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Simultaneous determination of the lactone and carboxylate forms of irinotecan (CPT-11) and its active metabolite SN-38 by high-performance liquid chromatography: Application to plasma pharmacokinetic studies in the rat

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Abstract

Irinotecan (CPT-11) and its main metabolite SN-38 are potent anticancer derivatives of camptothecin (CPT), with active lactone and inactive carboxylate forms coexisting. A simple and sensitive HPLC method using the ion-pairing reagent tetrabutylammonium hydrogen sulfate (TBAHS) was developed to simultaneously determine all four analytes in rat plasma samples. Camptothecin (CPT) was used as internal standard. The mobile phase was 0.1 M potassium dihydrogen phosphate containing 0.01 M TBAHS (pH 6.4)–acetonitrile (75:25, v/v). Separation of the compounds was carried out on a Hypersil C_{18} column, monitored at 540 nm (excitation wavelength at 380 nm). All four compounds gave linear response as a function of concentration over 0.01–10 μM. The limit of quantitation in rat plasma was 0.01, 0.008, 0.005 and 0.005 μM for CPT-11 lactone, CPT-11 carboxylate, SN-38 lactone and SN-38 carboxylate, respectively. The method was successfully used in the study on the effect of coadministered thalidomide on the plasma pharmacokinetics of CPT-11 and SN-38 in rats. Coadministered thalidomide (100 mg/kg body weight by intraperitoneal injection) significantly increased the $AUC_{0–10h}$ values of CPT-11 lactone and CPT-11 carboxylate by 32.6% and 30.3 %, respectively, ($P < 0.01$), but decreased the values by 19.2% and 32.4% for SN-38 lactone and carboxylate, respectively, ($P < 0.05$). Accordingly, the value of total body clearance (CL) of CPT-11 lactone was significantly lower in combination group compared to the control (1.329 versus 1.837 L/h/kg, $P = 0.0002$). Plasma $t_{1/2\beta}$ values for SN-38 lactone and carboxylate were significantly ($P < 0.01$) smaller in rats with coadministered thalidomide, as compared to rats receiving CPT-11 alone. Further studies are needed to explore the underlying mechanisms for the observed kinetic interaction between CPT-11 and thalidomide.

Keywords: HPLC; Irinotecan; SN-38; Lactone; Carboxylate

1. Introduction

Irinotecan hydrochloride (CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin, see Fig. 1), a semisynthetic derivative of camptothecin (CPT) inhibiting DNA topoisomerase I [1,2], has been widely used for the treatment of colorectal cancer and many of other solid tumours [3–5]. CPT-11 is activated by carboxylesterases to the

Abbreviations: AUC, area under the concentration–time curve; CL, clearance; C_0, maximum plasma concentration; CPT, camptothecin; CV, coefficient of variation; DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; IS, internal standard; LOQ, limit of quantification; TBAHS, tetrabutylammonium hydrogen sulfate; $t_{1/2\beta}$, elimination half-life; V_d, volume of distribution

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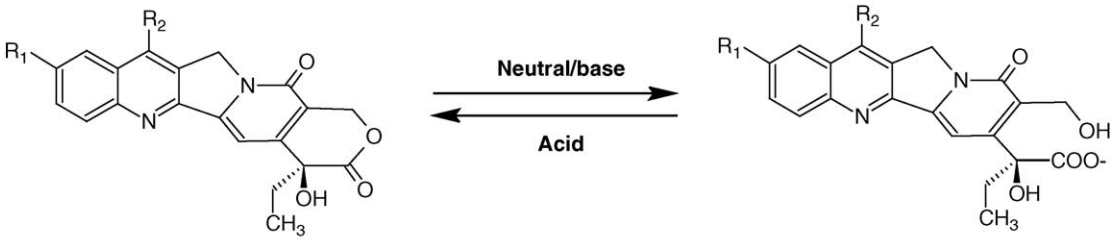


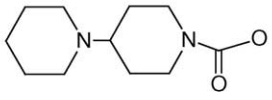
Compound	R_1	R_2
CPT	H	H
CPT-11		C_2H_5
SN-38	OH	C_2H_5

Fig. 1. Conversion of the lactone and carboxyl forms of CPT, CPT-11 and SN-38.

active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) which is 100–1000-fold more cytotoxic than the parent molecule [6–8]. SN-38 is further converted to glucuronide by uridine diphosphate glucuronosyltransferase 1A [6,9,10]. SN-38 is considered responsible for the major dose-limiting toxicities including myelosuppression and unpredictable severe diarrhea of CPT-11 [11,12]. The lactone form of CPT-11 and SN-38 has a closed α-hydroxy-δ-lactone ring, which can be hydrolyzed to form the open-ring hydroxyl acid (carboxylate form). The rate of hydrolysis is dependent on pH [4,13,14], ionic strength [13], and protein concentration [14,15]. Human serum albumin preferentially binds the carboxylate form over the lactone form with a 150-fold higher affinity. The lactone form has been found to be essential for the stabilization of the DNA–topoisomerase I complex and the tumor inhibitory activity of the lactone form is significantly greater than the carboxylate form [1,2,16]. Therefore, it is important to simultaneously quantitate both the carboxylate and lactone forms of CPT-11 and SN-38.

