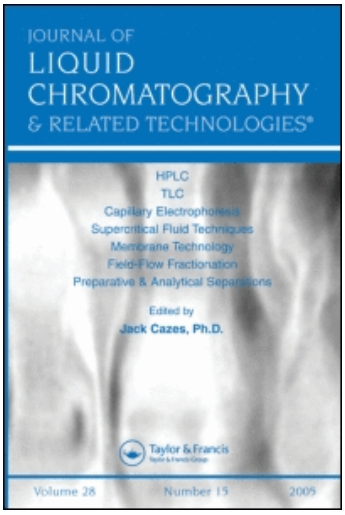

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Sensitive Determination of the Carboxylate and Lactone Forms of the Novel Antitumor Drug Irinotecan and Its Active Metabolite in Plasma by HPLC

V. M. M. Herben[a]; D. Mazee[a]; D. M. van Gortel-van Zomeren[a]; S. Zeedijk[a]; H. Rosing[a]; J. H. M. Schellens[a]; W. W. ten Bokkel Huinink[a]; J. H. Beijnen[a]

[a] Departments of Pharmacy, Pharmacology, and Medical Oncology Netherlands Cancer Institute/Slotervaart Hospital Louwesweg, Amsterdam, The Netherlands

To cite this Article Herben, V. M. M. , Mazee, D. , Zomeren, D. M. van Gortel-van , Zeedijk, S. , Rosing, H. , Schellens, J. H. M. , Huinink, W. W. ten Bokkel and Beijnen, J. H.(1998) 'Sensitive Determination of the Carboxylate and Lactone Forms of the Novel Antitumor Drug Irinotecan and Its Active Metabolite in Plasma by HPLC', Journal of Liquid Chromatography & Related Technologies, 21: 10, 1541 — 1558

To link to this Article: DOI: 10.1080/10826079808000533

URL: http://dx.doi.org/10.1080/10826079808000533

J. LIQ. CHROM. & REL. TECHNOL., 21(10), 1541–1558 (1998)

SENSITIVE DETERMINATION OF THE CARBOXYLATE AND LACTONE FORMS OF THE NOVEL ANTITUMOR DRUG IRINOTECAN AND ITS ACTIVE METABOLITE IN PLASMA BY HPLC

V. M. M. Herben, D. Mazee, D. M. van Gortel-van Zomeren, S. Zeedijk, H. Rosing, J. H. M. Schellens, W. W. ten Bokkel Huinink, J. H. Beijnen

Departments of Pharmacy, Pharmacology, and Medical Oncology
Netherlands Cancer Institute/Slotervaart Hospital
Louwesweg 6
1066 EC Amsterdam, The Netherlands

ABSTRACT

A sensitive high-performance liquid chromatographic (HPLC) method has been developed and validated for the determination of the lactone and carboxylate forms of the novel antitumor drug irinotecan (CPT-11) and its active metabolite SN-38 in plasma. The instability of the compounds required immediate bed-side protein precipitation of plasma samples with an ice-cold mixture of methanol and acetonitrile. These methanolic extracts could be stored at -70°C for at least 3 months without degradation of the analytes. Separation of the lactone and carboxylate forms of CPT-11 and SN-38 was achieved on a C_{18} reversed phase column with a mobile phase composed of a mixture of 0.1 M ammonium acetate, triethylamine, and acetonitrile (800:1:156, w/v/w) and 5 mM tetra-butyl ammonium phosphate. Detection was performed fluorimetrically. Within-run and between-run precision was always less than 11% in the

concentration ranges of interest (1.0-100 ng/mL and 0.5-25 ng/mL, for CPT-11 and SN-38, respectively). The method was successfully implemented in a phase I clinical and pharmacokinetic trial in patients treated with a 14-days low dose continuous infusion of CPT-11.

INTRODUCTION

Irinotecan [7-ethyl-10-[4-(1-piperidino]carbonyloxycamptothecin (CPT-11)], a semisynthetic analogue of the alkaloid camptothecin, is a novel antitumor drug with promising activity, *e.g.* in colorectal cancer. CPT-11 inhibits DNA replication and RNA transcription by stabilizing the cleavable complexes formed between the nuclear enzyme topoisomerase I and DNA.[1,2] *In vivo*, CPT-11 acts as a prodrug. After intravenous administration it is converted by carboxylate esterase enzymes to SN-38 (Figure 1),[3] which has stronger cytotoxic activity (100-to-1000-fold) than CPT-11 in *in vitro* models.[4,5] However, this biotransformation is relatively inefficient in man. Most of SN-38 undergoes subsequent conjugation mediated by hepatic uridine diphosphate glucuronyl-transferases to the corresponding glucuronide (SN-38G), which is relatively devoid of antitumor activity.[6] Part of the SN-38 glucuronide excreted in the bile is deconjugated by ß-glucuronidase in the intestinal microflora.

