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## Determination of irinotecan (CPT-11) and its active metabolite SN-38 in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection

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### Abstract

Sensitive high-performance liquid chromatographic assays have been developed to determine the levels of the lactone and lactone plus carboxylate (total) forms of the antitumor agent irinotecan (CPT-11) and its active metabolite SN-38, in human plasma. The related compound camptothecin was used as the internal standard. The selective sample pretreatment for the lactone forms involved a single solvent extraction with acetonitrile–*n*-butyl chloride (1:4, v/v), whereas the sample clean-up for the total forms was a simple protein precipitation with aqueous perchloric acid–methanol (1:1, v/v), which results in the conversion of the carboxylate to the lactone forms. Chromatography was carried out on a Hypersil ODS column, with detection performed fluorimetrically. The methods have been validated, and stability tests under various conditions have been performed. The lower limits of quantitation are 0.5 and 2.0 ng/ml for the lactone and total forms, respectively. The assays have been used in a single pharmacokinetic experiment in a patient to investigate the applicability of the method in vivo. © 1997 Elsevier Science B.V.

**Keywords:** Irinotecan; CPT-11; SN-38

### 1. Introduction

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; CPT-11) (Fig. 1) is a synthetic derivative of the plant alkaloid camptothecin with increased aqueous solubility, that does not produce hemorrhagic cystitis associated with the parent compound [1]. CPT-11 has demonstrated pronounced antitumor activity against a variety of experimental tumors [2–5]. The mechanism of

action of CPT-11 involves inhibition of mammalian DNA topoisomerase-I [6,7], causing stabilization of cleavable complexes during DNA replication, transcription and repair, and, ultimately, cell death [8–11].

Extensive preclinical studies have shown that CPT-11 is in vivo rapidly converted into an active metabolite (7-ethyl-10-hydroxycamptothecin; SN-38) (Fig. 1) via endogenous carboxylesterases in the intestinal mucosa, plasma and liver [12]. In vitro studies demonstrated that SN-38 is significantly more cytotoxic than CPT-11 [13,14]. Similar to all other

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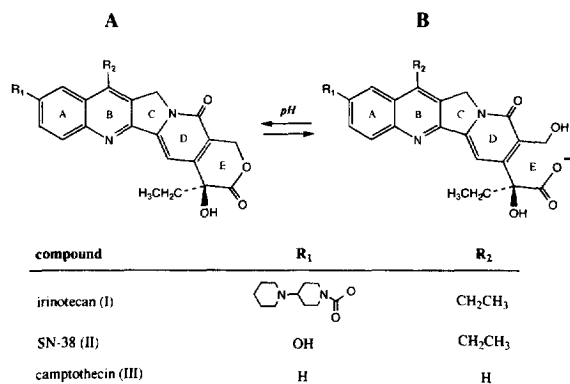


Fig. 1. Chemical structures of the lactone and carboxylate forms of CPT-11, its active metabolite SN-38, and the related compound CPT (used as internal standard for the HPLC assay).

camptothecin analogues, CPT-11 and SN-38 can undergo a reversible, pH-dependent hydrolysis in which the closed lactone form is converted to the open carboxylate form and vice versa [15].

Initial pharmacokinetic studies of CPT-11 involved assays based on high-performance liquid chromatography (HPLC) that measured only total levels, i.e. lactone plus carboxylate forms, of CPT-11 and SN-38 [16–21]. In more recent clinical trials attempts have been made to carefully detail the complex pharmacokinetics of the lactone and carboxylate forms by implementation of more selective assays that can discriminate between the two forms of both compounds [22]. The availability of such analytical methods is particularly important as only the closed lactone forms of CPT-11 and SN-38 are active as antitumor agents [23].

We now report on the development and validation of new, simplified and sensitive methods for the determination of the lactone and total forms of CPT-11 and SN-38 in human plasma. Compared to previously described methods, the assay sensitivity has been significantly improved with reproducible lower limits of quantitation of 0.5 ng/ml for CPT-11 and SN-38 lactone in plasma. The usefulness of the analytical method was subsequently tested by the determination of CPT-11 and SN-38 in plasma samples from a patient with advanced colorectal cancer given CPT-11 by a 90-min intravenous infusion.