Several high-performance liquid chromatographic (HPLC) methods have been developed for the simultaneous determination of CPT-11 and SN-38 in buffered solutions and in biological fluids including plasma [17–21], red blood cells [22], saliva [23], urine [24], feces [24], intestine [25], microsomes[26] and in tumor tissues [27,28] as well. In these studies, biological samples were prepared by liquid-liquid extraction [29], solid-phase extraction [30,31] or direct deproteneization [19,23,24]. Components were separated on C_{18}, C_8 or CN column at temperature from ambient to 60 °C in isocratic mode or gradient mode with fluorometric detector. However, most techniques were limited to determine the total concentrations of two forms of CPT-11 and SN-38 by transferring carboxylate forms to lactone forms through acidification of the sample [19,23,31] or by transferring lactone forms to carboxylate forms through basification of the sample [18]. Some of them were able to determine the carboxylate forms of CPT-11 and SN-38 in plasma by subtracting lactone form from the total form with appropriate process to stop the conversion of lactone form to carboxylate form [19,23,31]. However, these methods needed several different HPLC conditions for each sample to determine the lactone form and total form separately. Some methods could separate lactone and carboxylate forms at the same time by gradient [17,21] or isocratic elution [32,33], with or without the addition of iron-pairing regent [34]. However, most of them calculated the concentration of each compound by external standard [17,21,32] and were validated in human plasma [21,32,33].

Since SN-38 is the major metabolite of CPT-11, which has more significant anti-cancer activity than CPT-11 and both compounds are reversibly converted to their inactive open-ring carboxylate forms, it is necessary to develop simple and cost-effective HPLC methods for the determination of the two forms of CPT-11 and SN-38. Additionally, proper sample handling should be adopted to avoid the conversion between these two forms as well as photodegradation [35]. In this report, we described a simple and sensitive isocratic HPLC method for the simultaneous determination of the lactone and carboxylate forms of CPT-11 and SN-38 in rat plasma with CPT as the internal standard. The separation was carried out on a reversed-phase C_{18} column and monitored at appropriate excitation and emission wavelengths at which both compounds have good response. The validated HPLC methods have been used to study the effect of coadministered thalidomide, a potent tumor necrosis factor-α inhibitor [36,37], on the plasma pharmacokinetics of CPT-11 and SN-38 in the rat. Thalidomide ameliorated CPT-11-induced late-onset diarrhea and reduced the metabolism of CPT-11 to SN-38 in cancer patients [38]. Thus, it is worthwhile examining the pharmacokinetic response of CPT-11 and SN-38 to thalidomide.

2. Experimental

2.1. Chemicals and reagents

CPT-11 lactone form (diethyl-4,11 hydroxy-4 (piperidino-4 piperidino-carbonyloxy)-9 1H-pyrano (3′,4′,6,7) indolizino (1,2-b) quinolein-(4H,12H) dione-3,14 hydrochloride trihydrate, M_r 677.20, with a purity of 99.5%) and SN-38 lactone form (SN-38 monohydrate; M_r 410.43, with a purity of 100%) were kindly supplied by Pharmacia (Kalamazoo, MI, USA). CPT-11 and SN-38 carboxylate forms were prepared from their lactone forms respectively by dilution with acetonitrile–0.02 M borate buffer at pH 9.0 (50:50, v/v). Thalidomide was provided by Celgene Co. (MA, USA). The internal standard (IS) camptothecin (CPT, with a purity of 95.0%) and the ion-pairing reagent tetrabutylammonium hydrogen sulfate (TBAHS) were all purchased from Sigma Chemical (St. Louis, MO, USA). Acetonitrile was of HPLC-grade purchased from Fisher Scientific (Fair Lawn, NJ, USA). The water used was of Milli-Q grade purified by a Milli-Q UV Purification System (Millipore, Bedford, MA, USA). All other reagents were of analytical grade available commercially. Fresh blood was collected from healthy male Sprague–Dawley rats (provided by the Laboratory Animal Centre, National University of Singapore, Singapore) and the plasma obtained by centrifugation at 1500 × *g* for 10 min at 37 °C.

2.2. Equipment and chromatographic conditions

The HPLC system (Shimadzu, Nakagyo-ku, Kyoto, Japan) consisted of a LC-10AT pump, a FCV-10AL low-pressure gradient flow control valve, a DGU-14A on-line solvent degasser, a RF-10AXL fluorescence detector and a SIL-10AD sample injector. Shimadzu Class-LC10 Workstation was used for system control and data were monitored and analyzed using Shimadzu CLASS VP software. Separation of compounds was carried out at ambient temperature on a Hypersil ODS Column (200 mm × 4.6 mm i.d., 5 μm) from Agilent Technologies (Palo Alto, CA, USA), preceded by a phenomenex C18 guard column (Torrance, CA, USA). The mobile phase comprised of 0.1 M potassium dihydrogen phosphate containing 0.01 M TBAHS (pH 6.4)–acetonitrile (75:25, v/v). In all instances, a flow-rate of 0.8 ml/min was employed. The eluted peaks were monitored at an excitation and emission wavelengths of 380 and 540 nm, respectively.

