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Journal of Chromatography B, 856 (2007) 198–204
JOURNAL OF CHROMATOGRAPHY B
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Validation and implementation of a liquid chromatography/tandem mass spectrometry assay to quantitate ON 01910.Na, a mitotic progression modulator, in human plasma

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Received 27 February 2007; accepted 27 May 2007
Available online 7 June 2007

Abstract

A reverse-phase high performance liquid chromatographic method with tandem mass spectrometry (LC–MS/MS) was developed and validated for the quantitation of ON 01910.Na, a novel synthetic benzyl styryl sulfone, in human plasma. The assay involved a simple sample preparation with acetonitrile protein precipitation. ON 01910.Na and the internal standard temazepam were separated on a Waters X-Terra™ MS C_{18} column with mobile phase of acetonitrile containing 0.1% formic acid /10 mM ammonium acetate (55:45, v/v) using isocratic flow at 0.2 mL/min for 5 min. The analytes were monitored by tandem-mass spectrometry with electrospray positive ionization. Two calibration curves were generated over the range of 10–2000 ng/mL and 100–20000 ng/mL. The lower limit of quantitation (LLOQ) was 10 ng/mL for ON 01910.Na in human plasma. The accuracy and within- and between-day precisions were within the acceptance criteria for bioanalytical assays. ON 01910.Na was found stable in plasma at −70 °C for at least 1 year. The method was successfully applied to characterize the plasma concentration–time profiles of ON 01910.Na in the cancer patients in the Phase I study.
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Keywords: ON 01910.Na; High performance liquid chromatography (HPLC); Mass spectrometry (MS); LC–MS/MS; Pharmacokinetics

1. Introduction

ON 01910.Na is a novel synthetic benzyl styryl sulfone (Fig. 1), and currently under development as a potential anticancer agent. It is a potent anti-mitotic agent that induces selective G2/M arrest followed by apoptosis in a broad spectrum of cancer cells. [1]. ON 01910.Na has differential effects on cell cycle progression in tumor cells and normal cells. It induces reversible cell arrest at the G1 and G2 stage without apoptosis in normal cells [1]. ON 01910.Na has demonstrated significant antitumor activities in multiple xenograft animal models, and also showed synergistic activity when combined with any one of several other chemotherapeutic agents (e.g., paclitaxel, vincristine and irinotecan). ON 01910.Na is currently being evaluated in a Phase I clinical trial in patients with refractory solid tumors at several institutions including the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins [2].

To assess the pharmacokinetic behavior of ON 01910.Na in patients, a sensitive and reliable assay method for the quantitation of ON 01910.Na was necessary. Here, we developed and validated a high-performance liquid chromatography with tandem mass spectrometry (LC–MS/MS) method for the determination of ON 01910.Na in human plasma.

2. Experimental

2.1. Chemicals and reagents

ON 01910.Na (HPLC purity, 98.6%) (Lot number CHP030104ON19R) was provided by ChemPacific Co. (Bal-

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doi:10.1016/j.jchromb.2007.05.047

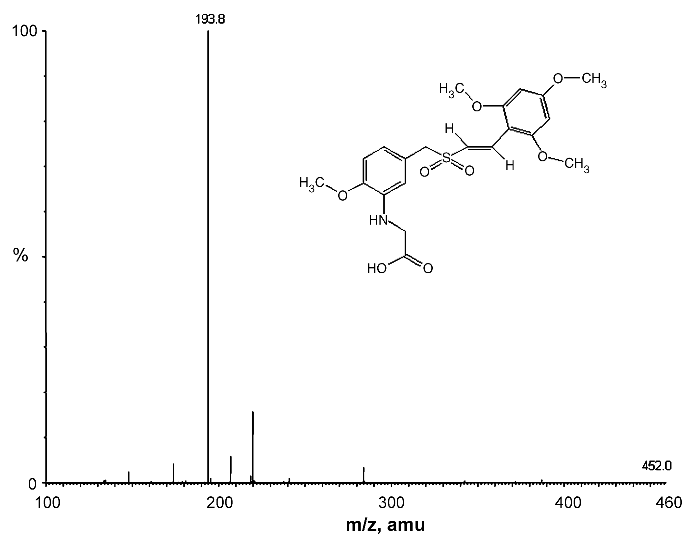

Fig. 1. Daughter mass spectrum and structure of ON 01910.Na (free acid) with monitoring at *m/z* 452.0 → 193.8.