## 2. Experimental

### 2.1. Chemicals

CPT-11 (batch KO16; purity 99.6%) and SN-38 (batch LIE 783; purity: 100%) were kindly donated by Rhône-Poulenc Rorer (Vitry-sur-Seine Cedex, France). The internal standard camptothecin (CPT; batch 93K05A; purity 100%) was obtained from Pharmacia (New Mexico, USA). Acetonitrile, *n*-butylchloride, dimethyl sulfoxide (DMSO) and methanol were purchased from Rathburn (Walkerburn, UK). Ammonium acetate, hydrochloric acid and perchloric acid were obtained from Baker (Deventer, The Netherlands) and sodium chloride from Merck (Darmstadt, Germany). Tetrabutylammonium sulphate came from Serva (Heidelberg, Germany). All chemicals were of the highest purity available and were used as received. Lyophilized type IX-A  $\beta$ -D-glucuronide glucuronosohydrolase (EC 3.2.1.31 from *Escherichia coli*; activity 1560 000 units/g) was purchased from Sigma (St. Louis, MO, USA). Throughout the study filtered and deionized water obtained from a Milli-Q system was used (Millipore, Milford, USA). Blank human plasma was obtained from healthy volunteers via the Central Laboratory of the Blood Transfusion Service (Amsterdam, The Netherlands).

### 2.2. Drug solutions

Separate stock solutions of CPT-11, SN-38 and CPT (at 1.00 mg/ml each) were prepared in triplicate by dissolving the appropriate amount of the drug, accurately weighed, in 10.0 ml of DMSO. A mixture of CPT-11 and SN-38 was obtained by dilution of the standard solutions in DMSO, yielding a final concentration of 20  $\mu$ g/ml for both compounds. Working solutions for the lactone forms of CPT-11 and SN-38 were prepared daily at 10, 20, 50, 100, 200 and 300 ng/ml by serial dilution in a mixture of methanol–0.01 *M* aqueous hydrochloric acid (2:3, v/v) from the stock mixture. Similarly, working solutions for CPT-11 and SN-38 total were made at concentrations of 80, 200, 800, 2000, 4000 and 8000 ng/ml. Spiked human plasma samples used for the construction of calibration curves were prepared by addition of 25 or 50  $\mu$ l of the methanolic working

solutions to 1000  $\mu\text{l}$  q.i.d. of drug-free human plasma to obtain standards of 0.5, 1, 2.5, 5, 10 and 15 ng/ml for CPT-11 and SN-38 lactone, and 2, 5, 20, 50, 100 and 200 ng/ml for CPT-11 and SN-38 total.

Drug solutions for interference analysis were obtained from the hospital pharmacy, either as solutions or after dissolving solid material in saline, e.g. tablets or capsules (final concentration: 1.0–50  $\mu\text{g}/\text{ml}$  in plasma).

### 2.3. Sample pretreatment

All frozen samples were thawed at 4°C in a water-bath and were homogenized by vortex-mixing. For determination of the lactone forms of CPT-11 and SN-38, the internal standard CPT (100  $\mu\text{l}$  of 25 ng/ml in methanol–0.01 *M* aqueous hydrochloric acid (2:3, v/v)) and 0.8 g of solid sodium chloride were added to 1000  $\mu\text{l}$  of human plasma in a 12-ml glass tube supplied with PTFE-covered screw caps. The samples were extracted with 7.5 ml of acetonitrile–*n*-butyl chloride (1:4, v/v) by vigorous mixing for 5 min, followed by centrifugation at 4000 *g* for 2 min. The resulting gels were broken by a fast manual rotation of the tubes, and next, the layers were allowed to separate again by centrifugation at 4000 *g* for 5 min. The organic layer was pipetted into a clean glass tube containing 50  $\mu\text{l}$  of DMSO, and dried at 60°C under a gentle stream of nitrogen, until a volume of approximately 50  $\mu\text{l}$  remained. This residue was reconstituted in 100  $\mu\text{l}$  of methanol and 100  $\mu\text{l}$  of perchloric acid–water (1:500, v/v) by vortex-mixing for 5 s, and samples of 100  $\mu\text{l}$  were injected into the HPLC system.