The dose-limiting toxicity of CPT-11 therapy is diarrhea, which is believed to be secondary to the biliary excretion of SN-38. It may be extremely severe and treatment with conventional antidiarrheal agents is not always effective. Marked interpatient differences in degree of side effects were observed, which may at least partly be explained by large interpatient variability in disposition of CPT-11. Peak plasma concentrations and area under the plasma concentration versus time curve (AUC) of CPT-11 and SN-38 were significantly correlated with the severity of neutropenia and diarrhea.[7-12]

All camptothecin analogues, including CPT-11 and SN-38, are unstable at physiologic pH. They are known to undergo a pH-dependent, reversible hydrolytic dissociation of the lactone function into a hydroxy carboxylate form[13,14] (Figure 2). At acidic pHs (pH<4), the lactone structure predominates. More alkaline pHs, including physiological pH, favour the formation of the carboxylate forms. Only the lactone forms are effective topoisomerase inhibitors; the carboxylate forms are inactive.[15] Tenfold differences in the lactone-to-total (lactone plus carboxylate forms) ratio of SN-38 have been reported.[16] Therefore, for a complete understanding of the pharmacology of CPT-11 and SN-38, a bio-analytical method in which the lactone and carboxylate forms can be measured separately is required.

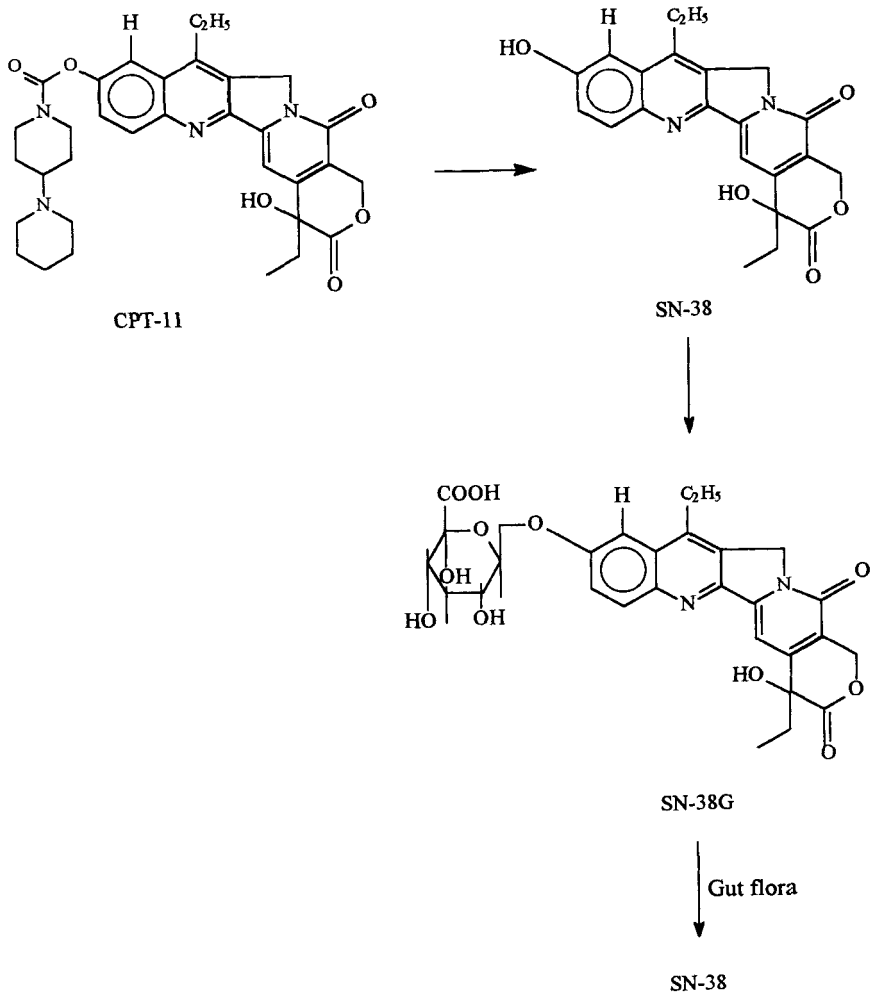

Figure 1. Chemical structures of CPT-11, its active metabolite SN-38 and the glucuronide conjugate of SN-38 (SN-38G).

Several methods have been described for the determination of CPT-11 and SN-38 using HPLC and fluorescence detection.[17-19] In general, however, these methods could only estimate concentrations as the total of lactone and carboxylate forms. To date, only Rivory et al. have reported an HPLC method for the measurement of the lactone and carboxylate forms of both compounds.[18]

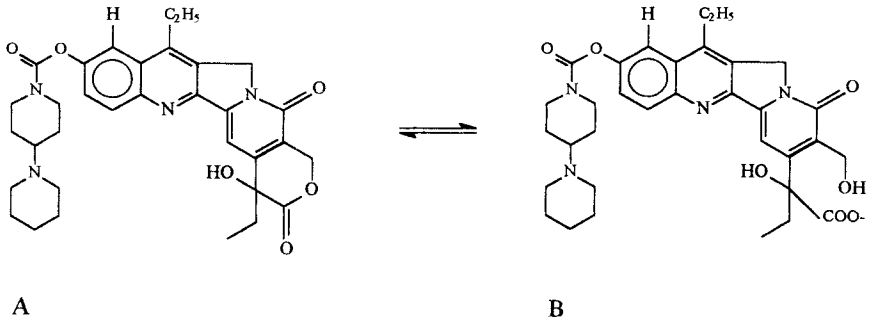

Figure 2. Schematic representation of the reversible hydrolysis of the lactone ring (A) into the ring-opened carboxylate form (B) of CPT-11.