timore, MD, USA). The internal standard, temazepam was purchased from Sigma (St. Louis, MO, USA). All other chemicals were HPLC grade and obtained from EM Science (Gibbstown, NJ, USA). Water was filtered and deionized with a Milli-Q-UF system (Millipore, Milford, MA, USA) and used throughout in all aqueous solutions. Drug-free (blank) human plasma from healthy donors was obtained from Pittsburgh Blood Plasma Inc. (Pittsburgh, PA, USA).

2.2. Stock solutions and standards

Stock solution of ON 01910.Na was prepared in methanol at a concentration of 1 mg/mL, and stored in glass vials at −20 °C. Working stock solutions were prepared fresh on each day of analysis as serial dilutions in acetonitrile/water (50:50, v/v). Two sets of the standards were prepared according to the following concentrations: 10, 20, 50, 100, 200, 500, 1000, and 2000 ng/mL for the low concentration calibration curve, and 100, 200, 500, 1000, 2000, 5000, 10000, 20000 ng/mL for the high concentration calibration curve. Quality control (QC) samples were prepared in blank plasma at ON 01910.Na concentrations of 10 (lower limit of quantitation, LLOQ), 30, 800, and 1600 ng/mL, or of 100, 300, 3000, and 16000 ng/mL for the low and high concentration curve, respectively. Prior to the high concentration curve being constructed, an additional dilutional QC was prepared at 80,000 ng/mL and diluted 1:100 (v/v) in pooled human plasma for quantitation and analyzed on the low calibration curve. Since dose escalation was still occurring, the high calibration curve may not encompass the range intended. Therefore, an additional dilutional QC was prepared at 200,000 ng/mL and diluted 1:100 (v/v) in pooled human plasma for quantitation and analyzed on the high curve. For cross-validation of the low and high curves, QC samples were prepared in blank plasma at ON 011910.Na concentrations of 150, 1500, and 16000 ng/mL. All standards and QC samples were prepared fresh daily. For long-term and freeze–thaw stability, QC samples were prepared as a batch and stored at −70 °C.

2.3. Sample preparation

Prior to extraction, frozen samples were thawed in a water bath at ambient temperature. A 100 μL aliquot of plasma was added to a borosilicate glass tube (13 mm × 100 mm) followed by 300 μL of acetonitrile containing internal standard temazepam (50 ng/mL). The mixture was vortex-mixed for 30 s, and centrifuged at 2000 rpm for 5 min at ambient temperature. An aliquot of 100 μL of the supernatant was collected and transferred into a borosilicate glass test tube (13 mm × 100 mm) containing 100 μL of Milli-Q water for the low curve and 500 μL of acetonitrile/water (50:50, v/v) for the high curve. The mixture was vortex-mixed for 30 s and then transferred into a 250-μL polypropylene autosampler vial, sealed with a Teflon crimp cap. For the low calibration curve, a volume of 20 μL was injected into the HPLC instrument using a temperature-controlled autosampling device (set at 10 °C). For the high calibration curve, a volume of 10 μL was injected into the HPLC instrument.