For determination of CPT-11 and SN-38 total, a 250- $\mu\text{l}$  aliquot of plasma was mixed for 10 min with 500  $\mu\text{l}$  of cold (–20°C) perchloric acid–water–methanol (1:20:20, v/v/v) in 1.5-ml polypropylene tubes (Eppendorf, Hamburg, Germany). After centrifugation for 5 min at 24 000 *g*, 100  $\mu\text{l}$  of the clear supernatant were injected into the HPLC system from autosampler vials with polypropylene inserts.

### 2.4. HPLC instrumentation and equipment

The chromatographic equipment consisted of a constaMetric 4100 solvent delivery system, an au-

toMetric 4100 autosampling device and a fluoriMonitor 4100 fluorescence detector (all from LDC Analytical, Rivera Beach, USA). A stainless steel (100×4.6 mm I.D.) analytical column packed with 5  $\mu\text{m}$  Hypersil ODS material (LC Service, Emmen, The Netherlands), protected by a LiChroCART 4–4 endcapped pre-column (4×4 mm I.D.) packed with 5  $\mu\text{m}$  LiChrospher 100 RP-18 material (Merck), was used for chromatographic separation. The analytical columns were maintained at a temperature of 50°C using a Model SpH99 column oven (Spark Holland, Meppel, The Netherlands). The mobile phases were composed of methanol–0.1 *M* ammonium acetate containing 0.01 *M* tetrabutylammonium sulphate (40:60 (v/v) for the assay of the lactone forms and 35:65 (v/v) for the assay of the total forms), with the pH adjusted to 5.50 using hydrochloric acid. Prior to use, the mobile phases were filtered under reduced pressure through a 0.45- $\mu\text{m}$  cellulosic acetate filter (Millipore), and degassed by ultrasonication. The mobile phases were delivered isocratically at a flow-rate set at 1.00 ml/min, and the column eluent was monitored fluorimetrically at an excitation wavelength of 355 nm and an emission wavelength of 515 nm, with a bandwidth of 30 nm.

Detection and integration of chromatographic peaks was performed by the Fisons ChromCard data analysis system (Milan, Italy), connected to an ICW workstation. Calibration curves were computed using the ratio of the peak height of CPT-11 or SN-38 and the internal standard versus the nominal concentration ( $x$ ), by using weighted ( $1/x^2$ ) least squares linear-regression analysis.

### 2.5. Precision and accuracy

A validation run of human plasma samples included the six-point calibration curves in duplicate and determination of quality control (QC) samples at four to five concentrations (spiked at 2, 7.5, 12, 120 and 5000 ng/ml for the lactone forms, and at 10, 75, 150 and 750 ng/ml for the total forms) of both CPT-11 and SN-38 in quintuplicate. The QC samples containing the highest concentration of CPT-11 and SN-38 were used to investigate the effect of sample dilution and/or limited volume injection. The complete validation procedure was performed on four separate days. The precision and accuracy of the

assays was assessed by the between-day and within-day precision and the percentage deviation (% DEV) from the nominal concentrations, respectively. Estimates of the between-day precision were obtained by one-way analysis of variance (ANOVA) using the run day as the classification variable. The between-groups mean square ( $MS_{bet}$ ), the within-groups mean square ( $MS_{wit}$ ) and the grand mean (GM) of the observed concentrations across run days were calculated using the NCSS package (Version 5.0, J.L. Hinze, East Kaysville, USA; 1991). The between-day precision, expressed as percentage R.S.D., was defined as:

$$\text{Between-day precision} = 100(SD_{day}/GM) \quad (1)$$

where  $SD_{day} = [(MS_{bet} - MS_{wit})/n]^{0.5}$  and  $n$  represents the number of replicates within each run. For each concentration, the estimate of the within-day precision, also expressed as R.S.D. (%), was calculated:

$$\text{Within-day precision} = 100[(MS_{wit})^{0.5}/GM] \quad (2)$$

In cases where the  $MS_{wit}$  is greater than the  $MS_{bet}$ , the resulting variance estimate is negative, implying that no significant additional variation was observed as a result of performing the assay on different days. The accuracy or mean percentage DEV for each concentration was calculated as:

$$\text{Mean \%DEV} = 100(\text{mean conc} - \text{nominal conc}/\text{nominal conc}) \quad (3)$$

## 2.6. Lower limit of quantitation

For determination of the lowest standard concentration in the analytical run quantitated with a definite level of certainty, i.e. the lower limit of quantitation (LLQ), plasma samples of five individuals were spiked at 0.25 and 0.5 ng/ml, and at 1.0 and 2.0 ng/ml for the lactone and total forms of CPT-11 and SN-38, respectively. For the concentration to be accepted as the LLQ, the mean percentage DEV of at least 80% of the samples assayed should be  $\leq 20\%$ , with a resulting within-day precision of  $\leq 20\%$ .

## 2.7. Extraction recovery

The recovery of CPT-11 and SN-38 was established at concentrations of 0.5, 1, 2.5, 5, 10 and 15 ng/ml for the lactone forms, and of 2, 5, 20, 50, 100 and 200 ng/ml for the total forms, by comparing peak areas of samples prepared in plasma with those for non-processed samples prepared in the mobile phase. The recovery was determined in four analytical runs.

## 2.8. Specificity and selectivity

The interference from endogenous components was investigated by the analysis of six different blank human plasma samples in duplicate. The following substances were investigated for interference with the analytical methods: acetaminophen, alizapride, codeine, dexamethasone, domperidon, metoclopramide, morphine and ranitidine.

## 2.9. Stability

The stability of the lactone and total forms of CPT-11 and SN-38 was evaluated at  $-80^\circ\text{C}$  for two months, and at  $37^\circ\text{C}$  for 20 h using the concentrations of the QC samples. After the storage period, the samples were analyzed immediately. The stability of both compounds was also investigated separately at lactone and total concentrations of 7.5 and 150 ng/ml, respectively, at (i) room temperature and (ii) five consecutive freeze–thaw cycles.

## 2.10. Analysis of patient samples

Fourteen plasma samples from a male patient treated for advanced colorectal cancer with a 90-min intravenous infusion of 200 mg/m<sup>2</sup> of CPT-11 followed by a 3-h intravenous infusion of 60 mg/m<sup>2</sup> of cisplatin have been analyzed. The samples were taken immediately before infusion, at 30 min and 3 h after beginning of the infusion and at 10, 20 and 30 min and 1, 1.5, 2, 4, 5, 8.5, 24 and 32 h post-infusion. The blood samples were collected in heparinized tubes, and cells were directly separated by centrifugation. The plasma supernatant was kept at  $-20^\circ\text{C}$ , until storage at  $-80^\circ\text{C}$  within 2 h.

Thawing of the samples directly prior to HPLC analysis was done at 4°C.

To investigate the presence of glucuronidated CPT-11 metabolites, plasma samples obtained at 2 and 5 h post-infusion were analyzed following enzyme-digestion as described previously with minor modifications [24]. Briefly, 250- $\mu$ l aliquots of plasma were incubated for 1 h at 37°C after addition of 1000 units of  $\beta$ -glucuronidase dissolved in 10  $\mu$ l of water. Next, samples were treated and extracted as outlined above for freshly thawed patient plasma.

