



# Quantitation of TL0901 Oligonucleotide in Human Plasma Using LC/MS/MS

## AUTHORS

**Laixin Wang, Weiwei Yuan, Yue Zhao, Jian Chen, Gregory vonArx, Min Meng and Patrick Bennett**  
Tandem Labs, Salt Lake City, UT

## INTRODUCTION

Antisense oligonucleotide compounds, including siRNAs and aptamers, are becoming more and more important as therapeutic agents. As a fairly new and large class of therapeutics, there is still no well established bioanalytical method for studying PK, PK/PD correlations. Early studies mainly used radiotracer, anion-exchange chromatography (AX-HPLC) or hybridization-based ELISA. These methods, however, are not specific [1]. Recently, we have reported validated UPLC-PDA methods for analyzing oligonucleotides in biological matrix [2,3]. Although these assays are very rugged for GLP analysis, the LLOQ is only approximately 1.00 µg/mL. Here we are presenting a sensitive and high throughput LC/MS/MS method to quantify TL0901 in human plasma (K<sub>2</sub>EDTA). TL0901 is a randomly picked 18-mer phosphorothiate oligonucleotide (sequence is 5'-ACTGTACGATTTCGACCTA) by Tandem Labs just for analytical method evaluation purpose.

## METHODOLOGY

### SAMPLE PREPARATION:

1. Thaw the samples in a wet ice-bath.
2. Aliquot 200 µL of samples into the corresponding labeled 2.0 mL Eppendorf centrifuge tubes.
3. Add 50.0 µL ice-cold working internal standard solution [10.0 µg/mL of TL090 (n-6) in water].
4. Add 200 µL of pH=8.0 extraction buffer to all samples.
5. Add 200 µL of the phenol:chloroform:isoamyl alcohol (25:24:1 v/v/v) solution to all samples.
6. Vortex-mix the samples thoroughly.
7. Centrifuge the samples in the microcentrifuge at 13,000 rpm for 15 minutes.
8. Transfer the clear supernatant to corresponding wells of 96-well plate and dried down.
9. Reconstitute the samples with 200 µL mobile phase A.



## METHODOLOGY *continued*

### CHROMATOGRAPHIC CONDITIONS:

Column: Phenomenex C18, 2x50 mm

Mobile Phase: A: HFIP and TEA buffered water  
 B: HFIP and TEA buffered MeOH  
 C: 90:10 MeCN:water (backflush at 0.600 mL/min)  
 Gradient with column backflush  
 (back flush column time: 2.0' to 3.0')