2.4. Chromatographic and mass-spectrometric conditions

Chromatographic analysis was performed using a Waters model 2690 separations system (Milford, MA, USA). Separation of the analytes from potentially interfering material was achieved at 40 °C using Waters X-Terra™ MS column (150 mm × 2.1 mm i.d.) packed with a 3.5 μm C_{18} stationary phase, protected by a guard column packed with 3 μm RP18 material (Milford, MA, USA). The mobile phase used for the chromatographic separation was composed of acetonitrile/10 mM ammonium acetate (pH 3.5) (55:45, v/v) containing 0.1% formic acid, and was delivered isocratically at a flow rate of 0.2 mL/min. The column effluent was monitored using a Micromass Quattro LC triple-quadrupole mass-spectrometric detector (Beverly, MA, USA). The instrument was equipped with an electrospray ionization source, and controlled by the Masslynx version 3.4 software (Micromass), running under Microsoft Windows NT on a Compaq AP200 Pentium III computer. The samples were analyzed using an electrospray probe in the positive ionization mode operating at a cone voltage of 15 V for ON 01910.Na and 25 V for temazepam. Samples were introduced into the ionization source through a heated nebulized probe (350 °C). The spectrometer was programmed to allow the $[MH]^+$ ion of ON 01910.Na at *m/z* 452.0 and temazepam at *m/z* 301.2 to pass through the first quadrupole (Q1) and into the collision cell (Q2). The collision energy was set at 20 eV for ON 01910.Na and temazepam. The product ions for ON 01910.Na (*m/z* 193.8) and temazepam (*m/z* 255.0) were monitored through the third quadrupole (Q3). Argon was used as collision gas at a pressure of 0.0027 mBar, and the dwell time per channel was 0.3 sec for data collection.

2.5. Method validation

2.5.1. Specificity

The specificity of the method was tested by visual inspection of chromatograms of extracted human plasma samples from six

different donors for the presence of endogenous or exogenous interfering peaks. The interfering peak area needed to be less than 20% than the peak area for ON 01910.Na at the lower limit of quantitation in an aqueous solution.

2.5.2. Calibration curve

Linearity was assessed at ON 01910.Na concentration ranging from 10 to 2000 ng/mL for the low calibration curve and 100 to 20000 ng/mL for the high calibration curve. Calibration curves were built by fitting ON 01910.Na concentrations of the calibrators versus peak area ratios of the analyte to internal standard using least-squares non-linear regression analysis with different weighting scheme (i.e., 1, $1/x$, and $1/x^2$). The selection of weighting scheme was guided by evaluation of goodness-of-fit criteria including correlation coefficient (R^2), % recovery of back-calculated calibrators and QCs, and residual plots.

2.5.3. Accuracy and precision

Validation runs for the calibrator standards (in duplicate) and QCs including LLOQ, low, medium, high, and dilutional QC (in quintuplicate) were performed on 4 and 3 consecutive days for the low and high calibration curves, respectively. In addition, a cross-validation for the low and high curves was performed on three consecutive days. The accuracy was assessed as the relative percentage of the back-calculated to nominal concentration, which was equal to determined concentration/nominal concentration × 100%. The within- and between-day precisions were estimated by one-way analysis of variance (ANOVA) using the JMP™ statistical discovery software version 4 (SAS Institute, Cary, NC, USA). The between-day variance (VAR_{bet}), the within-day variance (VAR_{wit}), and the grand mean (GM) of the observed concentrations across runs were calculated from ANOVA analysis. The within-day precision (WDP) was calculated as:

$$\mathrm{WDP} = 100 \times \left(\frac{\sqrt{(\mathrm{VARwit})}}{\mathrm{GM}}\right)$$

The between-day precision (BDP) was defined as:

$$\mathrm{BDP} = 100 \times \left(\frac{\sqrt{((\mathrm{VARbet} - \mathrm{VARwit})/n)}}{\mathrm{GM}}\right)$$

where n represents the number of replicate observations within each day.

2.5.4. Recovery

Extraction recovery was assessed by comparing peak area ratios of the ON 01910.Na to internal standard obtained from the extracted QC samples versus unextracted solvent standards at the concentrations of 30, 800, and 1600 ng/mL.