### 3. Results and discussion

#### 3.1. Chromatography and detection

Chromatography was initially performed using Nova-Pak Radial-Pak ODS or Inertsil ODS reversed-phase analytical columns, in combination with mobile phases composed of ammonium acetate buffer and methanol or acetonitrile. Applying several methods of sample pretreatment, blank human plasma samples contained remaining interfering substances around the retention times of CPT-11 or SN-38 and/or sufficient separation between the analytes was associated with unacceptably long run times. Alternatively, analysis on a Hypersil ODS column with tetrabutylammonium sulphate as an ion-pairing agent added to the mobile phase resulted in no interfering substances in blank plasma samples combined with acceptable analyte separation (selectivity factors and resolution were always  $\geq 1.32$  and  $\geq 1.22$ , respectively). The composition and the pH of the mobile phases were selected in order to optimize separation factors and peak shapes of the analytes. At pH 5.50, the conversion of lactone to carboxylate was slow, while at a pH higher than 5.50, significant (on-column) conversion of lactone to carboxylate forms was observed. For the determination of CPT-11 and SN-38 using CPT as the internal standard, the fluorescence wavelength couple (excitation at 355 nm and emission at 515 nm) was selected according to data published previously [22].

An organic-solvent extraction was chosen as the sample pretreatment procedure, as this procedure had resulted earlier in an effective clean-up of human plasma samples for the analysis of related com-

pounds, such as 9-aminocamptothecin [26], without the need for elaborate solid-phase extraction techniques. Among the tested extraction and protein-precipitation procedures, a mixture of acetonitrile-*n*-butylchloride (1:4, v/v) for analysis of the lactone forms, and a mixture of aqueous perchloric acid-methanol (1:1, v/v) for analysis of the total forms resulted in minimal base-line distortion and optimal extraction recovery. Based on these results, validation of the analytical methods was performed using the Hypersil ODS column, mobile phases containing tetrabutylammonium sulphate, and sample pretreatment procedures consisting of liquid-liquid extraction with acetonitrile-*n*-butyl chloride or protein-precipitation with aqueous perchloric acid-methanol.

#### 3.2. Validation

Blank human plasma samples from six different donors showed no interfering substances for both analytical procedures [Fig. 2A (lactone forms) and Fig. 3A (total forms)]. Interference analysis with drugs potentially co-administered with CPT-11, including acetaminophen, alizapride, codeine, dexamethasone, domperidon, metoclopramide, morphine and ranitidine, did not reveal the presence of chromatographic peaks with retention times similar to that of CPT-11 or SN-38. Typical chromatograms of plasma samples spiked with known amounts of CPT-11 and SN-38 in the assay for the lactone and total forms are shown in Fig. 2B and Fig. 3B, respectively, in which the selectivity of the analytical procedures for the analytes is shown by the sharp resolution of the peaks. The retention times of CPT-11, SN-38 and CPT in the assay of the lactone forms were 4.9, 8.1 and 6.5 min, while the retention times of CPT-11 and SN-38 in the assay for the total forms were 8.3 and 15.0 min, with overall chromatographic run times of 10 and 20 min, respectively.

At spiked concentrations of 0.5 and 2.0 ng/ml for lactone and total forms of CPT-11 and SN-38, respectively, the mean percentage deviation and the within-day precision were less than 20%. Therefore, these concentrations were estimated to be the LLQs. The lower value of the LLQ for the determination of the lactones resulted from a concentration step in the sample pretreatment versus dilution of the sample in the assay for the total forms. At the upper limits of