Time	0.01'	2.0'	2.5'	3.5'	5.5'
B%	15	45	45	15	end

Injection volume: 5– 20µL

Column temperature: 55°C

Flow rate: 0.300 mL/min

AS Temperature: RT

Needle wash 1: 0.1%TEA and 0.01% EDTA in 50:50 DMF:water

Needle wash 2: Mobile phase A

### MASS SPECTROMETER CONDITIONS

Instrument: API5000

Ionization Mode: Turbo ionspray, Negative ion mode

Source Temperature: 500°C

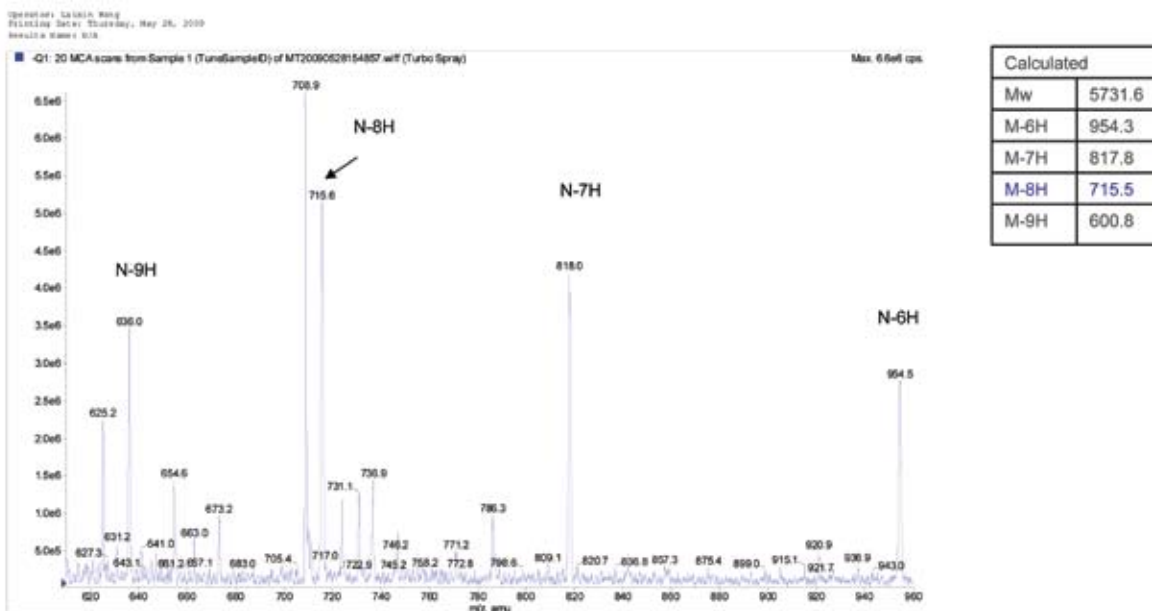
SRM Transitions:

Analyte	Internal Standard	Transitions (±0.5 amu)	Typical Retention Times (min.)
TL0901		715.4 →319.0	1.5
	TL0901(n-6)	631.7 →319.0	1.3

## RESULTS AND DISCUSSION

- Δ The molecular weight of TL0901 is 5731.6. A typical multiple charged Q1 scan was obtained under optimized negative ESI conditions (Figure 1).
- Δ The full length TL0901 can be completely separated chromatographically from its n-6 or shorter metabolites, but not the n-4 or longer metabolites under selected LC conditions (Figure 3). Fortunately, the analyte and each metabolite have multiple MRM transitions to choose to avoid interfering each other (Figure 1, 2 and 4).
- Δ The addition of EDTA to samples can improve the sample stability.