2.3. Standard samples

Stock solutions (500 μM) of lactone forms of CPT-11 and SN-38 were prepared in dimethyl sulfoxide (DMSO). Working solutions (10, 50 and 100 μM) of lactone forms of both compounds were prepared by diluting stock solutions with acetonitrile–0.01 M citric acid (pH 3.0) (50:50, v/v) [39]. Acetonitrile–0.02 M borate buffer, pH 9.0 (50:50, v/v) was used for the carboxylate forms of 2 analytes [17]. All stock and working solutions were stored at −20 °C under dark condition. IS stock solution (287 μM) was prepared by dissolving 0.50 mg CPT in 5 ml DMSO and stored at −20 °C under dark condition. The stock solution of IS was diluted with ice-cold acetone. An aliquot (100 μl containing 0.05 μg CPT) was added to a clean polypropylene tube (Axygen, CA, USA). The solution was evaporated under a stream of nitrogen and the residues were stored at −20 °C until analysis.

Plasma standards were prepared by adding 10 μl of the appropriate standard solution to 100 μl blank plasma in a tube containing 0.05 μg IS. Typical standard samples contained the lactone and carboxylate forms of CPT-11 and SN-38 ranging from 0.01 to 10 μM. Because of the wide range of the concentrations studied, we constructed the calibration curves at two concentration ranges (0.01–0.5 and 0.5–10 μM). Plasma proteins were then precipitated by the addition of 200 μl ice-cold methanol and acetonitrile (1:1, v/v). After rigorous vortex-mixing for 1 min, the mixtures were centrifuged at 3000 × *g* for 7 min at 4 °C. An aliquot of 100 μl supernatant was transferred to a fresh polypropylene tube and 20 μl was injected onto the column for analysis.

2.4. Calibration curves

The calibration curves were constructed by plotting the peak area ratio of the analytes over IS versus the concentrations spiked. The range of reliable response was established on the basis of at least six triplicate standards in rat plasma covering the concentration range of 0.01–0.5 and 0.5–10 μM for all compounds studied. The equations were calculated using linear regression. The linearity of the assay procedure was determined by calculation of a regression line using the method of unweighted least squares analysis. Concentrations in unknown samples were calculated from the resulting peak area ratios and the regression equation of the calibration curve. The limit of quantification (LOQ) was defined as the lowest drug concentration that could be determined with acceptable precision [i.e. coefficient of variation (CV) ≤ 20%] and accuracy (i.e. recovery of 100 ± 20%). The limit of detection was the amount which could be detected with a signal to noise ratio of 3.

2.5. Method validation

All validation runs were performed on three consecutive days and all samples used for validation were prepared as standard samples. Three different plasma concentrations (0.01, 0.5 and 10 μM) of both forms of CPT-11 and SN-38 were investigated for recovery, whereas CPT lactone (the internal standard) was measured at the concentration used in sample preparation. The recovery was determined by comparing the analytical results for extracted samples with unextracted standards that represent 100% recovery.

Quality control samples containing each analyte were prepared from weighing independent of those used for preparing calibration curves. Within-day and between-day precision

and the mean accuracy were determined by analysis of both lactone and carboxylate forms of CPT-11 at 0.01, 0.25, 2.5, 5, 10 and 50 μM (1:10 dilution) and SN-38 at 0.01, 0.25, 2.5, 5, 10 μM in triplicate on a single day and on 3 consecutive days respectively. The quality control samples were prepared on the day of analysis in the same way as calibration standards. During each analytical run, QC samples were included and processed as the calibration and unknown samples.

2.6. Pharmacokinetic studies in rats

Healthy Sprague–Dawley rats (200–230 g, $n = 5$, purchased from Laboratory Animals Centre, National University of Singapore, Singapore) were used in the kinetic studies and all animal procedures were approved by the Animal Ethnical Committee of the National University of Singapore. An injectable formulation of CPT-11 was prepared by dissolving CPT-11 (20 mg/ml), D-sorbitol (45 mg/ml), and D-lactic acid (0.9 mg/ml) in Milli-Q water heated to 70–90 °C for 5–10 min. The pH of this clear solution was adjusted to 3.5 by 1 M NaOH. The resulting solution was sterile-filtered (0.22 μm, Millipore, MA, USA) and stored at 4 °C under dark condition until use [40]. Thalidomide was dissolved in dimethyl sulfoxide (DMSO) and injected intraperitoneously (i.p.) at 100 mg/kg body weight at 1 μl per g body weight 30 min before the administration of CPT-11. This dose of thalidomide was adopted as it induced marked biological responses without showing any significant toxicity [41,42]. Rats were randomized to two groups receiving CPT-11 (60 mg/kg, i.v.) alone or in combination with thalidomide. The use of high dose (60 mg/kg) of CPT-11 to rats was to induce significant early and late-onset diarrhea [43] that could be alleviated by coadministered thalidomide. Blood samples (200 μl each time point) were collected by snipping the tail vein 0.25, 0.5, 1, 2, 4, 6, 8, and 10 h following drug administration using heparinized tubes. Plasma was obtained by immediate centrifugation at 1500 × *g* for 10 min at 4 °C. A 100-μl aliquot of the plasma was processed as described above in Section 2.3. The supernatant after protein precipitation was transferred to a clean polypropylene tube and stored at −70 °C in dark until analysis. When analyzed, the tubes were transferred to −20 °C for 1 h first and then vortex-mixed for 5 s and centrifuged at 3000 × *g* for 5 min at 4 °C. An aliquot (20 μl) of the solution was injected onto HPLC for analysis. All samples were put on ice to minimize the conversion of lactone to carboxylate form.

