SHIMADZU
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Application News

No. C145B

nSMOL™ Antibody BA Kit

LCMS Bioanalysis of Antibody Drugs Using Fab-Selective Proteolysis nSMOL - Trastuzumab 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.

LCMS Bioanalysis Solved with the nSMOL Method

Pharmacokinetic information provides some of the most fundamental indicators. The effective drug discovery is supported by the overall pharmacokinetic profile such as drug efficacy and toxicity.

While the enzyme-linked immunosorvent assay (ELISA) has been the current way to measure blood concentration until now, there are essential issues due to the effects of cross-reactivity and inhibitory substances. On the other hand, mass spectrometry may be able to solve these issues because of structure-indicated analysis.

Nevertheless, mass spectrometry has several issues. In particular, direct quantitation analysis (top-down proteomics) of complex matrices, such as plasma, is not suitable for repeat analysis because the ESI interface cannot be maintained due to the large excess analytes.

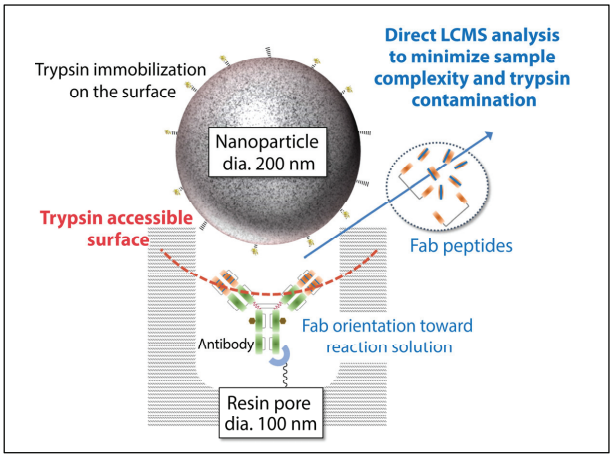
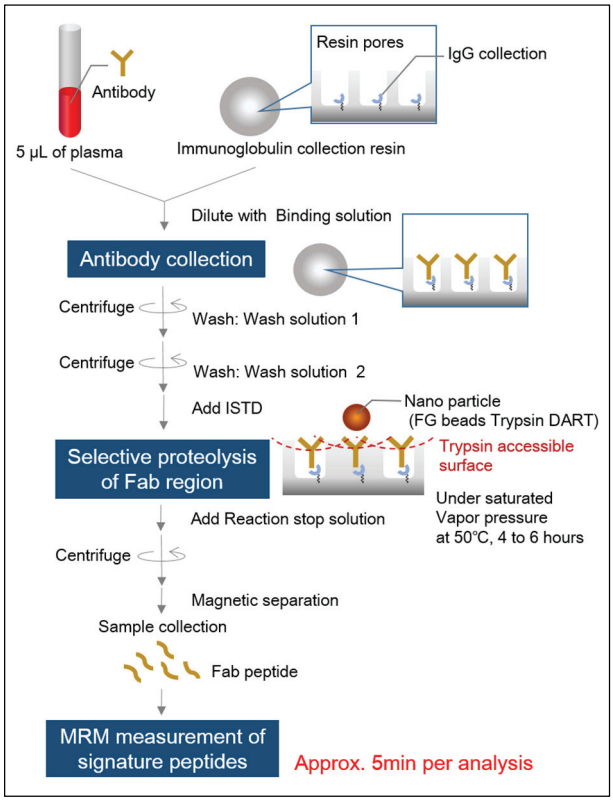


Fig. 1 Principle of the nSMOL Technique

Sample Processing Protocol and Analysis Conditions for Trastuzumab Using the nSMOL

<Sample Processing Protocol>

In the nSMOL protocol, the same sample processing protocol can be applied to all antibody drugs. The procedure is described below.



<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)/1-25 %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

Quantitation Peptides of Trastuzumab

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
IYPTNGYTR	542.8>404.7 (y7++)	For quantitation
	542.8>808.4 (y7+)	For structure confirmation
	542.8>610.3 (y5+)	For structure confirmation

* Quantitation range in human plasma : 0.0610 to 250 µg/ml
Averaged accuracy : 100.7 %

The quantitation peptide (signature peptide) is selected from tryptic peptides that contain a complementarity-determining region (CDR) with antibody specificity. However, there is not necessary that the CDR-containing peptide does not have the same amino acid sequence in the endogenous IgGs. For this reason, it should be confirmed that there is no competition with the signature peptide in the biological matrix.