2.5.5. Stability

The short-term stability of ON 01910.Na working solution in 50% acetonitrile (at the concentration of 10 μg/mL) and in plasma (at the concentration of 30 and 1600 ng/mL) was tested at ambient temperature (25 °C) for 4 and 6 h, respectively. The autosampler stability was examined at ON 01910.Na concentration of 30 and 1600 ng/mL at 10 °C for 12 h. The freeze–thaw stability of ON 01910.Na in plasma was assessed at concentrations of 30 and 1600 ng/mL through three freeze–thawing cycles. The long-term stability of ON 01910.Na in stock solution (1 mg/mL) at −20 °C and in plasma (30 and 1600 ng/mL) at −70 °C or below was investigated at 3-month intervals. All QCs were run in triplicate.

2.6. Pharmacokinetic analysis

ON 01910.Na is currently being evaluated in a Phase I clinical trial in patients with advanced cancers. ON 01910.Na was administered as a 2-h infusion on days 1, 4, 8, 11, 15, 18 followed by a 10-day observation period for a total of 28 days per cycle. The dose levels evaluated ranged from 80 to 4370 mg. The protocol was approved by the Institutional Review Board of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (Baltimore, MD, USA), and the patients provided written informed consent.

Blood samples were collected in heparinized tubes at the following time points on day 1 and 15 during the first cycle: pretreatment (within 15 min prior to infusion), during infusion at 15, 30, 60 min, and 1 h and 59 min (immediately before the end of infusion), and after the infusion at 10, 20, 30 min, 1, 2, 4, 8, 24, and 48 h; additionally, a pretreatment trough level was obtained on days 4, 8, 11, 18, and 28. The blood samples were immediately placed in an ice bath and then centrifuged at 1000 × *g* at 4 °C for 10 min. Plasma was separated and split into two aliquots, and store at −70 °C or below until analysis.

The low calibration curve at ON 01910.Na concentrations of 10–2000 ng/mL was used for the patients receiving ON 01910.Na dose of 80–2080 mg, while the high curve over the concentration range of 100–20000 ng/mL was used for those receiving ON 01910 dose of 3120 and 4370 mg.

ON 01910.Na pharmacokinetic parameters were estimated using noncompartmental analysis with the computer software program WinNonlin version 5.0 (Pharsight Corporation, Mountain View, California). The maximum plasma concentration (C_{max}) and the time (s) of occurrence for maximum concentration (T_{max}) were obtained by visual inspection of the plasma concentration–time curves after the infusion. The total area under the plasma concentration–time curve from time zero to the last measurable time point (AUC_{0-t}) was calculated using the linear and logarithmic trapezoidal method for ascending and descending plasma concentrations, respectively. The total area under the plasma concentration–time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated as the sum of AUC_{0-t} and the extrapolated area, which was calculated by the last observed plasma concentration divided by the terminal rate constant (λ_z), where λ_z was estimated by terminal log-linear phase of the plasma concentration-time curve. Systemic clearance (Cl) on day 1 was calculated as Dose/$AUC_{0-\infty}$.

3. Results and discussion

3.1. Detection and chromatography

A LC/MS/MS method to quantitatively determine ON 01910.Na concentrations in human plasma was developed,

