Analytical Method Development of Nusinersen in Rat Plasma and Tissues using LC-MS/MS

LSI Medience Corporation

○ Rika Dosho, Masaki Hoshino, Tomoko Saito, Ryuta Hosogai, Shin-ichiro Nitta, Chika Kitajima, Yui Akagawa, Eiji Murata, Atsushi Iwai, Keiko Nakai, Tetsuya Ueda

Introduction

In drug development, evaluation of "drug metabolism and disposition" is important, and quantitative analysis of drug in biological samples is essential. Recently, oligonucleotides (ONs) have been extensively investigated, and LC-MS/MS method, which has advantage in specificity and characterization of metabolism, has also drawn attention for analysis of ONs. We have developed an analytical method of Nusinersen, one of the antisense ONs, using triple-quadrupole mass spectrometer. The method was applied to analyze the plasma and tissue samples obtained from rats after intravenous administration of Nusinersen. In addition, we tried identifying Nusinersen metabolites using hybrid triple quadrupole time-of-flight mass spectrometer.

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

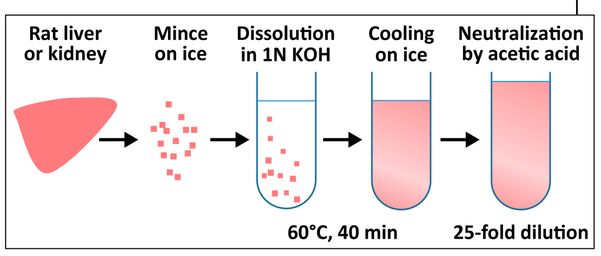
↓ Reconstitute with 50 µL of TE buffer/methanol (70:30, v/v)

↓ 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.	50°C		
Mobile phase A	Water/methanol/TEA/HFIP/acetylacetone (900:100:2:30:0.05, v/v/v/v/v)		
Mobile phase B	Methanol/water/TEA/HFIP/acetylacetone (900:100:2:30:0.05, v/v/v/v/v)		
Run time	14.0 min		
MS	**QTRAP®5500** and **Analyst®**(ver. 1.6.2) (SCIEX, Framingham, MA)		
Ionization mode	Turbo ion spray		
Scan type	MRM		
Polarity	Negative		
Ion spray voltage	-4500 V		
TEM	600°C		
Monitoring ions	Analyte	Q1 (*m/z*)	Q3 (*m/z*)
	Nusinersen	1017.0	94.9
	I.S.	881.7	94.9

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.

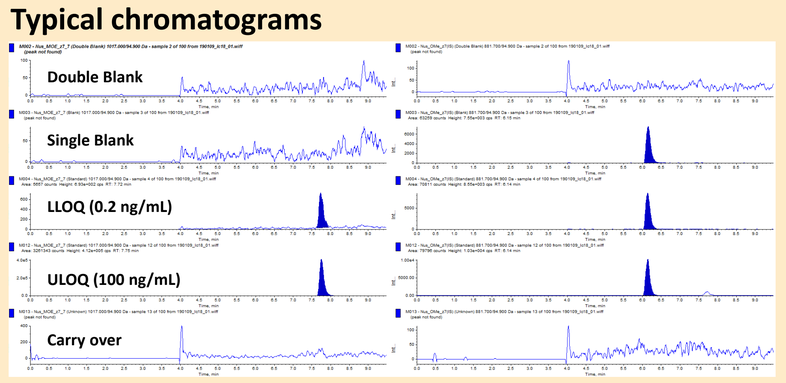
Result

Validation Study

Result:

Nusinersen in rat plasma and tissues was analyzed with the same analytical conditions of LC-MS/MS. Good linearity, precision and accuracy were observed over the concentration range of 0.2 to 100 ng/mL in plasma and 5 to 2500 ng/g in tissues. Nusinersen in plasma and tissues (data not shown) was stable under various conditions. S/N ratio of 0.2 ng/mL peak was enough for LLOQ and carry over peak was not observed.