This assay has been used in clinical pharmacokinetic studies, in which patients received dosages ranging from 300-500 mg/m^2 administered by a 30- or 90-minute infusion.[20] The lower limits of quantification in human plasma were 10 and 2 ng/mL for CPT-11 and SN-38, respectively.

In our Institute a phase I study exploring a 14-days continuous infusion schedule of CPT-11 was initiated, with dosages as low as 10 mg/m^2/day. It was therefore necessary to devise an HPLC method with improved sensitivity. This paper describes a sensitive and validated assay with a lower limit of quantification of 1.0 and 0.5 ng/mL for CPT-11 and SN-38, respectively, and reports preliminary pharmacokinetic results obtained during the phase I study of low dose CPT-11 infusion to demonstrate the applicability of the presented assay.

EXPERIMENTAL

Chemicals

CPT-11 (RP 64174-A), delivered as the hydrochloride salt, and SN-38 (RP 101645) were provided by Rhône-Poulenc Rorer (Centre de Recherche de Vitry-Alfortville, Vitry sur Seine cedex, France). Stock solutions of CPT-11 and SN-38 (1.0 μg/mL) were prepared in acetonitrile-0.01 M sodium tetraborate (pH~9) (50:50, v/v). Standard solutions were prepared by appropriate dilution with acetonitrile-0.01 M sodium tetraborate (pH~9) (50:50, v/v) for the carboxylate forms and acetonitrile-0.01 M citric acid (pH~2.5) (50:50, v/v) for the lactones. These procedures ensured quantitative conversion (>99%) in the carboxylates and lactones, respectively, as

verified by HPLC. The standard solutions were stored in 2.0 mL polypropylene tubes at 4°C and fresh solutions were prepared every 3 months. Camptothecin (CPT) was obtained from the National Cancer Institute (Bethesda, USA). Tetrabutylammonium phosphate (TBAP) was purchased from Waters Assoc. (Milford, MA, USA) as a ready-to-use solution (PIC-A®). Solvents and reagents were of analytical grade and double-distilled water was used.

Chromatography

The chromatographic system consisted of a solvent delivery system type 510 (Waters Assoc.) and an automatic sample injection device model ISS-100 (Perkin-Elmer, Norwalk, CT, USA), which was thermostatically cooled at 1°C for the determination of separate lactone and carboxylate forms of CPT-11 and SN-38. Separation of the compounds was performed on a Zorbax SB-C_{18} column (150 x 4.6 mm I.D.; particle size 3.5 μm) (Rockland Technologies Inc., Newport, DE, USA). To protect the analytical column, a guard column (10 x 3 mm I.D.) packed with reversed phase material (C_{18}) (Chrompack, Middelburg, The Netherlands) was used. The mobile phase consisted of a mixture of 0.1 M ammonium acetate (pH 6.4)-triethylammine-acetonitrile (800:1:156, w/v/w) to which one vial of PIC-A® solution was added. The final concentration of TBAP was 5 mM. The flow rate was 1.5 mL/min.

Detection was performed fluorimetrically using a FP-920 Intelligent Fluorescence Detector (Jasco International Co., Tokyo, Japan). The excitation wavelengths were set at 375 nm for CPT-11 and 385 nm for SN-38 and the emission wavelengths (bandwidth 40 nm) were set at 460 nm for CPT-11 and 525 for SN-38, in order to gain maximum response for each compound.

Sample Pretreatment

Lactone and carboxylate forms of CPT-11 and SN-38

During sample preparation, samples and solutions were constantly kept in ice-water. Blank human plasma (500 μL) was spiked with 50 μL of each appropriate standard lactone and carboxylate solution. Plasma proteins were precipitated with 900 μL of cold acetonitrile-methanol (1:1, v/v, -20°C). After vortex-mixing for 10 sec and centrifugation at 10,000 g for 3 min at 4°C, 70 μL of the clear supernatant was mixed with 70 μL of the mobile phase buffer (0°C). After brief vortex-mixing this solution was immediately transferred to an HPLC vial with insert and a volume of 20 μL was injected onto the HPLC column.

Total SN-38

For the determination of SN-38 as the total of lactone and carboxylate forms, blank human plasma (500 μL) was spiked with 100 μL of the appropriate standard carboxylate solution. Plasma proteins were precipitated with 900 μL of acetonitrile-methanol (1:1, v/v). After vortex-mixing for 10 sec and centrifugation at 10,000 g for 3 min, 70 μL of the clear supernatant was mixed with 70 μL 0.01 M sodium tetraborate (pH~9). After brief vortex-mixing this solution was transferred to an HPLC vial with insert and a 20 μL volume was injected onto the HPLC column.