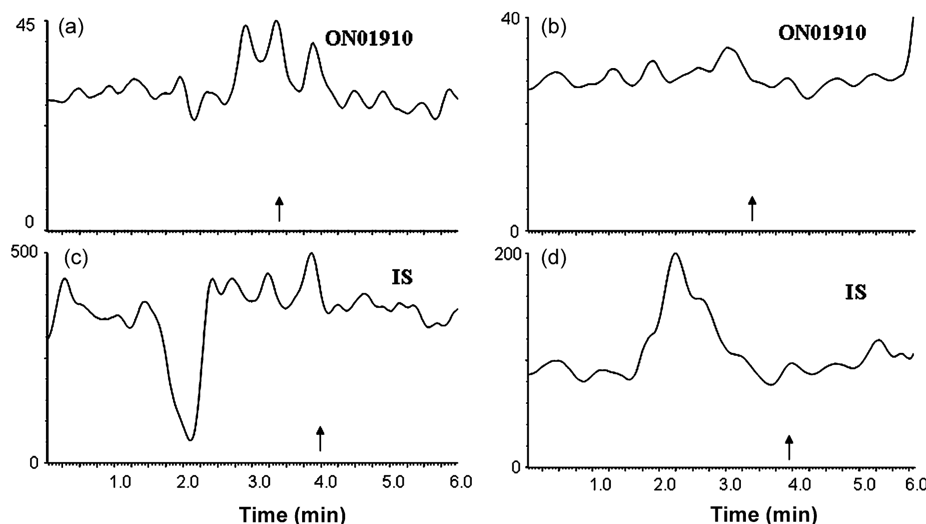

Fig. 2. Chromatograms of blank plasma that was monitored at m/z 452.0 → 193.8 for ON 01910.Na (a and b) and at m/z 301.2 → 255.1 for temazepam (c and d). The chromatograms of the blanks using 20 μL injection for the low calibration curve are (a) and (c); while the 10 μL injection for the high curve are (b) and (d).

validated, and implemented to quantitate drug in plasma from patients receiving treatment. The mass spectrum of ON 01910.Na showed protonated molecules ([MH$^+$]) at m/z 452.0. The collision energy fragmented ON 01910.Na into several fragments. The major fragments observed were at m/z 193.8 and were selected for subsequent monitoring in the third quadrupole (Fig. 1). The internal standard, temzepam, had protonated molecules ([MH$^+$]) at m/z 301.2 and produced a major fragment at m/z 255.0 (data not shown).

Representative chromatograms of blank and spiked human plasma samples that were monitored at m/z 452.0 → 193.8 (for ON 01910.Na) and m/z 301.2 → 255.1 (for temazepam) are shown in Figs. 2 and 3. The mean (± standard deviation) retention times for ON 01910.Na and temazepam under the optimal conditions were at 3.36 ± 0.06 and 3.91 ± 0.07 min, respectively, with an overall chromatographic run time of 5 min (Fig. 3). The selectivity for the analysis was shown by symmetrical resolution of the peaks, with no significant chromatographic interference around the retention times of ON 01910.Na and temazepam in human plasma (Fig. 2) from six different donors. During implementation of this assay, pre-treatment plasma samples from 16 cancer patients were analyzed with this assay with no interferences noted.

3.2. Calibration curves

A low and high concentration calibration curve was established over ON 01910.Na concentration range of 10–2000 ng/mL and 100–20000 ng/mL, respectively. The relationship between peak area ratios of analyte to internal standard versus ON 01910.Na concentrations was best fitted by a quadratic equation, expressed as $y = a \times x^2 + b \times x + c$, where y is peak area ratio, x is ON 01910.Na concentration, a, b, and c are fitted parameters. A weighting function of $1/x$ and $1/x^2$ was utilized for the low and high curve, respectively. For both low and high curves, a correlation coefficient (R^2) of >0.99 was

