LSI Medience

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日本核酸医薬学会第6回年会

Development of Simultaneous Quantification Method for Nusinersen and its Metabolites in Rat Plasma and Tissues using LC-MS/MS

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Introduction

Because drugs are often metabolized to active/toxic metabolites, it is important to quantify the drug and its metabolites in biological matrices for the evaluation of drug safety.

The LC-MS/MS method is useful for quantification of oligonucleotide drugs and its metabolites, because the method has high selectivity and can be quantified simultaneously.

Previously, we administered nusinersen, one of the antisense oligonucleotide drugs, to rats and identified 3'N-1 and 3'N-2 as its metabolites.

Therefore, we developed simultaneous quantification method for nusinersen and identified metabolites using LC-MS/MS.

We determined concentrations of nusinersen and its metabolites in rat samples using this method.

Precise Metabolite Identification

Equipment and software:

Q Exactive™ Focus & Xcalibur™ ver. 4.1
(Thermo Fisher Scientific, Waltham, MA)

Nusinersen (18mer):	5′-TCACTTTCATAATGCTGG -3′
3′N-1 (17mer):	5′-TCACTTTCATAATGCTG□ -3′
3′N-2 (16mer):	5′-TCACTTTCATAATGCT□□ -3′

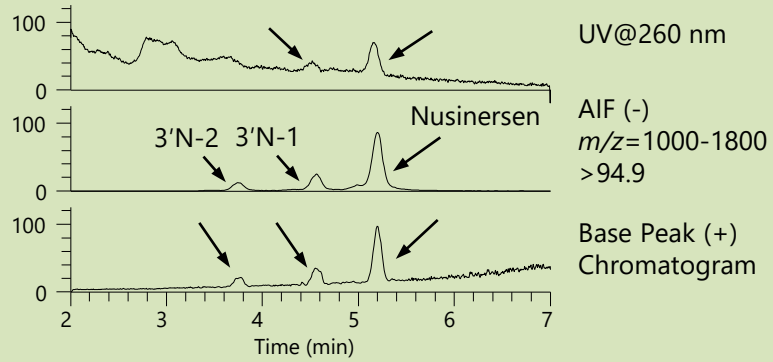
Result:

The metabolites were identified more precisely using UV detection, HILIC condition for achieving baseline separation, PSO_2^- detection with an AIF[1)] method, positive MS and MS/MS spectra.