FIGURE 1. Typical Q1 Scan Mass Spectrum of TL0901



RESULTS AND DISCUSSION *continued*

FIGURE 2. Typical Product Ion Scan Spectrum of TL0901

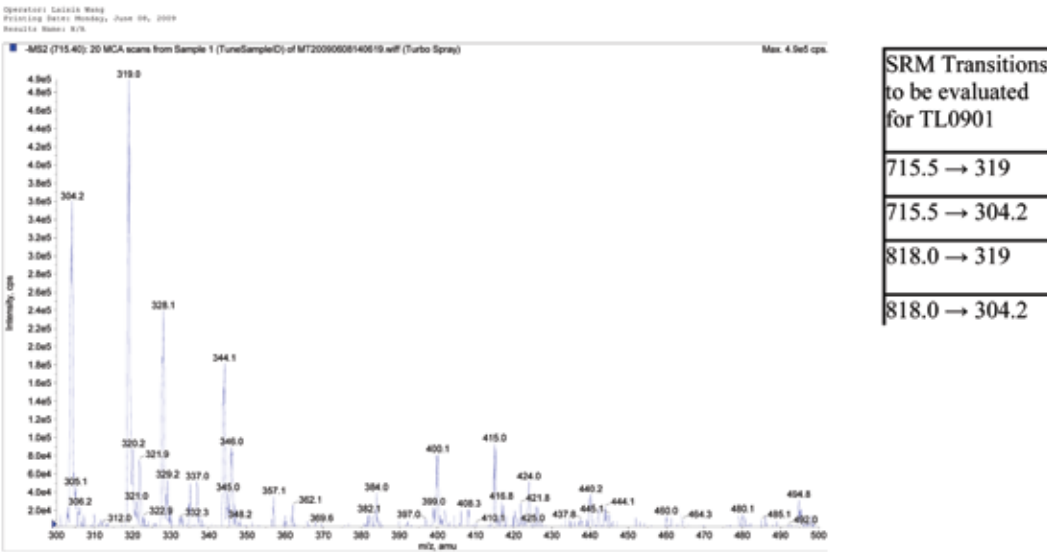
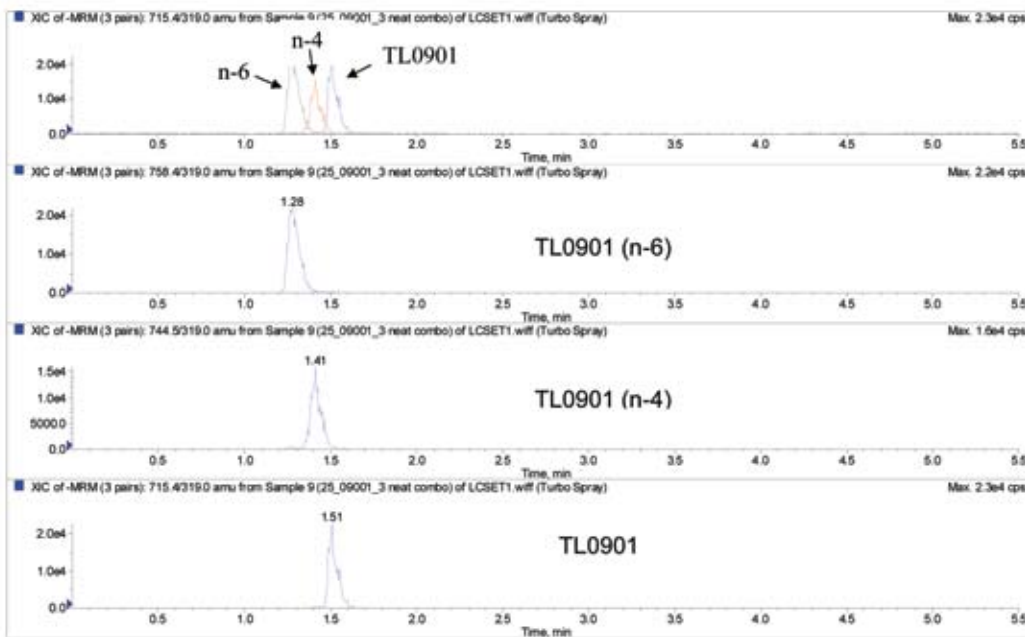


FIGURE 3. Liquid Chromatographic Separation of TL0901 and Its Metabolites





RESULTS AND DISCUSSION *continued*

FIGURE 4. MS/MS Selectivity and LC Separation of TL0901 and Its n-1 and n-2 Metabolites