2.7. Pharmacokinetic calculations

The concentration–time curves were obtained by plotting the mean concentrations of each analyte at each time point versus time on a semi-logarithmic scale. Pharmacokinetics parameters were calculated by non-compartment model using WinNonlin R program (Scientific Consulting Inc., North Carolina, USA). The half-life ($t_{1/2\beta}$) was calculated as $0.693/k'$ where k' is the elimination rate constant calculated from the terminal linear portion of the plasma log concentration–time curve. The area under the concentration–time curve (AUC_{0-10h}) was calculated using the log trapezoidal rule without extrapolation to infinity. The plasma clearance (CL) was calculated by dividing the total administered dose by the $AUC_{0-\infty}$, which was calculated using the log trapezoidal rule with extrapolation to infinity. C_0 was the initial plasma concentration of drugs determined by back-extrapolation to the *y*-axis (i.e. time = 0).

2.8. Statistical analysis

Data are expressed as mean ± S.D. Statistical significance was assessed using a standard unpaired Student's *t*-test with $P > 0.05$ being considered not significant.

3. Results

Representative chromatograms for the two forms of CPT-11 and SN-38 are shown in Fig. 2. Under the chromatographic conditions used for the analysis of 4 analytes, the

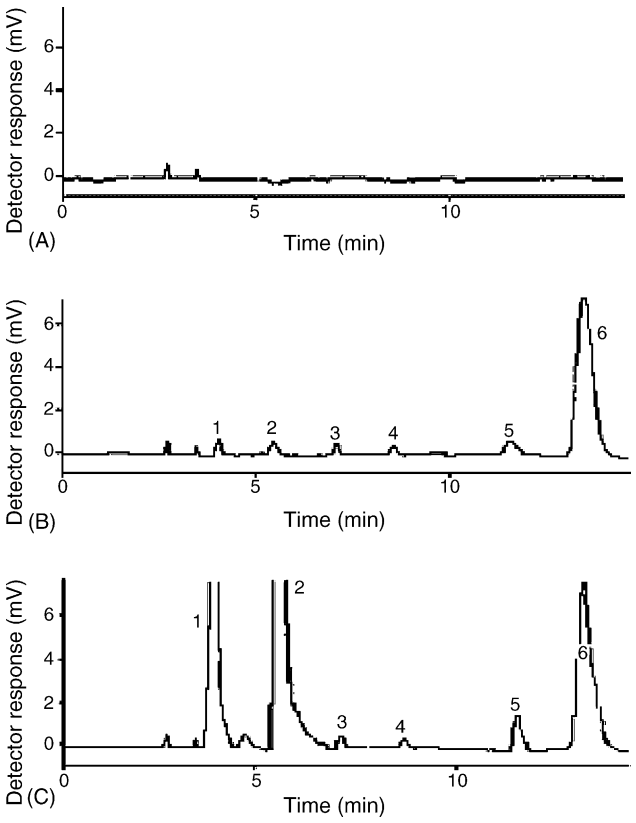


Fig. 2. (A) Chromatogram obtained from blank rat plasma; (B) representative chromatograms of blank rat plasma spiked with standards; and (C) rat plasma collected 6 h after administration of 60 mg/kg CPT-11 by intravenous injection in combination with 60 mg/kg thalidomide by intraperitoneous injection. Peaks: 1 = CPT-11 carboxylate; 2 = CPT-11 lactone; 3 = SN-38 carboxylate; 4 = CPT carboxylate; 5 = SN-38 lactone; and 6 = CPT lactone (internal standard). The concentration of all analytes in (B) is 0.025 μM.

Table 1
Recovery (%) of analytes from rat plasma (expressed as mean ± S.D.)

