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# Liquid chromatographic determination of irinotecan and three major metabolites in human plasma, urine and feces

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**Abstract**

A new simple reversed-phase high-performance liquid chromatographic method was developed for the determination of irinotecan (CPT-11) and three metabolites in human plasma, urine and feces homogenate. The metabolites of interest were 7-ethyl-10-hydroxycamptothecin (SN-38), its β-glucuronide derivative (SN-38G) and 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (RPR 121056A; also referred to as APC). Sample pretreatment from the various biological matrices involved a rapid protein precipitation with simultaneous solvent extraction of 250-μl aliquots of sample with 500 μl of methanol–5% (w/v) aqueous perchloric acid (1:1, v/v). Separation of the compounds was achieved on an analytical column packed with Hypersil ODS material (100×4.6 mm I.D., 5 μm), and isocratic elution with a mixture of methanol–0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid). The column effluent was monitored at excitation and emission wavelengths of 355 and 515 nm, respectively. Results from a 4-day validation study indicated that this single-run determination allows for simple, simultaneous and rapid quantitation and identification of all analytes with excellent reliability. The described procedure permits the analysis of patient samples, and will be implemented in future studies to investigate the complete metabolic fate and disposition of CPT-11 in cancer patients. 

*Keywords:* Irinotecan

## 1. Introduction

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; CPT-11) (Fig. 1) is a promising semisynthetic derivative of the topoisomerase-I poison 20-(*S*)-camptothecin with increased aqueous solubility [1–3]. Interesting response rates have been documented in patients with a variety of malignancies, including colorectal cancer, gynecologic cancers and refractory cervical cancer [4,5].

In recent years, the clinical pharmacokinetic behavior of CPT-11 has been the subject of intensive investigation. Along with these studies, the elucidation of the metabolic fate of CPT-11, its potential clinical therapeutic use, and its toxic properties were investigated. These studies revealed that CPT-11 is rapidly hydrolyzed by carboxylesterases to form the highly active metabolite 7-ethyl-10-hydroxy-

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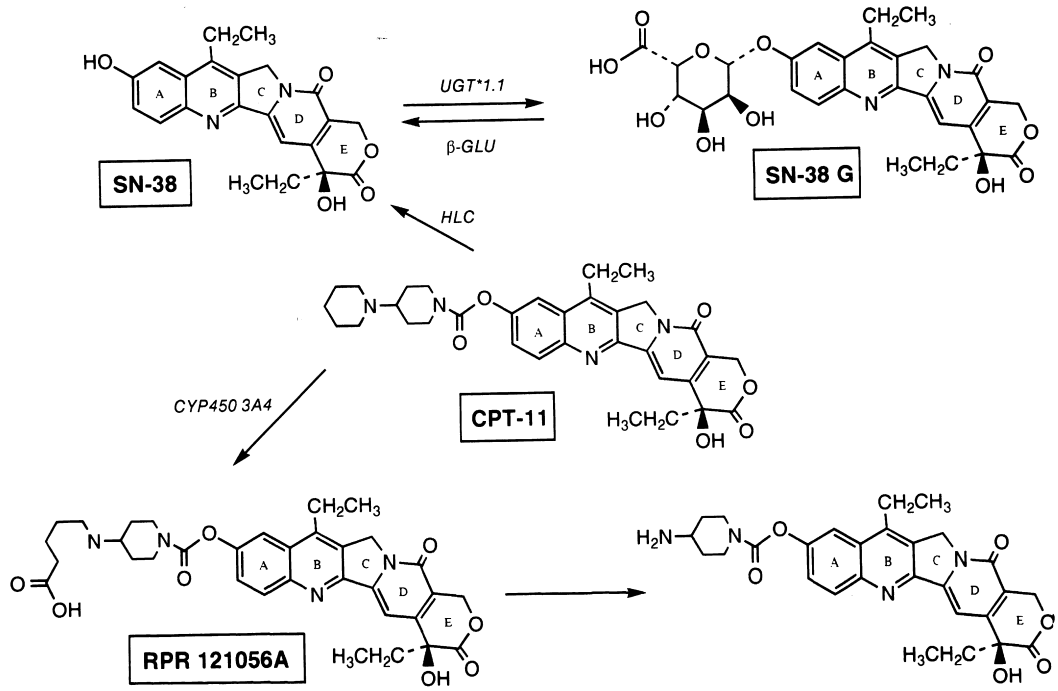