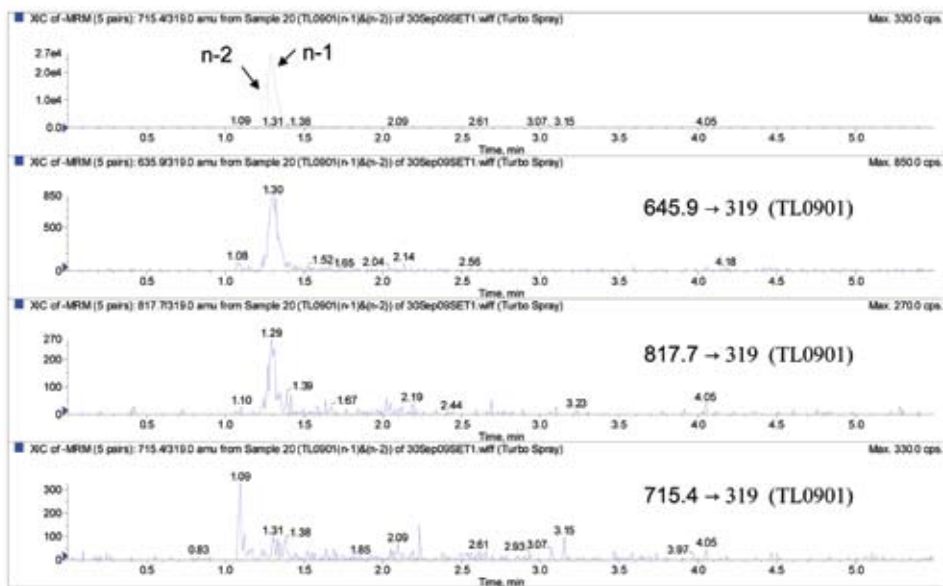
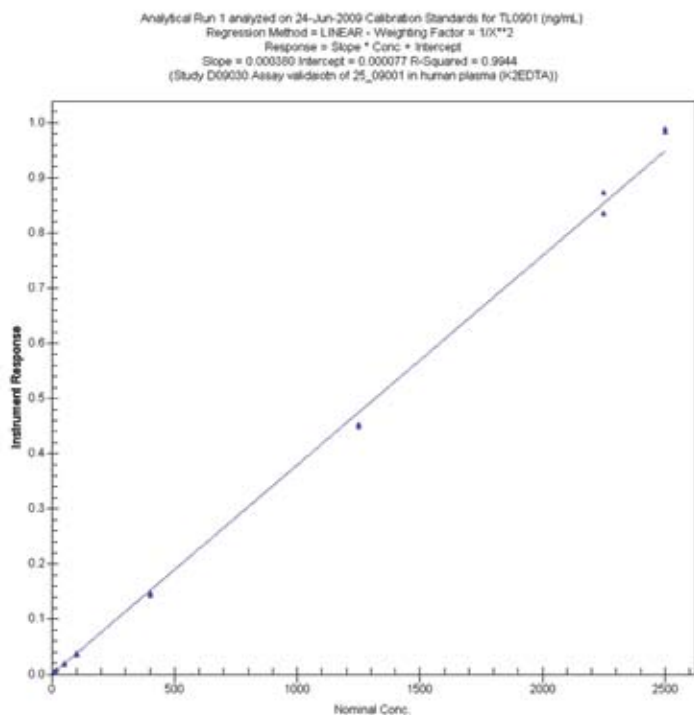


FIGURE 4. Representative Calibration Curve (10.0-2,500 ng/mL)



