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Application News

No. C176

Microflow Liquid Chromatography Mass Spectrometry System

Microflow LC-MS/MS Analysis of Monoclonal Antibody in Human Plasma at ng/mL Level with nSMOL™ Proteolysis

Abstract

nSMOL proteolysis has been reported as the novel technology to standardize the sample preparation workflow and to improve the sensitivity of mass spectrometric assay of therapeutic monoclonal antibodies in human serum or plasma.

We developed highly sensitive bioanalysis method to achieve low ng/ml sensitivity of Trastuzumab in human plasma with the combination of the nSMOL proteolysis and the newly developed Nexera Mikros™ system.

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Fig. 1 Nexera Mikros™, a Microflow LC-MS/MS System

Introduction

Mass spectrometric (LC-MS/MS) determination of therapeutic monoclonal antibodies in serum or plasma is increasingly used for pharmacokinetic studies in the preclinical, clinical, and therapeutic phases. One major advantage of this approach over conventional ligand binding assay (LBA) is high specificity for the target antibodies that can be achieved by selecting tryptic peptides derived from the complementarity-determining region (CDR) as the antibody signature peptide and subjecting it to LC-MS/MS quantitation. Moreover, LC-MS/MS approach requires much less assay developmental work than LBA, which completes within days rather than several months. Our recent advancement of sample preparation strategy, namely nano-surface and molecular-orientation limited (nSMOL) proteolysis, have further simplified the method development process. nSMOL proteolysis yields extremely clean CDR peptide mixture thereby alleviating the need to address interference from biological matrix.

Despite various advantages, one drawback of LC-MS/MS assays is that the level of sensitivity depends on the mass spectrometric response (efficiency of ionization and fragmentation) of the signature peptide, which is essentially unpredictable and uncontrollable. For example, recently reported bioanalyses of therapeutic mAb showed varying LLOQ levels ranging from 0.06-0.58 µg/mL in plasma. Currently there is risk that a newly-developed assay might not fulfil the sensitivity requirement for pre-clinical trials.

Here we aim to overcome this issue by implementation of a robust microflow LC-MS/MS system to measure signature peptides at increased sensitivity than conventional semi-microflow systems, while maintaining the same level of robustness, analysis turnaround time and ease of system configuration.

Sample and Pretreatment

Pooled human plasma sample was purchased from Kohjin Bio (Saitama, Japan). Trastuzumab was spiked at various concentrations (0, 0.00763, 0.0153, 0.0305, 0.0610, 0.122, 0.0244, 0.488, 0.977, 1.95, 3.91, 7.81, 15.6, 31.3, 62.5 µg/mL) for calibration curve and independently at four concentration set for QC samples. QC set 1 and 2 were prepared and ran on two separate days.

Spiked and blank plasma samples were pretreated after keeping at -80 °C for 24 h or longer using the nSMOL™ Antibody BA Kit (Shimadzu Corporation, Japan) in accordance with the instruction manual.

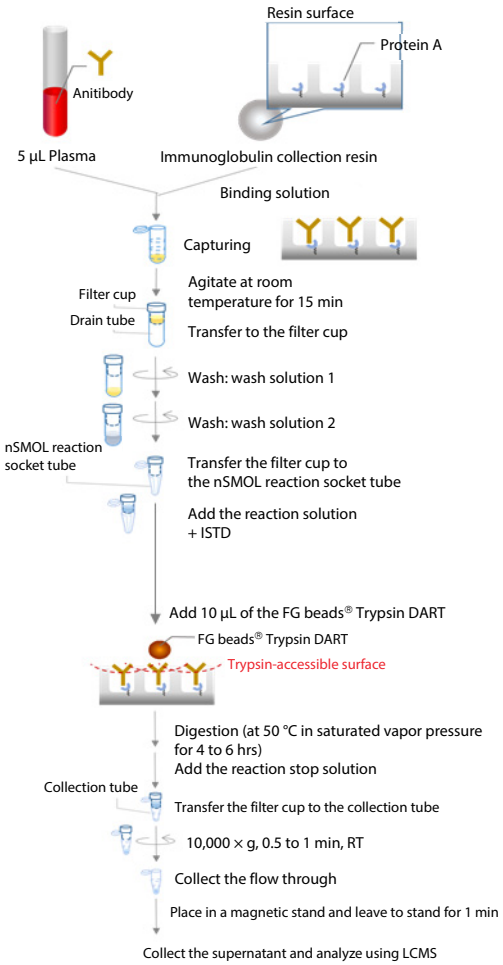

Fig. 2 Standard Protocols of nSMOL Workflow

Table 1 Analytical Conditions

[LC] Nexera Mikros System		
(Analytical)		
Column	:	Shim-Pack™ MC C18 (50 mm × 0.175 mm)
Flow Rate	:	4 µL/min.
Oven Temp.	:	50 °C
Solvent A	:	0.1 % Formic acid in water
Solvent B	:	0.1 % Formic acid in Acetonitrile
Gradient	:	0.00-0.50 min. 5 %B→4.50 min. 25 %B →4.51 min. 95 %B→5.00 min. 95 %B →5.01 min. 5 %B→11.00 min. STOP
(Trap)		
Trap column	:	L-column 2 ODS Micro (5 mm × 0.3 mm)
Oven Temp.	:	50 °C
Solvent A	:	0.05 % Trifluoroacetic acid in water
Solvent B	:	0.1 % Formic acid in Acetonitrile
Inj. Volume	:	10 µL
[MS] LCMS-8060 with Micro-ESI 8060		
Ionization	:	ESI Positive
DL Temp.	:	250 °C
Heat Block Temp.	:	400 °C
ESI Temp.	:	100 °C
Nebulizer Gas	:	2 L/min.
Drying Gas	:	OFF
Heating Gas	:	3 L/min.

