SHIMADZU
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Application News

No. C147A

nSMOL™ Antibody BA Kit

LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL - Part 3 - Nivolumab 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 the variety of antibody drug 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.

Method Validation for Nivolumab Bioanalysis

Cancer cells have been found to evade immune surveillance mechanism through the expression of immunosuppressive ligands, and avoid cytotoxicity from immune cells.

Nivolumab was developed by Dr. Honjo et al. as a breakthrough medicine that activate immune cells by blocking PD-1 mediated inhibitory signals*. Innovative drugs that apply these immunological mechanisms are named as immune checkpoint inhibitors, and many drug discovery for this field now continue to progress around the world.

These medicines are used in a cancer chemotherapy which act on advanced and complex immunological mechanisms. Therefore, it is important to progress integrative clinical trials in order to develop more efficient treatments by using many clinical indexes and biomarkers.

Shimadzu has applied the nSMOL and performed analytical validation of Nivolumab for the pharmacokinetic monitoring into early clinical implementations.

Quantitation Peptides of Nivolumab

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
ASGITFSNSG MHWVR	550.8>661.5 (y11++)	For quantitation
	550.8>746.4 (y13++)	For structure confirmation
	550.8>785.4 (y6 +)	For structure confirmation

* Quantitation range in human plasma : 0.15 to 300 µg/ml
Averaged accuracy : 100.4 %

Analysis Conditions for Nivolumab 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 (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)/1-40 %B (3 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

Structure Configuration of Nivolumab Candidate Signature Peptides Identified in nSMOL Reactions

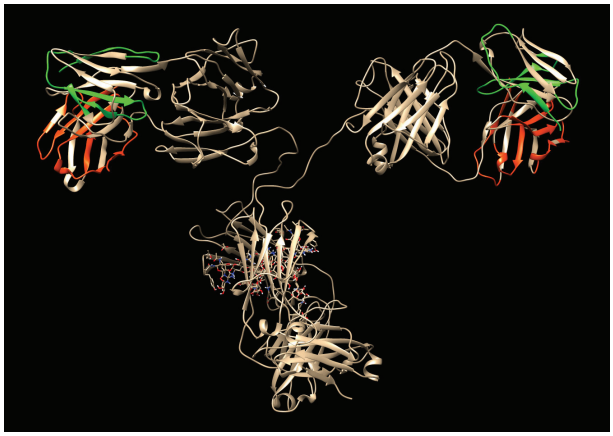

Fig. 1 Structure Configuration of Nivolumab Tryptic Peptides

Detected peptides are indicated in red (heavy chain) and green (light chain). Fv-selective proteolysis has been progressing by nSMOL.

MRM Chromatograms

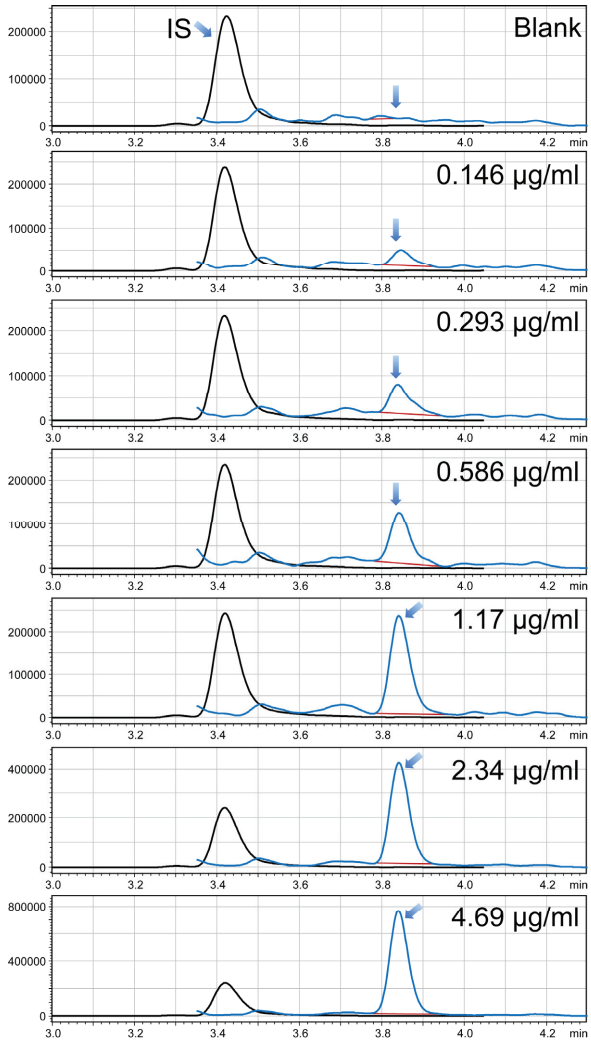

Fig. 2 MRM Chromatograms of ASGITFSNSGMHWVR (Blue), and $P_{14}R$ Internal Standard (Black) (in Human Plasma)

Full Validation Results for Nivolumab

<Precision and accuracy>

Set Concentration [µg/ml]	Data Average (N = 15)	Accuracy (%)	CV (%)
2.93	2.97	101	7.51
200	202	101	6.75

<Freeze-thaw test>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
2.93	2.73	95.6	-20
200	183	96.1	-20

<Long-term stability test>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
2.93	3.03	104	-20
200	213	107	-20

<Processed sample stability for 48 h>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
2.93	3.08	105	5
200	195	97.6	5

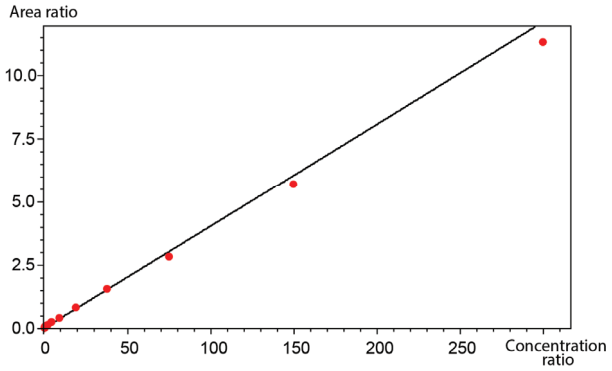

Fig. 3 Nivolumab Calibration Curve

Observations, Conclusions, and References

Although eight candidate signature peptides including CDRs were obtained using nSMOL, only the peptide ASGITFSNSGMHWVR indicated a positive correlation to drug concentration. This indicates that the sequence homology of fully human antibodies and endogenous IgGs is extremely similar.

In order to set suitable bioanalysis conditions, peptide candidates with structural specificity must be strictly selected. By utilizing Fv-selective reactions, Shimadzu nSMOL greatly facilitates the development of assay methods. The lower limit of quantitation is 0.15 µg/ml and the same assay method can be used from preclinical to clinical trials.

<References>
* Ishida Y, Agata Y, Shibahara K, and Honjo T., *EMBO J*, 1992, 11(11):3887
Iwamoto N et al. *Analyst*, 2014, DOI:10.1039/c3an02104a
Iwamoto N et al., *J. Chromatogr. B*, 2016, DOI:10.1016/j.jchromb.2016.04.038
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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.

First Edition: Mar. 2017

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