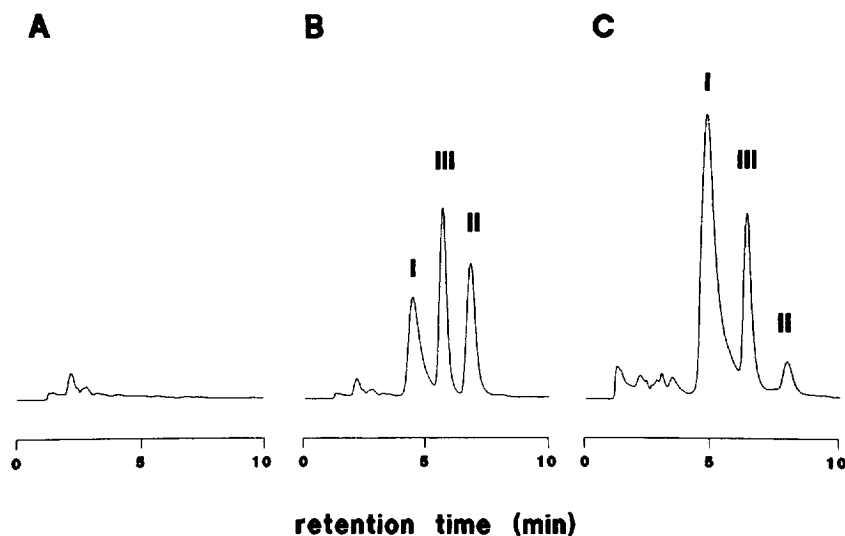


Fig. 2. HPLC chromatograms of (A) a blank human plasma sample; (B) a spiked human plasma sample containing 100 ng/ml of CPT-11 lactone and SN-38 lactone and the internal standard CPT; (C) a patient plasma extract prepared immediately following the collection of a blood sample at 1 h post-infusion of CPT-11. The mobile phase was composed of methanol–0.1 M ammonium acetate containing 0.01 M tetrabutylammonium sulphate (40:60, v/v). Peaks identified with pure standards include CPT-11 lactone (I), SN-38 lactone (II) and CPT lactone (III).

quantitation (ULQs; 15 ng/ml for the lactone forms and 200 ng/ml for the total forms), both the mean percentage deviation and the between-day precision

were also less than 20%. Initially, the ULQ for the lactone forms of CPT-11 and SN-38 was arbitrarily defined as 20 ng/ml for both compounds, but this

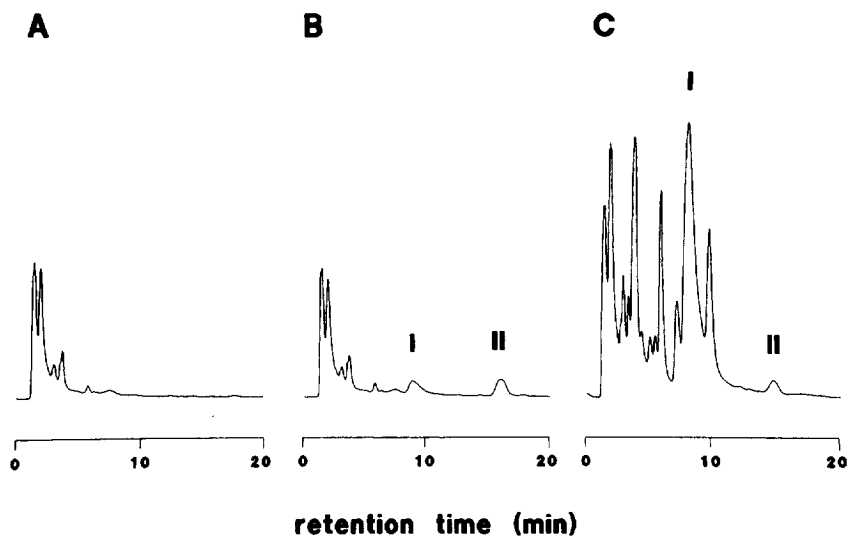


Fig. 3. HPLC chromatograms of (A) a blank human plasma sample; (B) a spiked human plasma sample containing 2 ng/ml of CPT-11 total and SN-38 total; (C) a patient plasma extract prepared immediately following the collection of a blood sample at 1 h post-infusion of CPT-11. The mobile phase was composed of methanol–0.1 M ammonium acetate containing 0.01 M tetrabutylammonium sulphate (35:65, v/v). Peaks identified with pure standards include CPT-11 total (I) and SN-38 total (II).