RESULTS AND DISCUSSION continued

FIGURE 5. Representative Chromatogram of a Plasma Blank

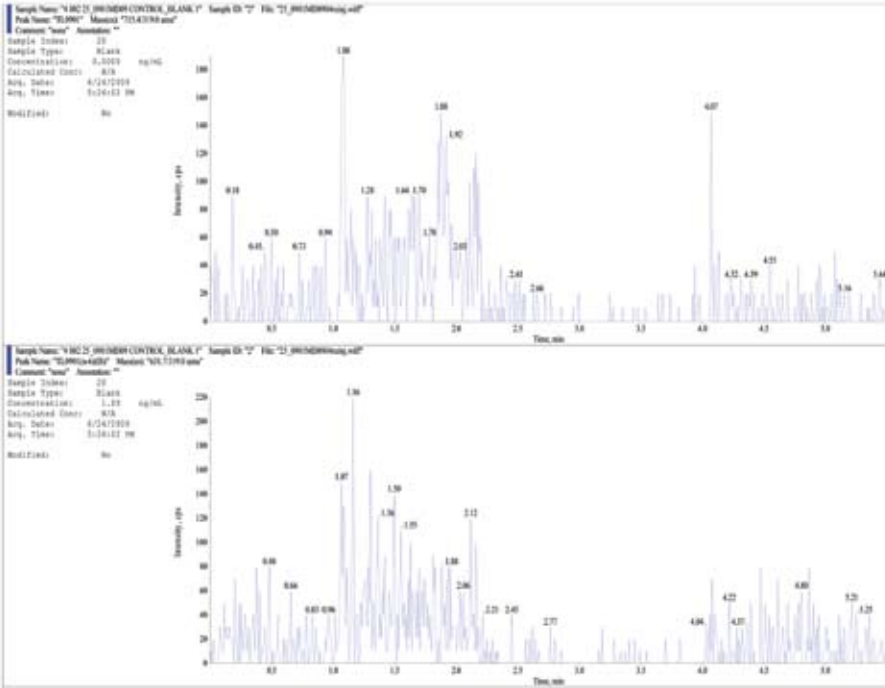
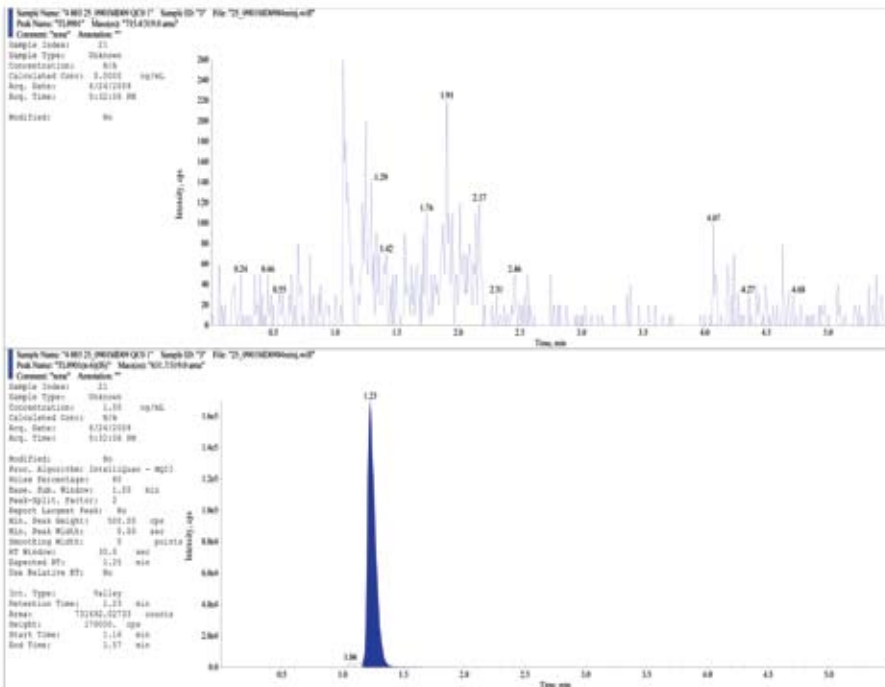


FIGURE 6. Representative Blank with Internal Standard (0-ng/mL QC)







## RESULTS AND DISCUSSION *continued*

### ASSAY VALIDATION

#### ACCURACY/PRECISION:

Demonstrated at n=6 at LLOQ, Low, Medium, High concentrations over 3 validation runs. (Tables 2)

#### SELECTIVITY:

Demonstrated with blank and LLOQ concentrations in ten sources of human plasma. (Tables 3)

#### ABILITY TO DILUTE:

Demonstrated above ULOQ at DF=10. (Table 4)

#### EXTRACTION RECOVERY:

Evaluated for analyte at Low, Medium, High concentrations (n=6). (Table 5)

#### CARRYOVER:

Evaluated in each run. No carryover present.

### STABILITY

#### STOCK SOLUTION:

Room temperature for analytes and the I.S., 6 hrs

96 days at 1-8°C in water

#### STABILITY IN MATRIX:

Freezing/thawing, 4 cycles

Ice-water bath, 6hrs

90 days at -70°C

#### EXTRACT:

Batch reinjection stability, 101 hours at RT

**TABLE 1. Back-Calculated Concentrations of Calibration Standards for TL0901**

All concentrations are expressed as ng/mL.