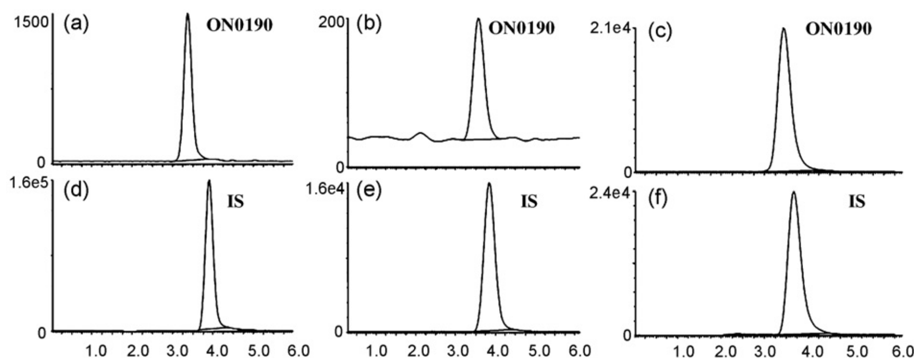

Fig. 3. Chromatograms of plasma spiked with (a) ON 01910.Na (10 ng/mL, 20 μL injection for the low calibration curve), (b) ON 01910.Na (100 ng/mL, 10 μL injection for the high calibration curve), (c) ON 01910.Na chromatogram from a select cancer patients receiving a 3120 mg dose administered as a 2-h intravenous infusion of ON 01910.Na obtained 20 min post-infusion (1:25 dilution, 10 μL injection for the high calibration curve), (d) internal standard temazepam (50 ng/mL, 20 μL injection), (e) internal standard temazepam (50 ng/mL, 10 μL injection), and (f) internal standard temazepam chromatogram from a select cancer patients receiving a 3120 mg dose administered as a 2-h intravenous infusion of ON 01910.Na obtained 20 min post-infusion (1:25 dilution, 10 μL injection for the high calibration curve). The following m/z ratios were monitored 452.0 → 193.8 for ON 01910.Na (a, b, and c) and 301.2 → 255.1 for temazepam (d, e, and f). The retention times for ON 01910.Na and temazepam were 3.36 ± 0.06 and 3.91 ± 0.07 min, respectively.

Table 1
Accuracy, within- and between-day precisions of calibrator standards in the low and high concentration calibration curves

Nominal concentration (ng/mL)	Accuracy (%)	Concentration (ng/mL)	Within-day (%)	Between-day (%)
Low curve QCs[a]				
10 (LLOQ)	97.1	9.7 ± 0.7	7.1	–[b]
20	101.2	20.2 ± 0.9	3.4	3.4
50	97.3	48.6 ± 1.9	5.2	–[b]
100	104.9	104.9 ± 3.7	4.6	–[b]
200	102.9	205.9 ± 11.7	4.9	3.2
500	94.5	472.7 ± 22.3	4.8	–[b]
1000	102.4	1024.0 ± 21.9	1.8	1.2
2000	99.7	1994.1 ± 80.5	5.3	–[b]
High curve QCs[c]				
100 (LLOQ)	105.1	105.1 ± 6.3	7.3	–[b]
200	92.1	184.1 ± 6.9	1.3	4.0
500	97.5	487.3 ± 12.8	2.3	1.4
1000	95.9	959.5 ± 41.0	2.7	3.7
2000	91.8	1835.8 ± 64.6	3.1	1.9
5000	111.1	5552.6 ± 225.3	2.6	3.5
10000	104.6	10455.6 ± 308.7	3.1	–[b]
20000	97.5	19507.2 ± 265.5	1.6	–[b]

[a] Each calibrator was evaluated in duplicate on 4 consecutive days.
[b] No additional variation was observed as a result of performing assay in different days.
[c] Each calibrator was evaluated in duplicate on 3 consecutive days.

obtained in all analytical runs, and the distribution of residuals was random and centered on zero (data not shown). As shown in Table 1, the average accuracy in terms of percent recovery of the back-calculated relative to nominal concentration ranged from 94.5% to 104.9% ($n = 8$) and from 91.8% to 111.1% ($n = 6$) for the low and high curve, respectively; the within- and between-day precisions were all less than 7.3% for all calibrators.