Concentration (μM)	No. of sample (*n*)	Recovery (%)	
		Carboxylate form	Lactone form
CPT-11			
0.01	3	108.9 ± 8.7	98.6 ± 11.3
0.5	3	96.3 ± 1.5	106.7 ± 3.2
10	3	94.4 ± 1.9	96.2 ± 7.8
SN-38			
0.01	3	112.6 ± 7.6	103.0 ± 10.7
0.5	3	98.2 ± 2.1	104.8 ± 3.7
10	3	97.6 ± 1.3	95.6 ± 8.5

retention times for lactone forms of CPT-11, SN-38 and CPT were 5.33 ± 0.14, 11.60 ± 0.20 and 13.52 ± 0.21 min ($n = 40$) and for carboxylate forms were 3.97 ± 0.10, 7.05 ± 0.15 and 8.12 ± 0.18 min ($n = 40$) respectively. The peaks for all analytes were slightly skewed to the right. We evaluated peak skew using the asymmetry coefficient $A_s = b/a$, where b is the distance after the peak maximum and a is the distance before the peak maximum, both a and b being measured at 10% of the total peak height. The asymmetry coefficients were between 1.05–1.26. These methods employed a simple protein precipitation step, with a recovery of > 94% at concentrations of 0.01–10 μM (Table 1). No concentration dependence was observed. The recovery of the IS, determined at the concentration used was 98.6 ± 4.7% ($n = 6$). Matrix-specific interfering peaks that required modification of the mobile phase composition were not observed in any cases, including in the presence of drugs such as thalidomide. In addition, single ion monitoring using liquid chromatography-mass spectrometry with an electrospray interface (Finnigan, Dreieich, Germany) did not detect the existence of oxidative metabolites in the plasma of rats treated with CPT-11.

All four compounds gave linear response as a function of concentration over 0.01–10 μM. The mean correlation coefficients (r) for the daily calibration curves were all >0.999 ($n = 5$) and the within- and between-run CVs of the response factors for each concentration assayed were below 10%. The mean y intercepts were 0.003–0.05 ($n = 5$) for all four analytes. For each point on the calibration curves for all 4 analytes, the concentrations back-calculated from the equation of the regression analysis were within acceptable limits for accuracy and precision of ±20%. A linear regression of the back-calculated concentrations versus the nominal values provided a unit slope and an intercept not significantly different from zero. The distribution of the residuals showed random variation, was normally distributed and centered on zero. The bias was not statistically different from zero, and the 95% confidence intervals included zero (data not shown).

The LOQ in rat plasma (100-μl aliquot) was 0.01, 0.008, 0.005 and 0.005 (M for CPT-11 lactone, CPT-11 carboxylate, SN-38 lactone and SN-38 carboxylate, respectively. The validation data in terms of within-day and between-day precision and accuracy are represented in Table 2. The differences between the theoretical and the actual concentration and the

Table 2
Within- and between-day precision and accuracy of the HPLC methods for the determination of the lactone and the carboxylate forms of CPT-11 and SN-38 in rat plasma

Concentration (μM)	Carboxylate form		Lactone form	
	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
Within-day				
CPT-11				
0.01	5.5	108.8	4.2	95.5
0.25	1.9	96.4	3.5	102.2
2.5	1.5	96.4	2.0	96.9
5	2.4	100.7	1.2	102.9
10	1.2	100.3	2.3	100.6
50	3.7	104.4	2.6	99.7
SN-38				
0.01	5.9	111.7	6.4	109.3
0.25	2.3	94.5	3.2	92.4
2.5	1.7	99.6	2.1	95.7
5	2.0	102.3	2.0	98.4
10	2.4	103.1	2.4	107.2
Between-day				
CPT-11				
0.01	4.7	106.2	3.6	97.2
0.25	1.6	97.8	2.4	100.5
2.5	2.4	98.2	1.3	97.8
5	1.9	96.5	1.3	95.5
10	1.3	100.4	3.2	94.3
50	2.9	97.3	1.5	103.2
SN-38				
0.01	3.9	111.8	5.2	107.7
0.25	1.3	95.9	3.2	99.6
2.5	1.6	99.3	2.2	95.3
5	2.1	96.4	1.8	102.4
10	1.7	100.5	2.2	104.3

CVs were less than 15% at any quality control sample concentrations. Dilution of CPT-11 in lactone or carboxylate form at 1:10 gave acceptable precision (CVs < 4%) and accuracy (recovery within 97.3–104.4%).

We applied this method to the study of plasma pharmacokinetics of CPT-11 and SN-38 in rat. Fig. 3 shows representative plasma concentration–time profiles for all analytes studied in rats receiving CPT-11 alone and in combination with thalidomide. Coadministered thalidomide (100 mg/kg body weight) significantly increased the AUC_{0-10h} values of CPT-11 lactone and CPT-11 carboxylate by 32.6% and 30.3% respectively ($P < 0.01$), but decreased the values by 19.2% and 32.4% for SN-38 lactone and carboxylate, respectively ($P < 0.05$; Table 3). Accordingly, the value of total plasma clearance (CL) of CPT-11 lactone was significantly lower in combination group compared to the control (1.329 versus 1.837 L/h/kg, $P < 0.001$). Plasma $t_{1/2\beta}$ values for SN-38 lactone and carboxylate were significantly ($P < 0.01$) smaller in rats with coadministered thalidomide, as compared to rats receiving CPT-11 alone. The volume of distribution (V_d) for CPT-11 lactone was decreased by 24.7% in rats receiving combination therapy than those receiving CPT-11 alone