Method Validation

Lactone and carboxylate forms of CPT-11 and SN-38

A three-run validation was completed for the determination of the separate lactone and carboxylate forms of CPT-11 and SN-38 in human plasma. The following parameters were determined: linearity, within-run and between-run precision, accuracy, specificity, selectivity, and absolute recovery. Seven plasma calibration standards were prepared and analyzed in singular, producing curves ranging from 1.0-100.0 ng/mL for CPT-11 and 0.5-25.0 ng/mL for SN-38. Linear least squares regression of the peak areas versus the concentration was performed with a weighting factor of $1/x$, the reciprocal of the concentration. The lack-of-fit test (α=0.05) was used to evaluate the linearity of the calibration curve.

Six replicates of quality control samples containing 1.0, 5.0, 50.0, and 100.0 ng/mL for CPT-11 and 0.5, 2.5, 10.0, and 25.0 ng/mL for SN-38 were prepared for three different runs with plasma calibration standards as described above to determine within-run and between-run precision and accuracy. The precision of the assay at each concentration was calculated as the coefficient of variation (%C.V.). The mean calculated concentration relative to the nominal concentration provided a measure of accuracy of the method.

Six batches of blank human plasma were processed and analyzed to test whether endogenous plasma constituents co-eluted with CPT-11 or SN-38 lactone and carboxylate forms. Co-administered drugs which may used by patients treated with CPT-11, like anti-emetics, benzodiazepines, coumarin derivatives, and paracetamol, were investigated for interference with the analytical method.

Absolute recovery from plasma was calculated by comparing the measured peak areas of plasma standards with peak areas of similarly prepared standards in which the plasma was replaced by an equal volume of distilled water. Recoveries were determined in three different runs.

Total SN-38

A three-run validation was completed for the determination of SN-38 as the total of the lactone and carboxylate forms in human plasma after complete conversion into the carboxylate form in a way identical as described in the previous section. Seven plasma calibration standards were prepared and analyzed in duplicate, producing curves ranging from 0.5-50.0 ng/mL. Quality control samples contained 0.5, 2.0, 10.0, and 50.0 ng/mL.

Stability

Lactone and carboxylate forms of CPT-11 and SN-38

The chemical stabilities of the lactone and carboxylate forms of CPT-11 and SN-38 in the methanol extract originating from spiked plasma were studied at two different ratios (lactone-to-carboxylate ratios of 1:3 and 3:1) during a period of 1 month at -30°C and 3 months at -70°C. Three replicates were analyzed at each time point. In addition, we re-analyzed extracts of patient plasma samples after 1 month of storage at -30°C.

The chemical stabilities of the lactone and carboxylate forms of CPT-11 and SN-38 in plasma methanol extracts kept in ice water and, prior to analysis, diluted in mobile phase buffer at -20°C and at 1°C in the autosampler were evaluated at a concentration of 100 ng/mL. Each of these samples contained CPT lactone as an indicator of lactone degradation. CPT was dissolved in acidified methanol and 50 μL (equal to 50 ng CPT) were added to 2.0 mL polypropylene tubes. The solution was evaporated under a nitrogen stream. In these tubes samples were prepared as described under Sample pretreatment.

Total SN-38

The chemical stability of SN-38 as the total of the lactone and carboxylate forms in alkalified methanol extracts during storage at room temperature and at 4°C has been studied at a concentration of 100 ng/mL.

Pharmacokinetics

The pharmacokinetics of CPT-11 and SN-38 were studied in a patient receiving daily doses of 10 mg/m^2 of CPT-11 (Campto®) over 14 days administered as a continuous infusion through an indwelling i.v. catheter and a portable device. The pump was filled once at the beginning of the cycle. Prior to the study the

stability of CPT-11 in the infusion fluid over 21 days was determined. Blood samples of 5 mL each were drawn pre-infusion and at various time points during infusion. The samples were collected in heparinized tubes (10 IU/mL) and immersed into icewater within 30 sec. Plasma was immediately isolated by centrifugation for 5 min at 3,000 g at 4°C. Subsequently, a volume of 500 μL of plasma was added to 1000 μL cold acetonitrile-methanol (1:1, v/v, -20°C).

After vortex-mixing for 10 sec and centrifugation at 10,000 g for 3 min at 4°C the supernatant was transferred to another tube with screw cap and briefly immersed into a dry ice/ethanol bath.

Samples were stored at -20°C and within 12 hours transferred to -70°C. Prior to analysis, the samples were placed in a -20°C freezer for 1 h, vortex-mixed and centrifuged briefly (10,000 g, 4°C) and put in icewater for 15 min. Aliquots of 70 μL of the supernatant were diluted with 70 μL of the mobile phase buffer (0°C). After brief vortex-mixing this solution was transferred to an HPLC vial with insert and a volume of 20 μL was directly injected onto the HPLC column.

Rivory et al. have shown that due to the addition of the ion-pairing agent TBAP to the mobile phase, potential metabolites other than SN-38 elute early in the chromatogram. One of these was identified as the ß-glucuronide of SN-38.[18] We determined SN-38G concentrations as the difference in total SN-38 levels before and after incubation of plasma samples with ß-glucuronidase.