3.3. Accuracy, precision, and recovery

The LLOQ for ON 01910.Na was established at 10 ng/mL, at which the mean signal-to-noise ratio was 129.9. For the low calibration curve QCs at ON 01910.Na concentrations of 10 (LLOQ), 30, 800, 1600, and 80000 ng/mL, the average accuracy in terms of percent recovery of the back-calculated relative to nominal concentration ranged from 90.9% to 99.1%, and within- and between-day precisions were all less than 5.3% (Table 2). Similarly, for the high calibration curve QCs at ON 01910.Na concentrations of 100, 300, 3000, 16000, and 200000 ng/mL, the average accuracy ranged from 95.0% to 103.7%, and within- and between-day precisions were all less than 10.9% (Table 2). The cross validation for the low and high calibration curves at ON 01910.Na concentrations of 150, 1500, and 16000 ng/mL demonstrated that within- and between-day precisions for all QCs within 6.7%, and accuracy of the determined concentrations from the low and high curve were all within 8.7% at the three QC concentrations (Table 3).

The mean relative extraction recovery of ON 01910.Na from human plasma was determined as 89.4%, 109.3%, and 95.1% for the triplicate QCs at the concentrations of 30, 800, and 1600 ng/mL, respectively.

Table 2
Accuracy, within- and between-day precision of the low and high calibration curve QC samples

Nominal concentration (ng/mL)	Accuracy (%)	Concentration (ng/mL)	Within-day (%)	Between-day (%)
Low curve QCs[a]				
10 (LLOQ)	90.9	9.1 ± 0.5	5.3	2.2
30	92.6	27.8 ± 1.2	3.3	3.3
800	97.5	780.0 ± 45.2	3.4	5.3
1600	99.1	1586.3 ± 79.0	4.9	0.9
80000	93.3	74604.0 ± 3440.4	3.0	3.9
High curve QCs[b]				
100 (LLOQ)	103.7	103.7 ± 10.3	3.6	10.9
300	95.0	285.1 ± 15.7	5.4	1.1
3000	96.8	2904.1 ± 135.5	2.5	4.7
16000	103.7	16584.5 ± 481.5	1.7	2.8
200000	93.3	186654.9 ± 19890.5	8.7	7.3

[a] Performed in quintuplicate on 4 consecutive days.
[b] Performed in quintuplicate on 3 consecutive day.

Table 3
Cross validation for the low and high concentration calibration curves

Nominal concentration (ng/mL)	Accuracy (%)	Concentration (ng/mL)	Within-day (%)	Between-day (%)
Low curve QCs[a]				
150	105.7	158.5 ± 9.6	2.3	6.6
1500	100.9	1513.4 ± 53.0	2.0	3.4
16000[b]	100.0	1599.8 ± 58.5	3.4	1.7
High curve QCs[a]				
150	97.4	146.1 ± 7.8	3.4	4.9
1500	91.3	1369.3 ± 99.5	4.5	6.7
16000	103.9	1662.6 ± 90.5	2.1	5.9

[a] Each QC was performed in quintuplicate on 3 days.
[b] The QC (16000 ng/mL) was diluted at 1:10 with blank plasma prior to extraction to fit the low curve.

3.4. Stability

The short- and long-term stability of ON 01910.Na was demonstrated in Table 4. At ambient temperature (∼25 °C), ON 01910.Na was stable for at least 4 h in 50% acetonitrile working solution and at least 6 h in plasma. Freeze–thaw stability, which was assessed at ON 01910.Na plasma concentrations of 30 and 1600 ng/mL, showed no significant (<6.1%) degradation of ON 01910.Na through three full cycles of freeze–thaws. In acetonitrile extraction supernatant, ON 01910.Na was stable in the autosampler (10 °C) for at least 11.5 h, allowing the assay to be performed continuously overnight for a large number of samples. The long-term stability tests suggested that ON 01910.Na was stable in methanol (stock solution, 1 mg/mL) at −20 °C for at least 3 months, and stable in plasma at −70 °C for at least 1 year, with degradation less than 15%.