Furthermore, in principle, mass spectrometry can only access the *m/z* and signal intensity. Accordingly, Shimadzu recommends the setting of structure confirmation MRM transition in addition to quantitative MRM in each bioanalysis. This ensures reliable and high quality analysis.

MRM Chromatograms and Calibration Curves

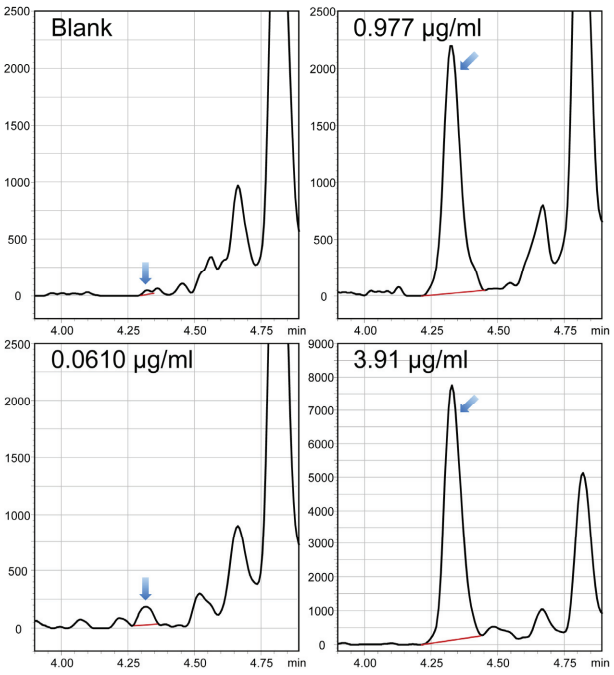


Fig. 2 MRM Chromatogram of Trastuzumab IYPTNGYTR in Human Plasma

Full Validation Results for Trastuzumab

<Precision and accuracy>

Set Concentration [µg/ml]	Data Average (N = 15)	Accuracy (%)	CV (%)
2.93	2.58	88.1	8.2
200	211	106	5.6

<Freeze-thaw test>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
2.93	2.87	98.1	-20
200	199	99.7	-20

<Long-term stability test>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
2.93	3.03	104	-20
200	203	101	-20

<Processed sample stability for 48 h>

Set Concentration [µg/ml]	Data Average (N = 5)	Accuracy (%)	Temperature (°C)
2.93	3.67	91.2	5
200	211	106	5

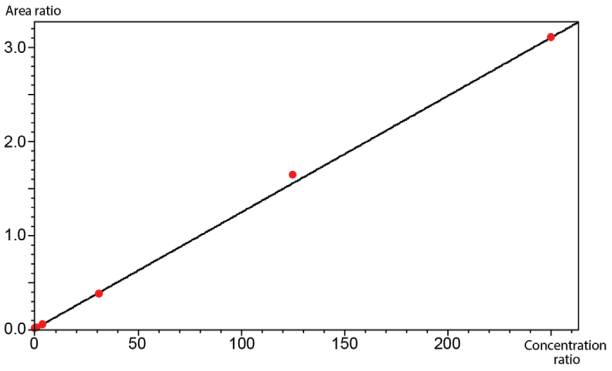


Fig. 3 Calibration Curve of Trastuzumab

Observations, Conclusions, and References

The nSMOL fulfills the guideline criteria for small molecule drug compounds and enabled quantitative analysis of Trastuzumab in human plasma.

The lower limit of quantitation is 0.06 µg/ml and the same assay method can be used from preclinical to clinical testing.

The nSMOL assay described here succeeded in shortening the analysis time by significantly decreasing the noise matrix.

<References>
Iwamoto N et al. *Analyst*, 2014, DOI:10.1039/c3an02104a
Iwamoto N et al., *Anal Methods*, 2015, DOI:10.1039/c5ay01588j
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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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