To 100 μL of plasma, 500 units of ß-glucuronidase (type X-A from *Escherichia coli*, Sigma Chemical, St. Louis, USA) dissolved in 25 μL of a 25 mM phosphate buffer (pH 6.8) was added, followed by incubation at 37°C for 2 hours. Subsequently, 200 μL cold acetonitrile-methanol (1:1, v/v, -200C) was added to both incubated and freshly thawed plasma.

After vortex-mixing for 10 sec and centrifugation at 10,000 g for 3 min, 70 μL of the clear supernatant was mixed with 70 μL 0.01 M sodium tetraborate (pH~9). After brief vortex-mixing this solution was transferred to an HPLC vial with insert and a volume of 20 μL was injected onto the HPLC column.

Statistical Analysis

Statistical analysis was performed using the computer program SPSS® (Statistical Package for Social Sciences, version 6.1 for Windows).

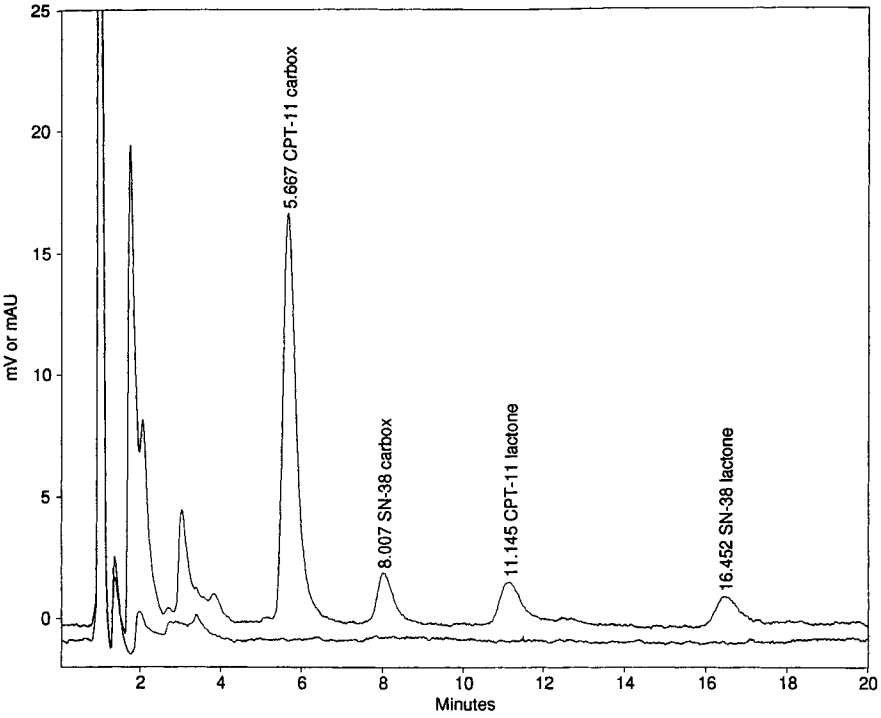

Figure 3. HPLC chromatograms of human plasma extracts obtained pre-infusion (lower line) and on the third day of a 14-days continuous infusion of CPT-11. The patient received an i.v. dose of 10 mg/m²/day. Fluorescence detection wavelengths were set at 385 nm (excitation) and 525 nm (emission).

RESULTS AND DISCUSSION

Chromatography and Detection

The selection of the wavelengths was based on scanning experiments of CPT-11 and SN-38 in the mobile phase buffer. Fluorescence intensities of SN-38 were very different from those of CPT-11 and CPT. Since very low concentrations were to be expected after the relative low doses administered to patients, it was decided to use the most favourable pair of wavelengths for each compound. Excitation and emission wavelengths were 375 and 460 nm for CPT-11 and 385 and 525 nm for SN-38, respectively. Figure 3 shows representative chromatograms of patient