Table 4
Assessment of stability of ON 01910.Na[a]

	ON 01910.Na concentrations[b]	
	30 ng/mL (QC)	1600 ng/mL (QC)
Short-term stability (in plasma) (25 °C)		
0.5 h	93.5	98.3
1.0 h	99.9	103.0
2.0 h	99.3	100.3
4.0 h	94.7	99.4
6.0 h	93.4	97.2
Freeze–thaw stability (in plasma) (−70 °C)		
Cycle 1	105.5	94.4
Cycle 2	106.1	94.9
Cycle 3	97.2	98.4
Autosampler stability (in 50% acetonitrile) (10 °C)		
4.1 h	108.5	109.0
8.0 h	111.2	99.5
11.5 h	104.5	93.6
Long-term stability (in plasma) (−70 °C)		
153 day	109.5	108.99
378 day	87.8	103.5

[a] Stability data were expressed as mean percentage of ON 01910.Na concentration determined at certain time point relative to that at time zero (%).
[b] Each concentrations were assessed in triplicate.

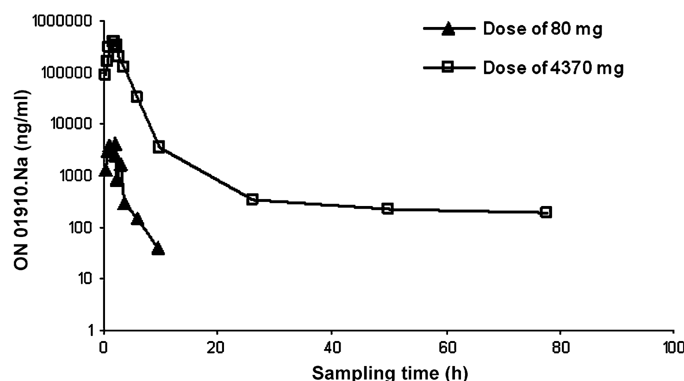

Fig. 4. Representative plasma concentration–time profiles of ON 01910.Na in two cancer patients receiving 2-h intravenous infusion of ON 01910.Na at the dose of 80 mg (▲) and 4370 mg (□).

3.5. Plasma concentration–time profile

The described LC–MS/MS method was successfully applied to study the pharmacokinetics of ON 01910.Na in the cancer patients enrolled in the dose-escalation Phase I trial. The patients received ON 01910.Na as 2-h intravenous infusion at doses ranging from 80 to 4370 mg. Fig. 4 shows the plasma concentration–time profiles of ON 01910.Na in two representative cancer patients receiving 2-h intravenous infusion at the dose of 80 and 4370 mg. ON 01910.Na plasma concentrations at 24 h and afterward (i.e., 48, 72, and 96 h) after the end of infusion at the dose of 80 mg were below the LLOQ (i.e., <10 ng/mL). The trough level at day 4 (i.e., 96 h) after ON 011910.Na infusion at the dose of 4370 was below the LLOQ (i.e., <10 ng/mL). In these two patients, following 2 h intravenous infusion of 80 and 4370 mg ON 01910.Na, the maximum plasma concentration achieved at the end of infusion were 4027.6 and 413554.0 ng/mL, respectively; the systemic clearance was estimated as 9.1 and 3.7 L/h, respectively.

4. Conclusion

In summary, a sensitive and reliable LC–MS/MS method was developed and validated for the determination of ON 01910.Na in human plasma. The LLOQ for ON 01910.Na was determined at 10 ng/mL in plasma, and the low and high calibration curves were established in the range of 10–2000 ng/mL and

100–20000 ng/mL, respectively. The low and high curves were cross-validated, and 1:100 dilution scheme was also validated. This method was easily applied for quantitation of ON 01910.Na in a large number of plasma samples and allowed characterization of pharmacokinetic profiles of ON 01910.Na over a wide dose range in the dose-escalation Phase I trial.

Acknowledgments

This work was supported by Onconova Therapeutics. We would like to thank Susan Davidson for quality assurance of the data contained in this manuscript and Sharyn Baker for her scientific input and discussions.

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