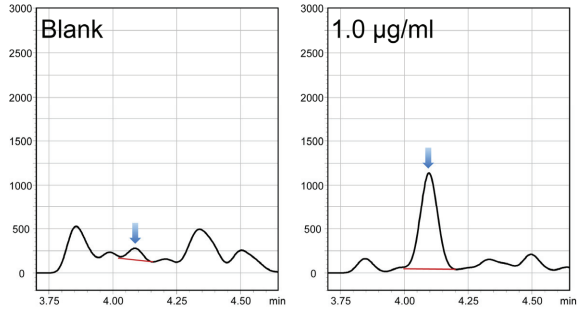

Fig. 3 MRM Chromatograms of STAYYLQMNSLR (in Human Plasma)

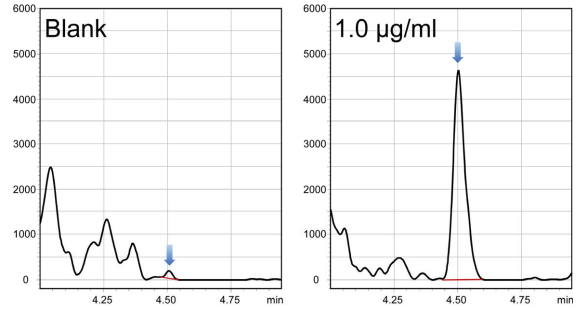

Fig. 4 MRM Chromatograms of VLIYFTSSLHSGVPSR (in Human Plasma)

Full Validation Results for Bevacizumab

<Precision and accuracy>

Set Concentration [µg/ml]	Data Average (N = 15)	Accuracy (%)	CV (%)
0.439	0.464	106	11.7
240	235	98.1	4.45

<Freeze-thaw test>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
0.439	0.395	89.9	-20
240	253	106	-20

<Long-term stability test>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
0.439	0.477	109	-20
240	223	93.1	-20

<Processed sample stability for 48 h>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
0.439	0.445	101	5
240	245	102	5

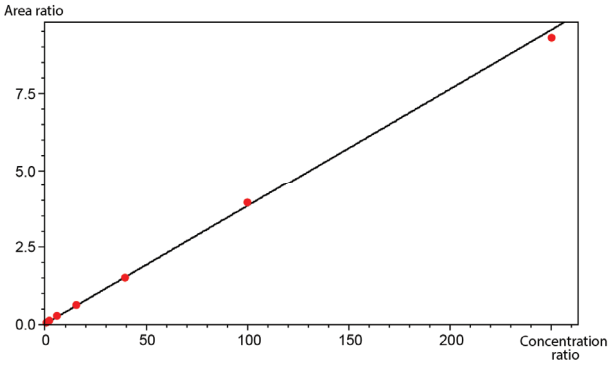

Fig. 5 Calibration Curve of Bevacizumab

Observations, Conclusions, and References

Although Bevacizumab quantitation peptide using nSMOL was obtained from the same region of Trastuzumab, the optimal peptide sequences for bioanalysis will depend on the interference with endogenous IgGs.

With respect to multiplexed quantitation of three sequences, nSMOL bioanalysis for Bevacizumab fulfilled the full validation criteria.

The lower limit of quantitation is 0.15 µg/ml and the same assay method can be used from preclinical to clinical trials.

<References>
Iwamoto N et al. *Analyst*, 2014, DOI:10.1039/c3an02104a
Iwamoto N et al., *Drug Metab Pharmacokinet*., 2016, DOI:10.1016/j.dmpk.2015.11.004
<Chief Scientists>
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Notes: 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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