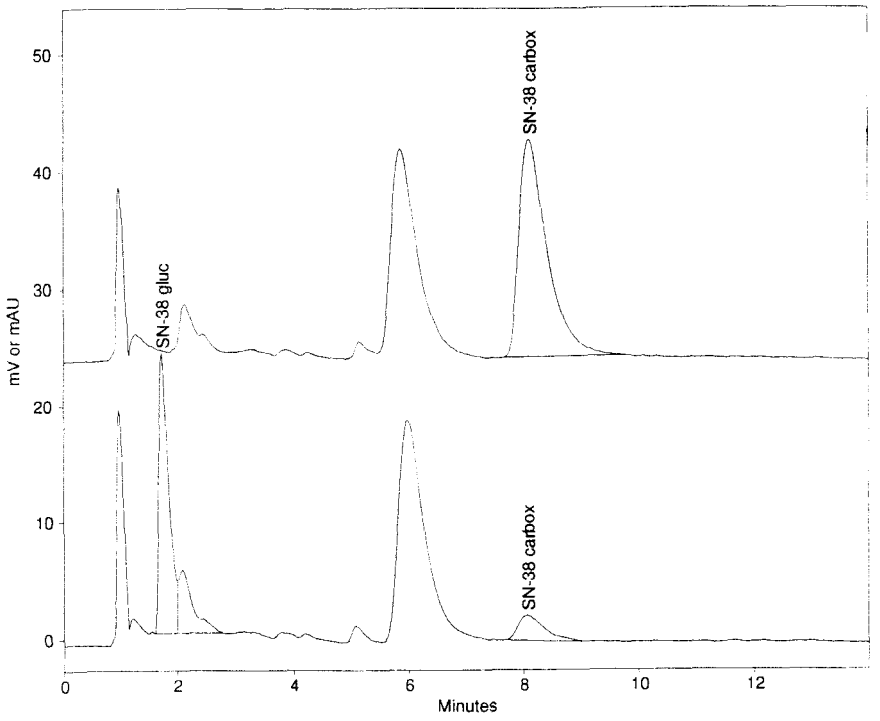

Figure 4. HPLC chromatograms of human plasma extracts with and without incubation (lower line) with ß-glucuronidase in a patient receiving an i.v. dose of CPT-11 10 mg/m²/day as a 14-days continuous infusion. Fluorescence detection wavelengths were set at 385 nm (excitation) and 525 nm (emission).

plasma extracts obtained pre-infusion and 3 days after the start of a 14-days continuous infusion of CPT-11. The retention times were 5.7 and 8.0 for the carboxylates and 11.1 and 16.5 for the lactones of CPT-11 and SN-38, respectively. The overall run time was 19 minutes. Alkalification of the samples with sodium tetraborate caused a quantitative conversion (>99%) of lactones into the carboxylate forms. Treatment of patient plasma samples with ß-glucuronidase resulted in almost complete disappearance of a chromatographic peak at 2 min and increase of the SN-38 peak (Figure 4).

In a previously described assay CPT was used both as internal standard and as an indicator of erroneous sample handling and storage.[17] Under our assay conditions the use of CPT lactone (100 ng/mL) as internal standard presented some difficulties. The peak of the carboxylate form of CPT appeared close to that of

SN-38 carboxylate in the chromatogram and interference of CPT carboxylate occurred at SN-38 concentrations lower than 2 ng/mL. The resolution of both compounds could be improved by increasing the amount of ammonium acetate in the mobile phase. However, this was associated with loss of sensitivity for SN-38 lactone as the increased run time resulted in peak broadening of the lactone species. Other camptothecin analogues, e.g. topotecan and lurtotecan (GI 147211) also proved to be unusable as internal standard under our assay conditions. Topotecan eluted with the solvent front whereas for lurtotecan the retention time was over 30 minutes and the fluorescence yield low. It was therefore decided to omit the internal standard.

Method Validation

Lactone and carboxylate forms of CPT-11 and SN-38

The validation procedure was in agreement with the conference report "Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies".[21] For the lactone and carboxylate forms of CPT-11 the assay was linear over a concentration range of 1.0-100 ng/mL in human plasma and correlation coefficients (r) of 0.999 or better were obtained. For every calibration curve the calibration concentrations were back-calculated from the peak areas. The deviation from the nominal concentration for all CPT-11 concentrations was less than 14%. For the lactone and carboxylate forms of SN-38 the assay was linear over a concentration range of 0.5-25 ng/mL in human plasma and correlation coefficients of 0.999 or better were obtained. For every calibration curve the calibration concentrations were back-calculated from the peak areas. The deviation from the nominal concentration for all SN-38 concentrations was less than 7%. The assay performance data for the determination of the lactone and carboxylate forms of CPT-11 and SN-38 are presented in Table 1. The average within-run precision and the between-run precision were less than 11% for all quality control samples. The average accuracies were within 94 and 120%. Chromatograms of six batches of blank human plasma samples contained no endogenous peaks co-eluting with CPT-11 or SN-38 lactone and carboxylate forms. No interferences with CPT-11 co-administered drugs were found. The extraction efficiencies of CPT-11 as the lactone and carboxylate form were 110.0 ± 6.0% and 106.7 ± 0.2%, respectively. For SN-38, the extraction efficiencies were 96.3 ± 6.0% and 94.5 ± 6.7%, for the lactone and carboxylate form, respectively.

Total SN-38

Total SN-38 concentrations were determined following the alkalification of the sample to convert all drug to the carboxylate form. Previous reports have used an

Table 1

Assay Performance Data for the HPLC Determination of CPT-11 and SN-38 in Plasma

Drug	Nominal conc. (ng/mL)	Lactone			Carboxylate		
		Within Run Precision (%)	Between run Precision (%)	Accuracy (%)	Within Run Precision (%)	Between Run Precision (%)	Accuracy (%)
CPT-11	1.0	7.0	10.6	114	8.0	8.0	108
	5.0	8.0	3.1	95	5.4	5.4	102
	50	4.4	1.4	94	4.6	3.0	106
	100	3.2	3.8	95	4.4	2.6	109
SN-38	0.5	4.8	4.0	108	6.2	8.2	99
	2.5	4.6	3.4	99	4.4	4.5	99
	10	4.2	2.1	102	2.7	1.1	102
	25	2.3	4.6	105	3.3	9.5	120