1) *Anal. Chem.* 2017, 89, 12, 6821–6826

Rat kidney, 360 min after dosing

Chromatograms

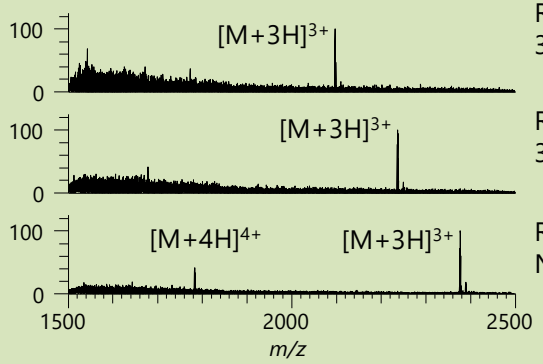


UV@260 nm

AIF (-)
m/z=1000-1800
>94.9

Base Peak (+)
Chromatogram

MS Spectra

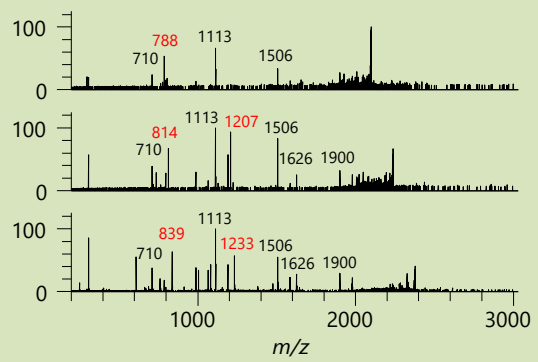


RT=3.8 min
3′N-2

RT=4.6 min
3′N-1

RT=5.2 min
Nusinersen

MS/MS Spectra from $[M+3H]^{3+}$

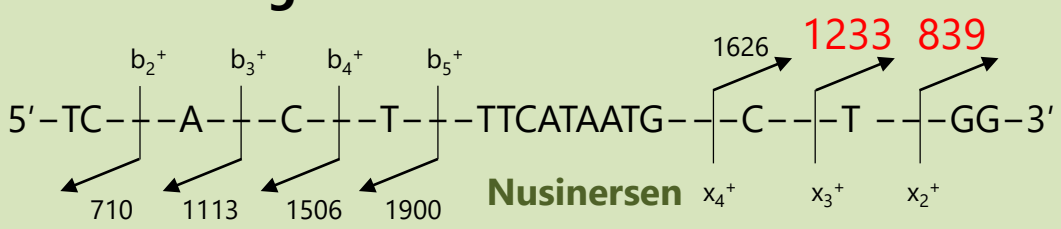


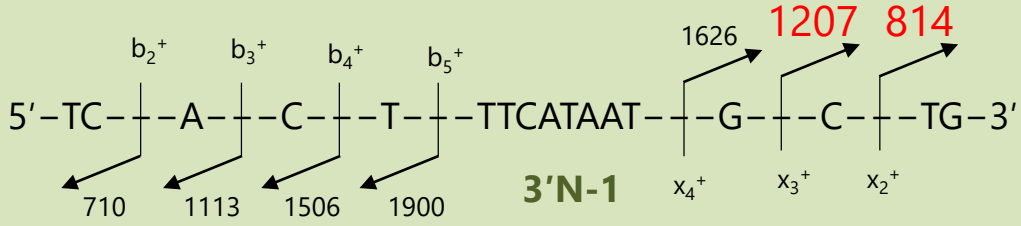
RT=3.8 min
3′N-2

RT=4.6 min
3′N-1

RT=5.2 min
Nusinersen

Observed fragments of nusinersen and 3′N-1 metabolite>





Pre-treatment

50 µL of rat plasma (EDTA-2K) or rat tissue lysate

↓ Add lysis buffer (Clarity®OTX* kit reagent)

Mixture

↓ Load to Clarity®OTX 25 mg plate
↓ Wash with 50mM NaH_2PO_4 (pH5.5)
↓ Wash with 500mM NaH_2PO_4 (pH5.5)/water/ACN (10:40:50, v/v/v)
↓ Elute with 400mM NH_4HCO_3/ACN (50:50, v/v)