Table 2 MRM Transitions

Peptide	MRM transition	Objectives
IYPTNGYTR	542.8 > 404.7 (y7++)	Quantifier
	542.8 > 808.4 (y7+)	Qualifier
	542.8 > 610.3 (y5+)	Qualifier
$P_{14}R$ (IS)	512.1 > 292.3 (b3+)	Quantifier
	512.1 > 389.3 (b4+)	Qualifier
	512.1 > 660.4 (b6+)	Qualifier

Results

Calibration curve in plasma matrix showed good linear response in the range 7.6 ng/mL to 62.5 µg/mL (Fig. 3). Compared to the LLOQ of 0.06 µg/mL as previously reported for Trastuzumab (also using nSMOL proteolysis and LCMS-8060), switching to the Nexera Mikros system contributed to sensitivity improvement by nearly one order of magnitude.

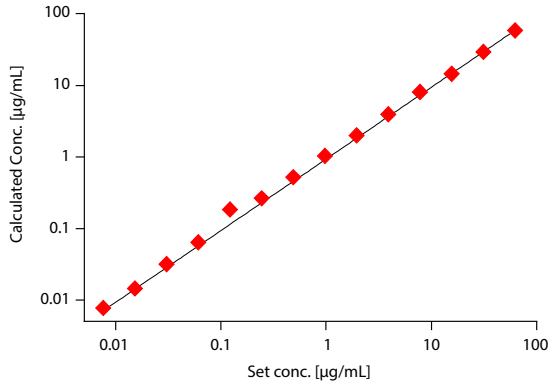

Fig. 3 Calibration Curve for Trastuzumab Bioanalysis

Table 3 Results of Assay Repeatability Evaluation Using QC Samples

Set conc. (µg/mL)	QC set 1 (N=5 for each level)			QC set 2 (N=5 for each level)		
	Deter-mined	Accuracy (%)	Repeat-ability (%)	Deter-mined	Accuracy (%)	Repeat-ability (%)
0.00763	0.00741	97.1	5.69	0.00762	100	11.3
0.0229	0.0234	102	6.68	0.0232	101	2.84
5.86	6.19	106	2.67	5.83	99.4	3.12
50.0	46.9	94.0	6.36	45.8	91.7	7.23

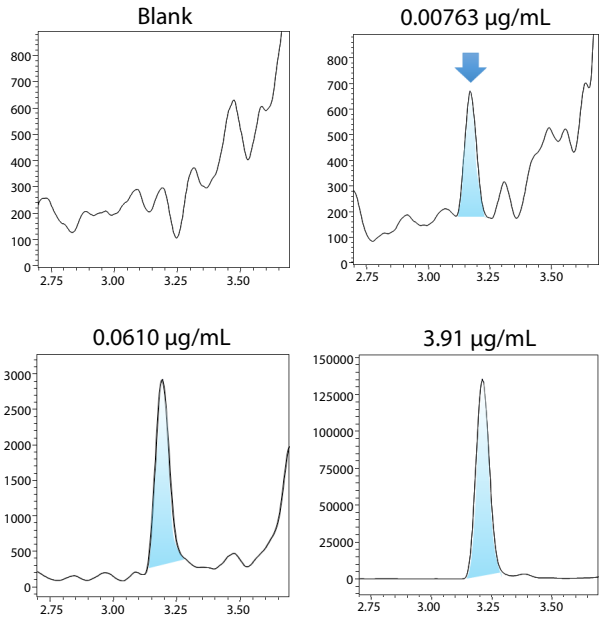

Fig. 4 Representative MRM Chromatograms (Flow Rare: 4 µL/min.)

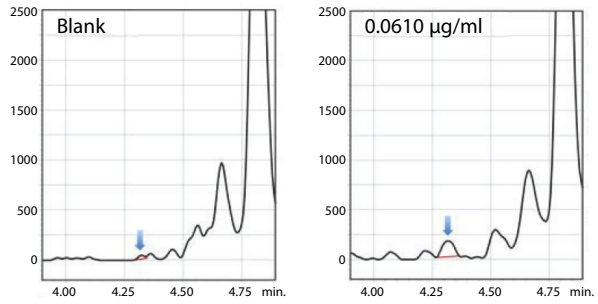

Fig. 5 Representative MRM Chromatograms (Flow Rare: 0.4 mL/min.)
***Cited from *Application News* No. C145A**

References
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