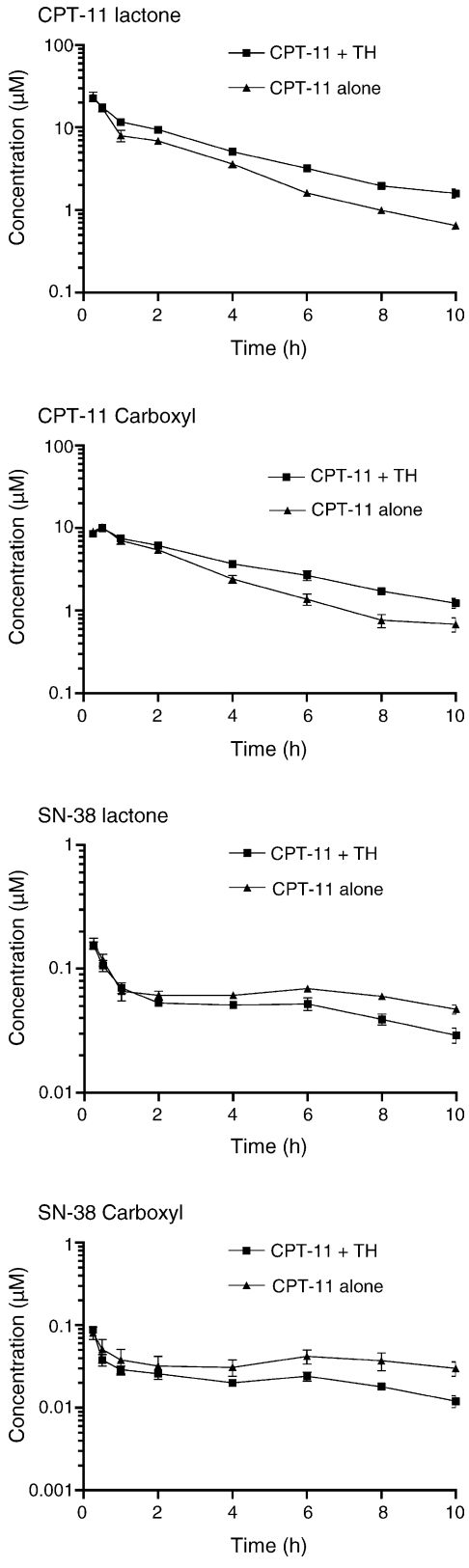


Fig. 3. Plasma concentration–time profiles for the lactone and carboxylate forms of CPT-11 and SN-38 in rats CPT-11 alone and in combination with thalidomide.

($P < 0.01$). However, coadministration of thalidomide did not significantly alter maximum plasma concentrations of all four analytes.

4. Discussion

We developed and validated a simple HPLC method for the determination of the lactone and carboxyl forms of CPT-11 and SN-38 in rat plasma. The reported method was fast and efficient, with simple sample preparation procedure and total running time of analytes and IS less than 16 min which was much shorter than those reported previously (about 30–35 min) [19,23,24]. Therefore, the risk of degradation and conversion of analytes can be significantly reduced during sample analysis. In the present study, we have chosen a mobile phase with 0.1 M potassium dihydrogen phosphate containing 0.01 M TBAHS at pH 6.4 which resulted in efficient separation between compounds with suitable retention time. The potassium dihydrogen phosphate buffer used in the mobile phase could control the pH value and ion strength and the ion-paring agent TBAHS would increase the affinity of the carboxylate forms to column C_{18} particles, which would facilitate their separation. Sano et al. [34] recently reported a simple and reliable HPLC method without an ion-pairing agent, which allows the simultaneous determination of both lactone and carboxylate forms of SN-38 and other camptothecin derivatives. The strength of the buffer and the buffer pH had a large influence on the retention of all compounds [33]. The lactone form was relatively stable at pH 6.4, whereas it started to decrease and rapidly convert to carboxylate in the pH range of 7.0–9.0. As to the wavelengths, we selected an excitation wavelength at 380 nm and set the emission wavelength at 540 nm in favor of SN-38 given that the plasma concentrations of CPT-11 are often higher than SN-38.

The validated HPLC method has been applied to a pharmacokinetic study when CPT-11 was combined with thalidomide in rats. The pharmacokinetic study revealed that thalidomide significantly increases the AUC of two forms of CPT-11 and decreased the AUC of two forms of SN-38. This may partially explain the finding that coadministered thalidomide reduced the gastrointestinal toxicity of CPT-11. Studies are ongoing at our laboratory to examine whether multiple dosing of thalidomide can also modulate the pharmacokinetics of CPT-11 and SN-38 in rats by employing our developed HPLC method.