Eluent

↓ Dry under N_2 stream

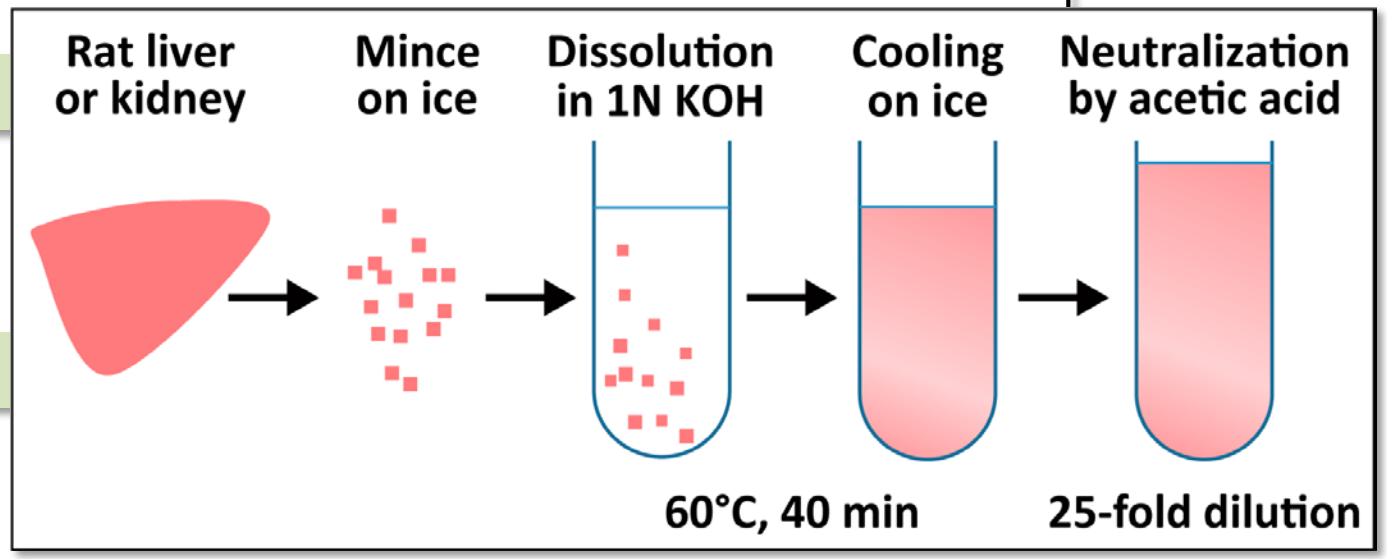
Residue

↓ Reconstitute with 50 µL of TE buffer/methanol (70:30, v/v)
↓ Filter

Filtrate

↓ Inject (7 µL) to LC-MS/MS

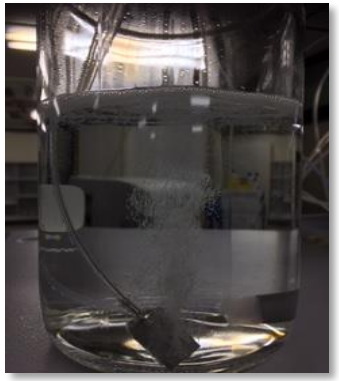
*: Phenomenex, Torrance, CA



LC-MS/MS condition

LC	**Nexera X2 system** (Shimadzu, Kyoto, Japan)
Column	X Bridge™ BEH C18 2.5µm, 2.1×50mm (Waters, Milford, MA)
Column temp.	60°C
Mobile phase A[*1]	Water/methanol/TEA/HFIP/acetylacetone (900:100:2:40:0.05, v/v/v/v/v)
Mobile phase B[*1]	Methanol/water/TEA/HFIP/acetylacetone (900:100:2:40:0.05, v/v/v/v/v)
Run time	27 min

*1: Under shading and nitrogen bubbling (Patent pending in Japan, patent application No. 2020-122679)



MS	**QTRAP®6500+** and **Analyst®**(ver. 1.7) (SCIEX, Framingham, MA)		
Ionization mode	Turbo ion spray		
Scan type	MRM		
Polarity	Negative		
Ion spray voltage	-4500 V		
TEM	500°C		
Monitoring ions	**Analyte**	**Q1 (*m/z*)**	**Q3 (*m/z*)**
	Nusinersen	1017.0	94.9
	3'N-1	957.1	94.9
	3'N-2	697.7	94.9
	I.S.[*2]	881.7	94.9

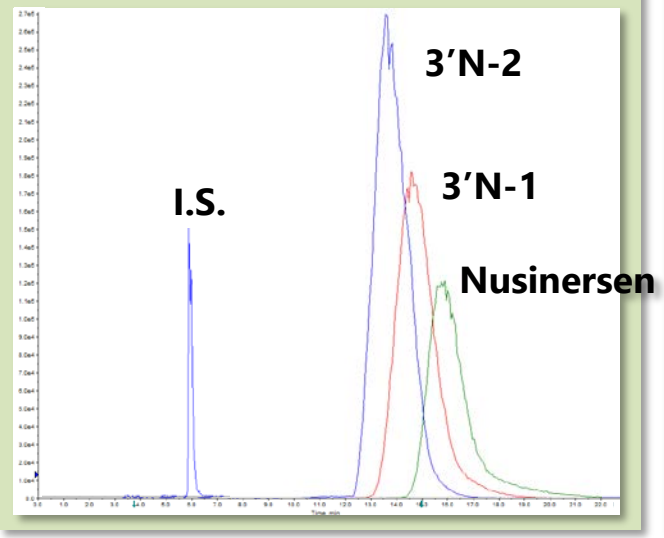
*2: Nusinersen which its chemical modification of 2'-MOE (2'-O-Methoxyethyl) is changed to 2'-OMe (2'-O-Methyl) is used as I.S. for this analytical method.

