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Application News

No. C148A

nSMOL™ Antibody BA Kit

LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL - Part 4 - Multiplex 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 Quantitation Peptide Selection

The development of antibody drugs, which are molecular target drugs, has progressed dramatically in recent years and most Fc regions has a structure derived from human IgG. The nSMOL enables collection of IgG fractions in plasma via Fc regions, and selective proteolysis on Fv of antibody drugs using trypsin immobilized on the surface of nanoparticles. This reaction field allows selection of quantitation peptides that reflect the structural characteristics of antibodies. Antibodies have three CDRs respectively on each heavy and light chain, and the collected peptides using the nSMOL are mainly peptides including CDRs.

In recent years, development of technology capable of quantifying many items of antibodies in a single analysis is required for due to the variety of antibody drugs and combination therapy. The nSMOL supports multiplex analysis and can quantify many antibodies in a single analysis with high precision because subject molecules of nSMOL are all IgGs in plasma. This indicates that the nSMOL can be applied in antibody pharmacokinetics for combination therapy.

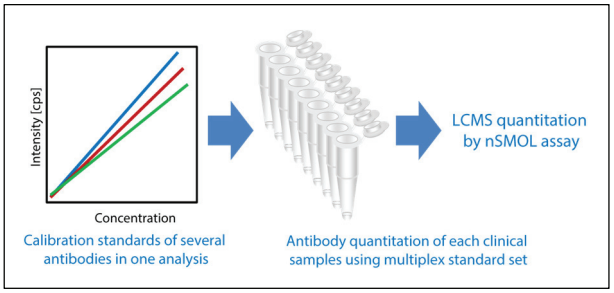

Fig. 1 Multiplex Analysis Using nSMOL

Conditions of Multiplex Analysis Using the nSMOL

<Sample Processing Protocol>

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

<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)/1-42 %B (4 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

Quantitation Peptides for Multiplex Analysis

Peptide	MRM transition	Purpose
$P_{14}R$	512.1>292.3 (b3+) 512.1>389.3 (b4+) 512.1>660.4 (b6+)	For quantitation (IS) For structure confirmation For structure confirmation
Brentuximab vedotin (VLIYAASNLE SGIPAR)	837.5>343.1 (y3+) 837.5>600.3 (y6+) 837.5>213.1 (b2+)	For quantitation For structure confirmation For structure confirmation
Rituximab (ASGYTFTSY NMHWVK)	598.1>817.5 (y13++) 598.1>707.5 (y11++) 598.1>657.1 (y10++)	For quantitation (IS) For structure confirmation For structure confirmation
Cetuximab (SQVFFK)	378.2>540.3 (y4+) 378.2>441.2 (y3+) 378.2>294.2 (y2+)	For quantitation For structure confirmation For structure confirmation

*	Quantitation range in human plasma	: 0.58 to 300 µg/ml
	Accuracy	: 91.5 to 115 %
	Precision	: 4.4 to 11.8 %

MRM Chromatograms

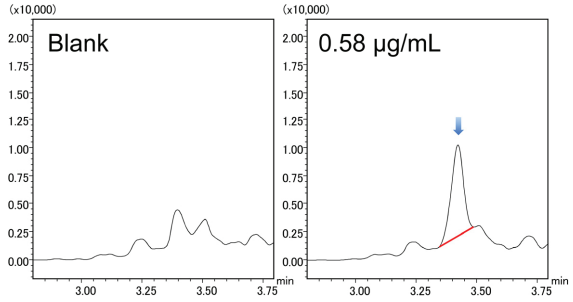

Fig. 2 Brentuximab MRM Chromatograms (in Human Plasma)

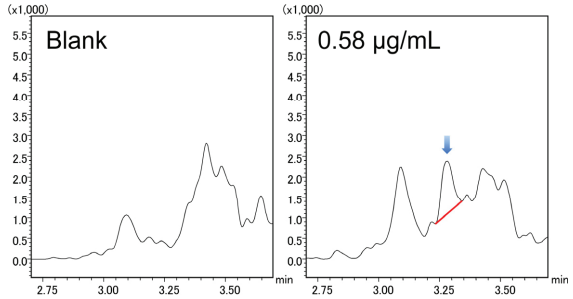

Fig. 3 Rituximab MRM Chromatograms (in Human Plasma)

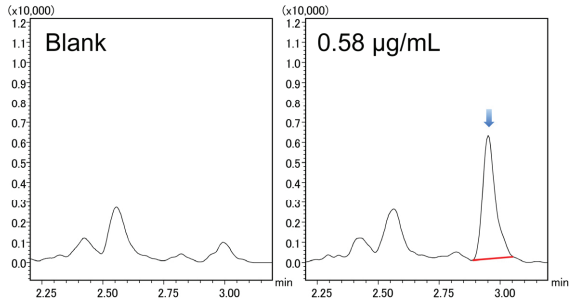

Fig. 4 Cetuximab MRM Chromatograms (in Human Plasma)

Precision of Multiplex Analysis

○ Brentuximab vedotin

Set Concentration [µg/ml]	Data Average (N = 9)	CV (%)	Accuracy (%)
1.76	1.74	7.60	99.2
240	249	7.12	104

○ Rituximab

Set Concentration [µg/ml]	Data Average (N = 9)	CV (%)	Accuracy (%)
1.76	1.77	5.44	101
240	247	9.65	104

○ Cetuximab

Set Concentration [µg/ml]	Data Average (N = 9)	CV (%)	Accuracy (%)
1.76	1.71	8.48	97.5
240	255	5.52	106

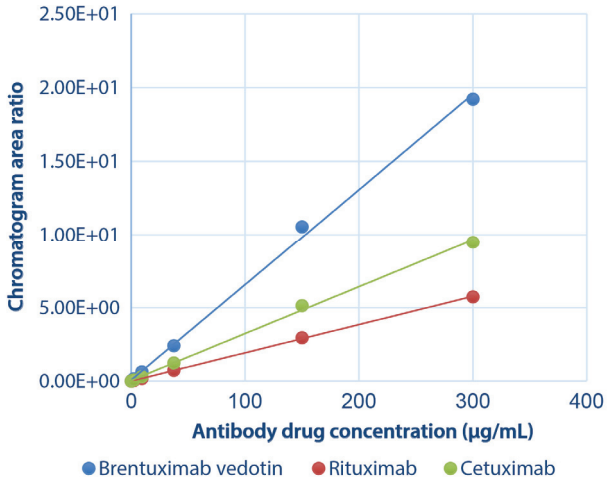

Fig. 5 Multiplex Calibration Curve

Observations, Conclusions, and References

By utilizing the nSMOL technique, it is possible to detect many antibody drugs in a single analysis. Therefore, it is also possible to create calibration curves of multiple antibody drugs in a single analysis by mixed multiple antibody drugs into plasma. Using the three antibodies presented this experimental model here, the precision and accuracy of quantitative values fulfilled the validation criteria of the guidelines issued by the FDA.

The lower limit of quantitation is 0.58 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., *Clinical Pharmacology & Biopharmaceutics*, 2016, DOI:10.4172/2167-065X.1000164
< Chief Scientists >
Noriko Iwamoto, Ph.D. and Takashi Shimada, Ph.D., Technology Research Laboratory

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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Shimadzu Corporation
www.shimadzu.com/an/