In summary, we reported on a simple and reliable HPLC method for simultaneous measurement of lactone and carboxylate forms of CPT-11 and SN-38 in rat plasma. The validated method has been applied to investigate the effect of thalidomide on the kinetics of all four analytes in the rat. The pharmacokinetic studies in the rat provided partial explanation for the finding that coadministered thalidomide reduced the gastrointestinal toxicity of CPT-11. Further studies are needed to explore the underlying mechanisms

Table 3
A comparison of pharmacokinetic parameters between rats receiving combination therapy or CPT-11 alone

Parameters	Treatment group		Comparison	
	CPT-11 + thalidomide	CPT-11 (control)	Combination vs. control (%)	*P*-value
CPT-11 lactone				
C_0 (μM)	29.03 ± 3.92	33.01 ± 5.29	−12.1	0.21
$t_{1/2\beta}$ (h)	3.00 ± 0.13	2.89 ± 0.36	3.7	0.56
AUC_{0-10h} (μM h)	60.89 ± 3.23	45.91 ± 4.08	32.6	<0.01
$AUC_{0-\infty}$ (μM h)	66.85 ± 3.70	48.55 ± 4.54	37.7	<0.01
V_d (L/kg)	5.74 ± 0.37	7.62 ± 0.80	−24.7	<0.01
CL (L/h/kg)	1.33 ± 0.07	1.84 ± 0.16	−27.7	<0.01
CPT-11 carboxylate				
C_0 (μM)	10.01 ± 0.27	10.29 ± 0.59	−2.7	0.366
$t_{1/2\beta}$ (h)	3.49 ± 0.21	2.34 ± 0.12	49.1	<0.01
AUC_{0-10h} (μM h)	38.34 ± 0.85	29.42 ± 3.02	30.3	<0.01
$AUC_{0-\infty}$ (μM h)	44.49 ± 1.70	31.13 ± 3.41	42.9	<0.01
SN-38 lactone				
C_0 (μM)	0.22 ± 0.01	0.22 ± 0.03	2.8	0.63
$t_{1/2\beta}$ (h)	5.00 ± 0.32	7.15 ± 0.82	−30.1	<0.01
AUC_{0-10h} (μM h)	0.55 ± 0.02	0.68 ± 0.04	−19.2	<0.01
$AUC_{0-\infty}$ (μM h)	0.76 ± 0.05	1.18 ± 0.13	−35.7	<0.01
SN-38 carboxylate				
C_0 (μM)	0.14 ± 0.02	0.13 ± 0.02	5.4	0.43
$t_{1/2\beta}$ (h)	4.56 ± 1.34	7.80 ± 0.97	−41.5	<0.01
AUC_{0-10h} (μM h)	0.26 ± 0.01	0.38 ± 0.09	−32.4	<0.05
$AUC_{0-\infty}$ (μM h)	0.34 ± 0.04	0.72 ± 0.16	−52.4	<0.01

for the observed kinetic and dynamic interactions between CPT-11 and thalidomide.

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References

[1] M.R. Redinbo, L. Stewart, P. Kuhn, J.J. Champoux, W.G. Hol, Science 279 (1998) 1504.
[2] L. Stewart, M.R. Redinbo, X. Qiu, W.G. Hol, J.J. Champoux, Science 279 (1998) 1534.
[3] P.J. Houghton, P.J. Cheshire, J.C. Hallman, M.C. Bissery, A. Mathieu-Boue, J.A. Houghton, Cancer Res. 53 (1993) 2823.
[4] M. Potmesil, Cancer Res. 54 (1994) 1431.
[5] T. Kunimoto, K. Nitta, T. Tanaka, N. Uehara, H. Baba, M. Takeuchi, T. Yokokura, S. Sawada, T. Miyasaka, M. Mutai, Cancer Res. 47 (1987) 5944.
[6] R.H. Mathijssen, R.J. van Alphen, J. Verweij, W.J. Loos, K. Nooter, G. Stoter, A. Sparreboom, Clin. Cancer Res. 7 (2001) 2182.
[7] Y. Kawato, M. Aonuma, Y. Hirota, H. Kuga, K. Sato, Cancer Res. 51 (1991) 4187.
[8] Y. Kawato, M. Aonuma, Y. Hirota, H. Kuga, K. Sato, Cancer Res. 51 (1991) 4187.
[9] N. Hanioka, S. Ozawa, H. Jinno, M. Ando, Y. Saito, J. Sawada, Xenobiotica 31 (2001) 687.
[10] A. Santos, S. Zanetta, T. Cresteil, A. Deroussent, F. Pein, E. Raymond, L. Vernillet, M.L. Risse, V. Boige, A. Gouyette, G. Vassal, Clin. Cancer Res. 6 (2000) 2012.
[11] Y. Sugiyama, Y. Kato, X. Chu, Cancer Chemother. Pharmacol. 42 (Suppl.) (1998) S44.
[12] E. Gupta, T.M. Lestingi, R. Mick, J. Ramirez, E.E. Vokes, M.J. Ratain, Cancer Res. 54 (1994) 3723.
[13] J. Fassberg, V.J. Stella, J. Pharm. Sci. 81 (1992) 676.
[14] T.G. Burke, Z. Mi, Anal. Biochem. 212 (1993) 285.
[15] T.G. Burke, C.B. Munshi, Z. Mi, Y. Jiang, J. Pharm. Sci. 84 (1995) 518.
[16] W.J. Slichenmyer, E.K. Rowinsky, R.C. Donehower, S.H. Kaufmann, J. Natl. Cancer Inst. 85 (1993) 271.
[17] D.F. Chollet, L. Goumaz, A. Renard, G. Montay, L. Vernillet, V. Arnera, D.J. Mazzo, J. Chromatogr. B Biomed. Sci. Appl. 718 (1998) 163.
[18] N.E. Schoemaker, H. Rosing, S. Jansen, J.H. Schellens, J.H. Beijnen, Ther. Drug Monit. 25 (2003) 120.
[19] J. Escoriaza, A. Aldaz, C. Castellanos, E. Calvo, J. Giraldez, J. Chromatogr. B Biomed. Sci. Appl. 740 (2000) 159.
[20] F.A. de Jong, R.H. Mathijssen, P. de Bruijn, W.J. Loos, J. Verweij, A. Sparreboom, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 795 (2003) 383.
[21] T.S. Owens, H. Dodds, K. Fricke, S.K. Hanna, K.R. Crews, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 788 (2003) 65.
[22] F.A. de Jong, R.H. Mathijssen, P. de Bruijn, W.J. Loos, J. Verweij, A. Sparreboom, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 795 (2003) 383.
[23] S. Poujol, F. Pinguet, F. Malosse, C. Astre, M. Ychou, S. Culine, F. Bressolle, Clin. Chem. 49 (2003) 1900.
[24] A. Sparreboom, P. de Bruijn, M.J. de Jonge, W.J. Loos, G. Stoter, J. Verweij, K. Nooter, J. Chromatogr. B Biomed. Sci. Appl. 712 (1998) 225.
[25] K. Arimori, N. Kuroki, A. Kumamoto, N. Tanoue, M. Nakano, E. Kumazawa, A. Tohgo, M. Kikuchi, Pharm. Res. 18 (2001) 814.