Typical chromatograms of validation samples

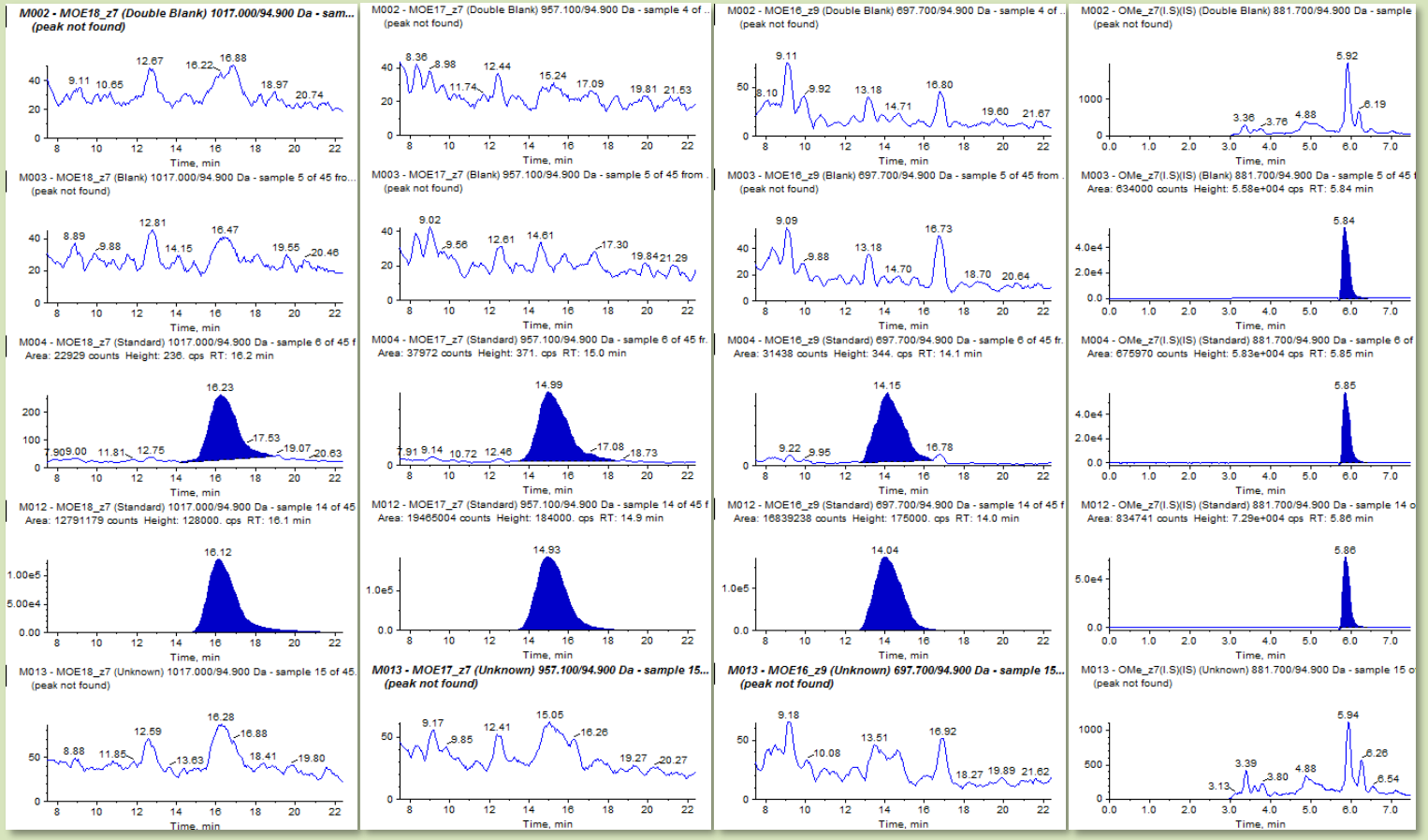
Nusinersen and its metabolites in rat plasma and tissues (data not shown) was analyzed with the same analytical conditions of LC-MS/MS.

S/N ratio of LLOQ peak was enough and carry over peak was not observed.

Overlay



Typical chromatograms (plasma)



Results of validation study (plasma)

Good linearity, precision and accuracy were observed over the concentration range of 1 to 400 ng/mL each in plasma. Nusinersen and its metabolites in plasma were stable under various conditions.

Items	Contents	Results
Selectivity	Male and female, n=3 each, total n=6	No interfering peak
Carry over	n=1	No interfering peak
Calibration curve	1-400 ng/mL, n=1 each	Accuracy: 90.3-113.0%, r≥0.9971
Within-run accuracy and precision	4 concentrations, n=5 each	Accuracy: 89.0-107.0% C.V.: 2.8-6.7%
Post-preparative stability	4°C, 72 hours, 1 concentration, n=3	Accuracy: 98.0-108.7%
Stability at room temperature in plasma	24 hours, 1 concentration, n=3	Accuracy: 104.0-109.0%
Freeze and thaw stability in plasma	5 cycles, 1 concentration, n=3	Accuracy: 88.0-94.3%

Results of validation study (tissue)

Good linearity, precision and accuracy were observed over the concentration range of 25 to 10000 ng/g in tissues (liver and kidney). Nusinersen and its metabolites in liver lysate were stable under various conditions.