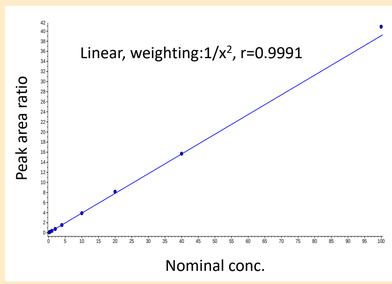
Typical chromatograms



Plasma

Linearity

Nominal conc. (ng/mL)	Back calculated conc. (ng/mL)	Accuracy (%)
Double blank	-	-
Single blank	-	-
0.2	0.198	99.0
0.4	0.418	104.5
1	0.954	95.4
2	1.90	95.0
4	3.91	97.8
10	9.92	99.2
20	20.8	104.0
40	40.1	100.3
100	105	105.0



Within-run

Nominal conc. (ng/mL)	Determined conc. (ng/mL)	Mean (ng/mL)	Standard deviation (ng/mL)	Accuracy (%)	Relative standard deviation (%)
LLOQ 0.2	0.199 0.172 0.188	0.186	0.014	93.0	7.5
QC-middle 4	3.94 3.90 3.77	3.87	0.09	96.8	2.3
ULOQ 100	98.6 99.5 97.9	98.7	0.8	98.7	0.8

Stability in plasma

Nominal conc. (ng/mL)	Storage condition	Determined conc. (ng/mL)	Mean (ng/mL)	Accuracy (%)	Residual ratio (%)
QC-low 0.6	Initial	0.572 0.618 0.589	0.593	98.8	-
	Room temp. [21 hours]	0.488 0.579 0.558	0.542	90.3	91.4
	4°C [21 hours]	0.532 0.543 0.563	0.546	91.0	92.1
	-80°C [27 days]	0.662 0.623 0.614	0.633	105.5	106.7
	Freeze and thaw [5 times]	0.549 0.576 0.528	0.551	91.8	92.9
	Processed sample 4°C [111 hours]	0.575 0.563 0.580	0.573	95.5	96.6

Liver

Linearity

Nominal conc. (ng/g)	Back calculated conc. (ng/g)	Accuracy (%)
Double blank	-	-
Single blank	-	-
5	5.11	102.2
10	9.87	98.7
25	23.6	94.4
50	48.0	96.0
100	97.9	97.9
250	248	99.2
500	504	100.8
1000	1050	105.0
2500	2650	106.0

Within-run

Nominal conc. (ng/g)	Mean (ng/g)	Accuracy (%)	Relative standard deviation (%)
LLOQ 5	4.70	94.0	11.3
QC-middle 100	96.9	96.9	2.8
ULOQ 2500	2460	98.4	2.8

Kidney

Linearity

Nominal conc. (ng/g)	Back calculated conc. (ng/g)	Accuracy (%)
Double blank	-	-
Single blank	-	-
5	4.64	92.8
10	10.1	101.0
25	26.4	105.6
50	47.1	94.2
100	106	106.0
250	248	99.2
500	487	97.4
1000	999	99.9
2500	2460	98.4

Within-run

Nominal conc. (ng/g)	Mean (ng/g)	Accuracy (%)	Relative standard deviation (%)
LLOQ 5	5.84	116.8	14.0
QC-middle 100	98.6	98.6	1.5
ULOQ 2500	2340	93.6	3.8

In Vivo Study | Administration: Crl:CD(SD), intravenous, 1 mg/kg, single dosing

Quantitative Analysis of Nusinersen

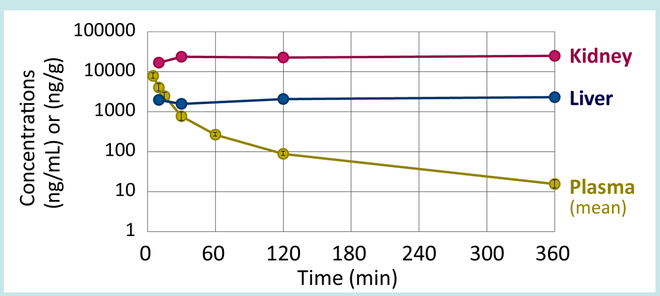
Result:

Concentrations of Nusinersen in plasma tend to decrease until 6 hours after dosing, whereas those in liver and kidney did not decrease or rather increased after dosing.