acidification procedure for the analysis of total CPT-11 and SN-38 levels.[17] We chose for alkalification in order to improve the peak shape and decrease the analysis time; the respective carboxylate and lactone forms of SN-38 elute at 8.0 and 16.4 minutes. For SN-38 as the total of lactone and carboxylate forms the assay was linear over a concentration range of 0.5-50.0 ng/mL in human plasma and correlation coefficients (r) of 0.998 or better were obtained. For every calibration curve the calibration concentrations were back-calculated from the peak areas. The deviation from the nominal concentration for all concentrations was less than 6%. The assay performance data for the determination of total SN-38 are presented in Table 2. The average within-run precision and the between-run precision were less than 12% for all quality control samples. The average accuracies were within 95 and 108%. The extraction efficiency was 97.9 ± 9.4%.

Stability

Lactone and carboxylate forms of CPT-11 and SN-38

The chemical stabilities of CPT-11 and SN-38 lactones, like all active camptothecin analogues, in biological matrices are limited. In methanolic extracts of plasma samples spiked with "watchdog" CPT lactone and kept in ice water, 6% of the analyte was converted into the carboxylate form within 60 minutes. A previously published study on the kinetics and mechanisms of lactonolysis demonstrated that at increased temperature the rate of conversion increases without

Table 2

Assay Performance Data for the HPLC Determination of SN-38 as the Total of Lactone and Carboxylate

Nominal Concn. (ng/mL)	Within-run Precision (%)	Between-run Precision (%)	Accuracy (%)
0.5	4.6	3.2	107
5.0	4.3	5.1	98
20	2.4	5.2	108
50	2.6	11.7	95

affecting the lactone-to-carboxylate ratio at equilibrium.[14] Therefore, it is of utmost importance that blood samples are immediately immersed into ice water at the bedside followed by centrifugation at 4°C and deproteinisation of the plasma by addition of cold methanol/acetonitrile. Protein-free plasma methanol extracts can be stored at -30°C for at least 5 days. The lactone-to-carboxylate ratio of CPT-11 and SN-38 was unchanged after 5 days and the conversion of CPT lactone into CPT carboxylate was less than 2%. However, CPT-11 appeared to degrade during prolonged storage at -30°C (Table 3). After 1 month the lactone-to-carboxylate ratio of CPT-11 in re-analyzed patient samples was changed from 0.28 to 0.37. SN-38 lactone in patient samples remained stable during this storage period, yet lactonolysis was evident in spiked plasma methanol extracts. The data for stability in plasma methanol extracts at -70°C are presented in Table 4. Both CPT-11 and SN-38 were stable for at least 3 months at -70°C.

Stability studies at -30°C in the diluted plasma methanol extracts, prior to analysis, indicated that both CPT-11 and SN-38 were stable for at least 3 h. Diluted plasma methanol extracts spiked with CPT were unstable in the autosampler at 1°C. After 25 minutes 5% of CPT lactone was converted to the carboxylate form. When the processed methanolic samples are taken from the freezer they should be analyzed immediately.

Total SN-38

SN-38 as the total of the lactone and carboxylate forms was stable in alkalified methanol extracts for at least 4 days when stored in the autosampler at room temperature or at 4°C.

Table 3

Stability Data of CPT-11 and SN-38 in Plasma Methanol Extracts at -30°C

Drug	Time (Months)	Lactone 25 ng/mL Carboxylate 75 ng/mL		Lactone 75 ng/mL Carboxylate 25 ng/mL		Patient Sample	
		C.V. (%)	Lactone-to Carboxylate Ratio	C.V. (%)	Lactone-to Carboxylate Ratio	C.V. (%)	Lactone-to Carboxylate Rateo
CPT-11	0	1.6	0.33	1.0	2.81	2.2	0.28
	0.25	8.8	0.31	2.7	2.54	n.d.	n.d.
	0.5	4.6	0.31	3.2	2.35	n.d.	n.d.
	1	5.3	0.30	2.8	2.21	1.4	0.37
SN-38	0	3.2	0.29	4.7	2.85	14.2	1.88
	0.25	2.9	0.28	8.7	2.30	n.d.	n.d.
	0.5	9.3	0.25	1.7	2.07	n.d.	n.d.
	1	6.8	0.23	3.1	2.00	10.5	1.88

C.V. = coefficient of variation, n=3; n.d. = no data.

Table 4

Stability Data of CPT-11 and SN-38 in Plasma Methanol Extracts at -70°C

Drug	Time (Months)	Lactone 25 ng/mL Carboxylate 75 ng/mL			Lactone 75 ng/mL Carboxylate 25 ng/mL		
		x (%)	C.V. (%)	Lactone-to-Carboxylate Ratio	*x* (%)	C.V. (%)	Lactone-to Carboxylate Ratio
CPT-11	0	100	2.6	0.33	100	1.9	2.85
	0.25	102	3.4	0.33	100	2.3	2.72
	0.5	101	2.9	0.33	100	4.0	2.83
	1	100	1.5	0.32	101	1.5	2.73
	2	100	2.6	0.32	103	5.9	2.69
	3	105	5.2	0.34	102	3.3	2.81
SN-38	0	100	1.8	0.29	100	2.8	2.82
	0.25	106	1.6	0.30	101	1.4	2.80
	0.5	102	2.1	0.28	98	1.1	2.66
	1	106	2.1	0.30	103	1.3	2.84
	2	109	2.2	0.30	105	2.1	2.77
	3	105	3.0	0.30	103	1.6	2.80

x = Concentration of lactone form as percentage of initial concentration; C.V. =coefficient of variation, n=3.