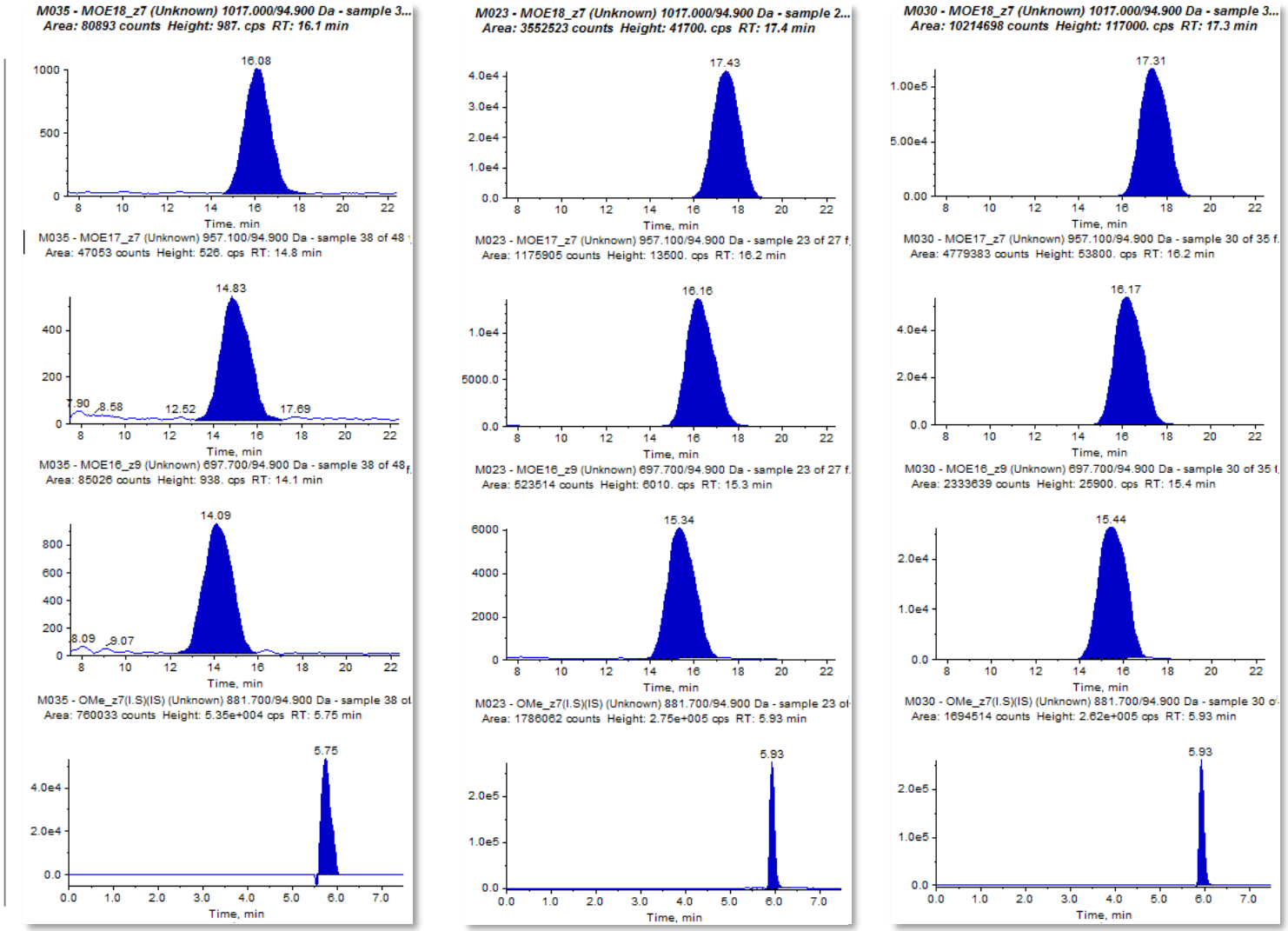
Items	Contents	Results
Carry over	n=1	No interfering peak (liver and kidney)
Calibration curve	25-10000ng/g tissue, n=1 each	Accuracy: 95.6-106.0%, r≥0.9992 (liver) Accuracy: 93.6-108.0%, r≥0.9986 (kidney)
Within-run accuracy and precision	4 concentrations, n=5 each	Accuracy: 94.2-101.6%, C.V.: 1.2-5.1% (liver) Accuracy: 89.0-107.0%, C.V.: 1.2-6.7% (kidney)
Post-preparative stability	4°C, 24 hours, 1 concentration, n=3	Accuracy: 94.8-103.1% (liver)
Stability at room temperature in tissue lysate	24 hours, 1 concentration, n=3	Accuracy: 101.5-103.9% (liver)
Freeze and thaw stability in tissue lysate	5 cycles, 1 concentration, n=3	Accuracy: 99.1-100.9% (liver)