Time (min) after administration	5	10	15	30	60	120	360
Mean of plasma conc. (SD) (ng/mL)	7800 (1250)	4020 (830)	2450 (470)	768 (182)	265 (28)	87.8 (10.3)	15.5 (3.6)
Liver conc. (ng/g)		1960		1570		2070	2310
Distribution in liver (%)		9.9		7.1		9.5	8.8
Kidney conc. (ng/g)		16800		23600		22600	24900
Distribution in kidney (%)		14.0		19.0		20.9	20.7

Plasma: n=3, Liver: n=1, Kidney: n=1

Distribution (%) = Conc. (ng/g) × tissue weight (g) / dose (mg) × 100



Metabolite Identification

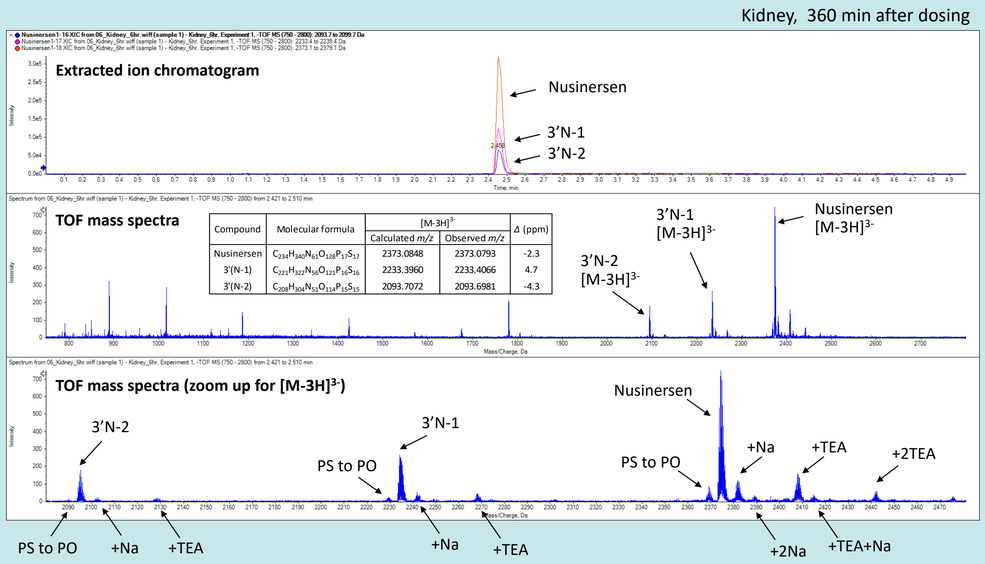
Equipment and Software:

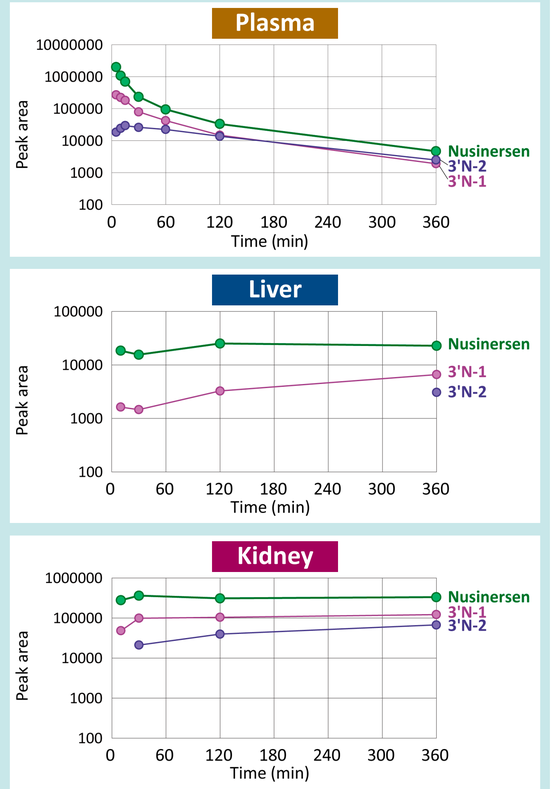
Triple TOF®5600, Analyst®TF (ver. 1.6), MetabolitePilot™ (ver. 2.0.4), PeakView™ (ver. 2.2), and MultiQuant™ (ver. 2.1.1) (SCIEX)

Result:

Two chain-shortened forms, 3'N-1 and 3'N-2, were identified as Nusinersen metabolites. The peak area ratios (Metabolites / Nusinersen, $[M-3H]^{3-}$) in plasma, liver and kidney tend to increase until 6 hours after dosing.

Kidney, 360 min after dosing





Conclusion

- **We have developed a LC-MS/MS method for analysis of Nusinersen in plasma, liver and kidney.**
- **Our results suggested that Nusinersen and its metabolites distribute in liver and kidney as commonly described for oligonucleotides.**