[26] K. Sai, N. Kaniwa, S. Ozawa, J. Sawada, Biomed. Chromatogr. 16 (2002) 209.
[27] H.M. Dodds, P.J. Tobin, C.F. Stewart, P. Cheshire, S. Hanna, P. Houghton, L.P. Rivory, J. Pharmacol. Exp. Ther. 303 (2002) 649.
[28] N. Kaneda, H. Nagata, T. Furuta, T. Yokokura, Cancer Res. 50 (1990) 1715.
[29] P. de Bruijn, J. Verweij, W.J. Loos, K. Nooter, G. Stoter, A. Sparreboom, J. Chromatogr. B Biomed. Sci. Appl. 698 (1997) 277.
[30] L.P. Rivory, M. Findlay, S. Clarke, J. Bishop, J. Chromatogr. B Biomed. Sci. Appl. 714 (1998) 355.
[31] I. Barilero, D. Gandia, J.P. Armand, A. Mathieu-Boue, M. Re, A. Gouyette, G.G. Chabot, J. Chromatogr. 575 (1992) 275.
[32] D.L. Warner, T.G. Burke, J. Chromatogr. B Biomed. Sci. Appl. 691 (1997) 161.
[33] L.P. Rivory, J. Robert, J. Chromatogr. B Biomed. Appl. 661 (1994) 133.
[34] K. Sano, M. Yoshikawa, S. Hayasaka, K. Satake, Y. Ikegami, H. Yoshida, T. Ishikawa, S. Sawada, S. Tanabe, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 795 (2003) 25.
[35] K. Akimoto, A.A. Kawai, K. Ohya, J. Chromatogr. A 734 (1996) 401.
[36] E.P. Sampaio, E.N. Sarno, R. Galilly, L. Jet, J. Exp. Med. 173 (1991) 699.
[37] C. Meierhofer, C.J. Wiedermann, Curr. Opin. Drug Discov. Devel. 6 (2003) 92.
[38] G. Allegrini, A. Di Paolo, A. Falcone, S. Cupini, G. Masi, E. Cerri, L. Marcucci, M. Lastella, R. Danesi, M. Del Tacca, Proc. Am. Soc. Clin. Oncol. (2003) 140.
[39] L.P. Rivory, E. Chatelut, P. Canal, A. Mathieu-Boue, J. Robert, Cancer Res. 54 (1994) 6330.
[40] O.C. Trifan, W.F. Durham, V.S. Salazar, J. Horton, B.D. Levine, B.S. Zweifel, T.W. Davis, J.L. Masferrer, Cancer Res. 62 (2002) 5778.
[41] S.F. Zhou, J.W. Paxton, M.D. Tingle, P. Kestell, L.-M. Ching, Cancer Chemother. Pharmacol. 47 (2001) 319.
[42] Q. Ding, P. Kestell, B.C. Baguley, B.D. Palmer, J.W. Paxton, G. Muller, L.-M. Ching, Cancer Chemother. Pharmacol. 50 (2002) 186.
[43] A. Kurita, S. Kado, N. Kaneda, M. Onoue, S. Hashimoto, T. Yokokura, Cancer Chemother. Pharmacol. 52 (2003) 349.