	<b>10.0</b>	<b>20.0</b>	<b>50.0</b>	<b>100</b>	<b>400</b>	<b>1250</b>	<b>2250</b>	<b>2500</b>
Mean	10.0	20.0	50.9	97.4	401	1240	2240	2550
S.D.	1.05	0.995	1.92	3.91	23.1	43.7	105	87.6
%CV	10.5	5.0	3.8	4.0	5.8	3.5	4.7	3.4
%Bias	0.0	0.0	1.8	-2.6	0.3	-0.8	-0.4	2.0
n	6	6	6	6	6	6	6	6

## RESULTS AND DISCUSSION *continued*

**TABLE 2. Intra- and Inter-Assay Accuracy and Precision for TL0901**  
 Accuracy and Precision Quality Control Samples from ANOVA

<b>Nominal Conc.</b>	<b>LLOQ QC 10.0 ng/mL</b>	<b>Low QC 30.0 ng/mL</b>	<b>Medium QC 250 ng/mL</b>	<b>High QC 2000 ng/mL</b>
Mean Observed Conc.	10.2	29.0	235	1950
%Bias	2.0	-3.3	-6.0	-2.5
Between Run Precision (%CV)	0.0	0.0	0.7	0.0
Within Run Precision (%CV)	7.6	6.0	2.7	3.4
Total Variation (%CV)	7.4	5.5	2.7	3.2
n	18	18	18	18
Number of Runs	3	3	3	3

**TABLE 3. Selectivity in 10 Lots of Human Plasma at the Lower Limit of Quantitation**  
 (10.0 ng/mL) for TL0901

	<b>LLOQ 10.0 ng/mL</b>
Mean	10.7
S.D.	0.651
%CV	6.1
%Theoretical	107.0
%Bias	7.0
n	10

**TABLE 4. Dilution Quality Control Samples (6,000 ng/mL at DF=10) for TL0901**

	<b>Dilution QC 6000 ng/mL DF=10</b>
Mean	6520
S.D.	296
%CV	4.5
%Theoretical	108.7
%Bias	8.7
n	6



## RESULTS AND DISCUSSION *continued*

TABLE 5. Relative Extraction Recovery for TL0901 at Low, Medium and High QC Concentration Levels

	<b>Low (30.0 ng/mL)</b>	<b>Medium (250 ng/mL)</b>	<b>High (380 ng/mL)</b>
Mean Recovery	87.2%	80.3%	83.0%
CV%	12.7	3.87	4.10
n	6	6	6

## CONCLUSION

A robust, sensitive and specific method was developed and validated to quantitatively determine oligonucleotide in human plasma. Similar assays have been successfully used to analyze pre-clinical and clinical samples. All ISR pass with great repeatability.

## REFERENCES

1. Zhongping John Lin, Wenkui Li and Guowei Dai, "Application of LC-MS for quantitative analysis and metabolite identification of therapeutic oligonucleotides", *Journal of Pharmaceutical and Biomedical Analysis*, 44: 330-341, 2007.
2. Yanhui Zhang, Laixin Wang, Jian Chen, Yue Zhao, Min Meng and Patrick Bennett (Tandem Labs) as well as Donna Dobinson and Colin Green (Antisoma Research Limited, Welwyn Garden City, UK, "Quantitative determination of AS1411 oligonucleotide in human urine using UPLC-PDA," the 11th Annual Symposium on Chemical & Pharmaceutical Structure Analysis, October 2008, Langhorne, PA.
3. Jian Chen, Laixin Wang, Yanhui Zhang, Ming Meng, Juan Wang and Patrick Bennett (Tandem Labs) as well as Donna Dobinson and Colin Green (Antisoma Research Limited, Welwyn Garden City, UK), "Quantitative determination of AS1411 oligonucleotide in monkey plasma using UPLC-PDA", the ISSX 15th Annual Meeting, October 2008, San Diego, CA.