Typical chromatograms of biological samples

Administration

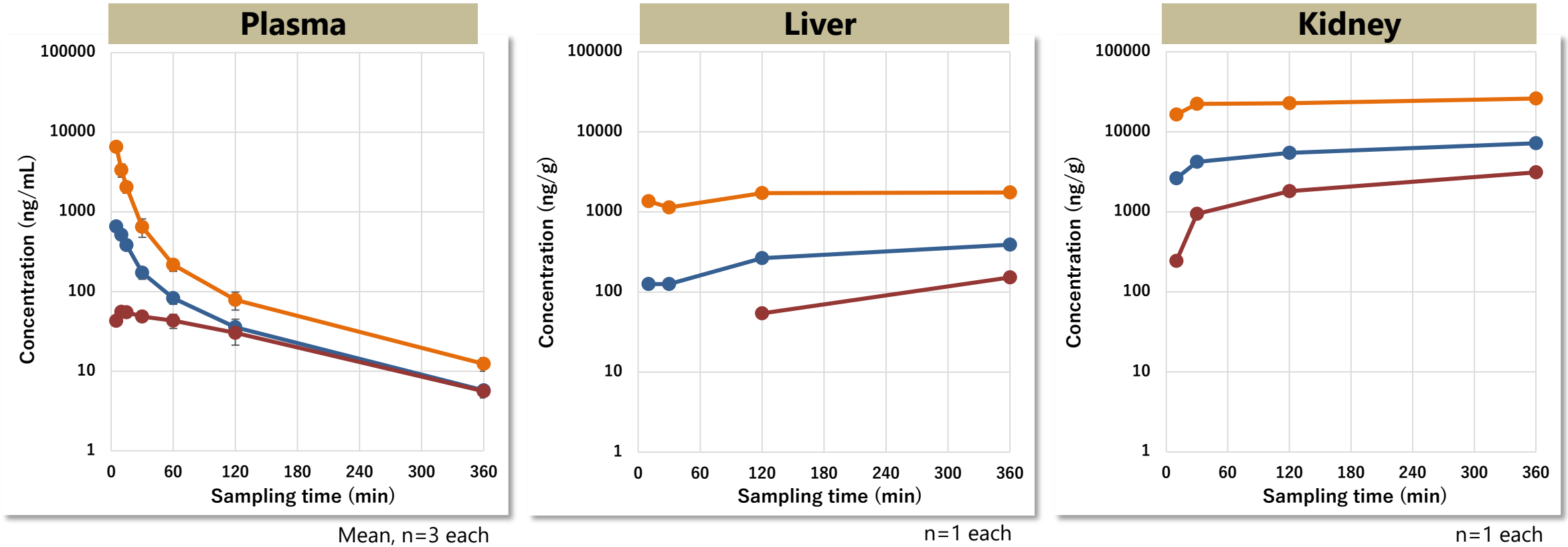
- Rat (Crl:CD(SD))
- Intravenous
- 1 mg/kg of nusinersen
- Single dosing

The chromatograms of the samples collected 360 minutes after dosing are shown.



Results of quantitative analysis

Concentrations of nusinersen and its metabolites in plasma tend to decrease until 360 minutes after dosing. Whereas nusinersen in liver and kidney did not decrease or rather increased after dosing, and its metabolites in liver and kidney tend to increase until 360 minutes after dosing.



Conclusion

- **The metabolites were identified more precisely using HILIC condition for achieving baseline separation and several mass spectrometric technique.**
- **We have developed an LC-MS/MS method for simultaneous quantification of nusinersen and its metabolites in plasma, liver and kidney.**
- **Our results showed that nusinersen and its metabolites distribute in liver and kidney as commonly described for oligonucleotides.**

We have no financial relationships to disclose for this presentation.