Table 1  
Accuracy (%DEV), within-day precision (WDP) and between-day precision (BDP) of the lactone forms of CPT-11 and SN-38 in human plasma samples

Compound	Nominal conc. (ng/ml)	DEV (%)	Precision (%)	
			WDP	BDP
CPT-11	0.5	+5.31	3.54	4.18
	2.0	-4.90	3.29	2.32
	7.5	-0.98	0.77	1.92
	12	+3.00	1.93	1.22
	120	+6.21	4.03	4.30
	5000	-13.1	2.84	9.32
SN-38	0.5	-0.06	2.63	2.51
	2.0	+2.20	1.92	2.05
	7.5	+0.92	0.57	2.41
	12	+1.70	0.95	2.05
	120	+8.01	1.45	4.11
	5000	-2.03	1.90	2.95

was subsequently diminished to 15 ng/ml as a result of detector overload at higher drug concentrations.

The results of the validation of the analytical methods in terms of precision and accuracy are listed in Tables 1 and 2. The use of the peak height in combination with a weight factor of  $1/x^2$  for the calibration resulted in minimal deviation from nominal concentrations, with linear-regression correlation coefficients (Pearson's  $r$ ) of  $\geq 0.995$  in all validation runs. Both methods were shown to be accurate

Table 2  
Accuracy (%DEV), within-day precision (WDP) and between-day precision (BDP) of the total forms of CPT-11 and SN-38 in human plasma samples

Compound	Nominal conc. (ng/ml)	DEV (%)	Precision (%)	
			WDP	BDP
CPT-11	2.0	+6.75	14.3	4.25
	10	-7.87	3.38	3.08
	75	-2.60	2.27	2.35
	150	+2.93	1.75	2.46
	750	+0.28	1.75	2.22
SN-38	2.0	+0.32	12.4	<sup>a</sup>
	10	+9.57	2.52	2.89
	75	+9.90	2.56	2.62
	150	+12.2	2.08	2.79
	750	+7.98	1.58	2.78

<sup>a</sup> No additional variation was observed as a result of performing the assay in different runs.

(average accuracy at four tested concentrations was between -13.1 and +12.2%) and precise (between-day and within-day variation were within 15%). The mean overall extraction efficiencies were  $91.5 \pm 3.9\%$  (CPT-11 lactone;  $n=48$ ),  $87.5 \pm 8.7\%$  (SN-38 lactone;  $n=48$ ),  $85.3 \pm 5.3\%$  (CPT-11 total;  $n=47$ ),  $99.3 \pm 9.2\%$  (SN-38 total;  $n=47$ ) and  $88.1 \pm 5.1\%$  (CPT lactone, used as internal standard;  $n=48$ ) and were independent of the concentration. Data from the stability studies indicated that the lactone forms of CPT-11 and SN-38 were unstable after storage at room temperature or at 37°C. This instability necessitates rapid freezing of clinical samples after blood collection to prevent significant degradation into the carboxylate forms. Lactone and total forms of CPT-11 and SN-38 were stable for at least two months after storage at -80°C. Repeated quick freeze-thawing cycles ( $n=5$ ) had no influence on the stability. Processed plasma samples were also found to be stable at room temperature upon standing in the autosampler tray for at least 20 h, allowing samples to be processed during the day with consecutive analysis overnight.

### 3.3. Patient samples

The described analytical methods were applied in our institute to a phase I and pharmacokinetic study of CPT-11 in combination with cisplatin in patients with advanced solid cancer. Immediately after sampling, tubes were immersed briefly into ice-water kept at the bedside, and centrifuged immediately and stored at -20°C to prevent significant degradation of the sample. Plasma collected from patients prior to drug administration were free from interfering endogenous compounds. However, several chromatographic peaks could be detected in processed samples obtained from patients after drug administration that were not present in pre-infusion specimens (Fig. 2C and Fig. 3C). In addition to the lactone forms of CPT-11, SN-38 and CPT in Fig. 2C, and the total forms of CPT-11 and SN-38 in Fig. 3C (positively identified with the aid of pure reference standards), several other major peaks were detected at 3.9, 5.2, 5.5, 6.0, 7.4 and 9.9 min in the assay for the total forms (Fig. 3C). Treatment of the plasma sample displayed in Fig. 3C with  $\beta$ -glucuronidase resulted in the disappearance of the peaks at 3.9 and 7.4 min,

and a concomitant increase in the SN-38 peak area which is indicative of the presence of a  $\beta$ -glucuronide conjugate of SN-38 (data not shown) [24,25]. The identity of other chromatographic peaks remains to be elucidated. Similar HPLC chromatographic profiles were obtained with plasma specimens from other cancer patients.