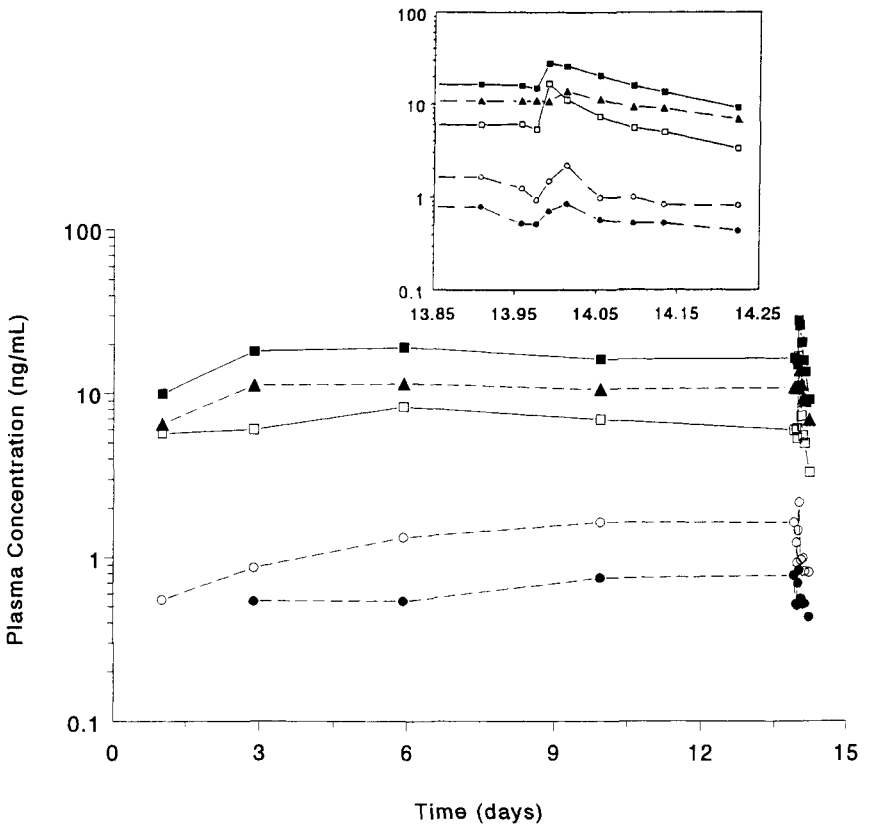

Figure 5. Representative plasma concentrations of the lactone (□ and ○) and carboxylate forms (■ and ●) of CPT-11 and SN-38, respectively, and total SN-38 glucuronide (▲) in a patient treated with 10 mg/m²/day of CPT-11 administered as a 14-days continuous infusion. Postinfusion levels are shown in inset.

Pharmacokinetics

We first determined the long-term stability of CPT-11 in the infusion fluid. After 24 days, CPT-11 was still present only in its lactone form. The pH of the solution was 3.7. Plasma concentration profiles of CPT-11 and SN-38 in a patient treated with 10 mg/m²/day as a continuous 14-days infusion are depicted in Figure 5. Drug levels remained constant throughout the infusion. 29% of CPT-11 was in the active lactone form, whereas for SN-38 the lactone form was the predominant form (67%).

Metabolite levels were on average 10-fold lower than corresponding CPT-11 concentrations. SN-38 was mainly present as the ß-glucuronide conjugate. Total plasma concentrations of SN-38 glucuronide were about 7-fold higher than those of SN-38.

CONCLUSION

We present a sensitive and validated assay for the quantification of the lactone and carboxylate forms of CPT-11 and SN-38 and demonstrated the applicability for pharmacokinetic evaluation of the drug in patients. To minimize the risk of analyte degradation, blood samples taken from patients treated with CPT-11 should be processed as quickly as possible. Stability data indicated that protein-free methanol extracts of plasma spiked with CPT-11 and SN-38 were stable at -70°C for at least 3 months. When methanol extracts were stored at -30°C, the lactone-to-carboxylate ratios of CPT-11 and SN-38 were changed. However, in processed patient samples SN-38 appeared stable for at least 1 month. We have also validated a sensitive method for the analysis of SN-38 as the total of lactone and carboxylate forms. Plasma methanol extracts were alkalified to convert all the drug into the carboxylate form. This method can be used for the estimation of SN-38 glucuronide concentrations from the increase in total SN-38 level following incubation of plasma samples with ß-glucuronidase.

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Received July 1, 1997
Accepted September 16, 1997
Manuscript 4535