Plasma concentration–time curves of the lactone and carboxylate forms of CPT-11 and SN-38 are displayed in Fig. 4. Concentrations of the lactone forms could be readily estimated from protein-free extracts after solvent extraction, whereas acidification of the samples, reconverting the carboxylate into the corresponding lactone, enabled determination of the total forms. The carboxylate forms of CPT-11 and SN-38 were quantitated from the difference in concentration of total and lactone forms. This indirect determination is justified as approximation of these concentrations and is defined by a combination of both methods, resulting in levels also within  $\pm 20\%$  of the nominal values. The observed three-exponential decline of the compounds, as well as the post-infusion predominance of the CPT-11 carboxylate form is in agreement with previous investigations [24,27]. The pharmacokinetic profile (e.g.

the terminal elimination half-life) of the as yet unidentified metabolites appeared to be similar to that of the parent drug (PdB and AS, unpublished data).

#### 4. Conclusion

Thoroughly validated analytical methods for the determination of CPT-11 and SN-38 in both their lactone and carboxylate forms in human plasma have been described. Although, over the last few years, various analytical procedures have been developed for the simultaneous determination of lactone and carboxylate forms of camptothecin analogues [22,28], clinical applicability of these assays is hampered by stability problems associated with lactone–carboxylate interconversion, which necessitates manual sample injection. In fact, our attempts to develop such a method for CPT-11 and SN-38 have been unsuccessful as a result of lack in stability, specificity and selectivity, and a poor extraction efficiency of the carboxylate forms. Ultimately, two methods were used in combination, with sample pretreatment procedures based on single

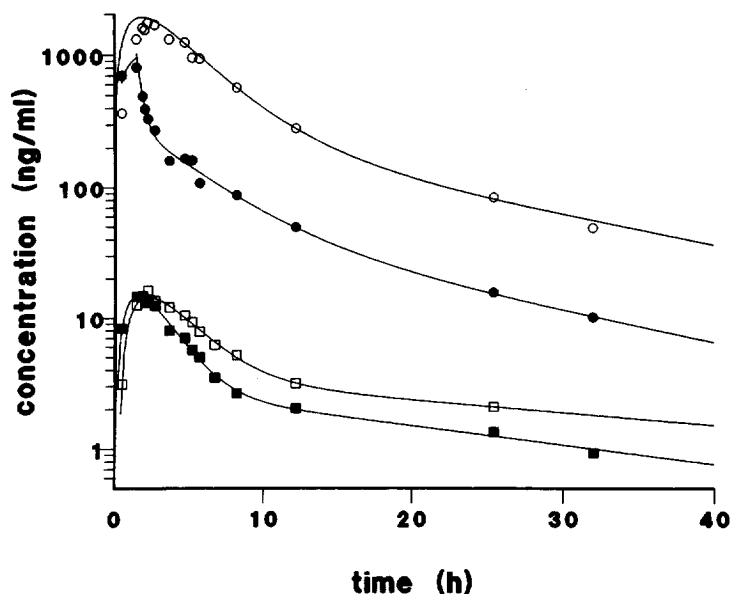


Fig. 4. Plasma concentration–time curves of CPT-11 lactone (closed circle), CPT-11 carboxylate (open circle), SN-38 lactone (closed square) and SN-38 carboxylate (open square) in a patient after a 90-min intravenous infusion of  $200 \text{ mg/m}^2$  of CPT-11. Pharmacokinetic curves were fitted to a three-compartment model using the MW\Pharm software package (MediWare, Groningen, The Netherlands).



solvent extractions. The resulting methodologies provide superior sensitivity as compared to previously described assays, with LLQs of 0.5 and 2.0 ng/ml for the lactone and total forms, respectively. Currently, elucidation of the structural identity and biochemical properties of the unknown metabolites is under further investigation.

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