Fig. 1. Chemical structures of irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) and three major human metabolites: (a) SN-38 (7-ethyl-10-hydroxycamptothecin); (b) SN-38G (7-ethyl-10-[3,4,5,-trihydroxy-tetrahydropyran-2-carboxylic acid]camptothecin); (c) RPR 121056A (7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin; (d) 7-ethyl-10-[4-(1-piperidino)-1-amino]carbonyloxycamptothecin (obtained from [28]). Abbreviations: UGT*1.1, uridine diphosphate glucuronosyltransferase isoform 1A1; β-GLU, human intestinal microflora β-glucuronidase; HLC, human liver carboxylesterase; CYP450 3A4, cytochrome *P* 450 isoform 3A4.

camptothecin (SN-38) [6–11], which is subsequently conjugated by liver uridine-diphosphate glucuronosyltransferase (isoform 1A1) to an inactive β-glucuronide derivative (SN-38G) [12–18]. Another pathway of CPT-11 metabolism consists of an oxidative attack at the terminal piperidine group on the $C_{10}$-side chain, presumably mediated by cytochrome *P* 450 3A, generating a major metabolite that was recently identified as 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (RPR 121056A) [19,20]. Although this compound was demonstrated to have weak inhibitory activity of cell growth in vitro, the potential contribution of this and other metabolites to biological effects is still unknown [19,21].

Preliminary examination of elimination pathways for CPT-11 have indicated that less than 50% of the delivered dose is excreted unchanged into the urine and bile, with a minor contribution from both SN-38 and SN-38G [15]. Hence, establishment of a complete mass balance for this drug, and determination of the metabolic fate and disposition of CPT-11 in humans is urgently required. To facilitate such studies, we now report on development, validation and use of a reversed-phase high-performance liquid chromatographic (HPLC) method for the quantitative determination of CPT-11 and its major metabolites in human plasma, urine and feces. The method is a modification of our previously reported procedure applied for the analysis of CPT-11 lactone plus CPT-11 carboxylate levels in plasma, and involves a rapid and highly selective protein precipitation step for sample clean-up [22]. A pilot study in a cancer patient receiving the drug by a 90-min intravenous

infusion was included to confirm the suitability of the method for clinical use.

## 2. Experimental

### 2.1. Chemicals and reagents

CPT-11 hydrochloride (batch: KO16) and reference standards of its metabolites SN-38 hydrochloride (batch: LIE783), SN-38G trifluoroacetate (batch: YEO265) and RPR 121056A hydrochloride (batch: EBO1143 [23]) were supplied by Dr. L. Vernillet (Rhône-Poulenc Rorer, Vitry-sur-Seine Cedex, France). A purity of ≥94.0% for each compound was confirmed by analytical HPLC as described in [22]. All other chemicals and HPLC solvents were of the highest grade available commercially. Milli-Q-UF quality water was used throughout (Millipore, Milford, MA, USA). Blank specimens of human plasma, urine and feces were obtained from healthy volunteers.

### 2.2. Sample extraction

Frozen plasma or urine samples were thawed in a waterbath and were homogenized by vortex-mixing. Next, 250 μl aliquots of plasma or 250 μl of plasma-diluted urine (1:1, v/v) were transferred to clean polypropylene microtubes (Eppendorf, Hamburg, Germany) containing 500 μl of methanol–5% (w/v) aqueous perchloric acid (1:1, v/v). The tubes were capped and mixed for 5 min on a multitube mixer, and then centrifuged at 24 000 *g* for 5 min. The upper aqueous layer from plasma and urine extracts was diluted 2-fold and 10-fold, respectively, in methanol–0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid). Finally, diluted extracts were transferred to limited-volume inserts, and 100 to 200-μl aliquots were subjected to chromatography. Feces specimens were accurately weighed and homogenized in 5 volumes of 5% (w/v) perchloric acid in water using five 1-min bursts of an Ystral X1020 tissue homogenizer (Dottingen, Germany) operating at 20 500 r.p.m. Next, aliquots of the feces homogenate were centrifuged at 24 000 *g* for 10 min, the resulting supernatant diluted with one volume of drug-free human plasma, and processed further as described for the urine samples.

### 2.3. Equipment and chromatographic conditions

Chromatographic analyses were performed using a constraMetric 4100 pump, an autoMetric 4100 autosampler and a fluoriMonitor 4100 fluorescence detector (LDC Analytical, Rivera Beach, USA). The analytical column used was packed with Hypersil ODS material (100×4.6 mm I.D., 5 μm) from LC Service (Emmen, The Netherlands), and protected by a LiChroCart 4-4 endcapped (RP-18) precolumn from Merck (Darmstadt, Germany). The column temperature was maintained at 50°C by using a Spark Holland Model SpH99 HPLC-column oven (Meppel, The Netherlands). The fluorescence detector operated at excitation and emission wavelengths of 355 nm and 515 nm, respectively, which yielded the optimum signal-to-noise ratio for all compounds. The mobile phase consisted of a mixture of methanol–0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid), and was delivered at a flow-rate of 1.0 ml/min. The mobile phase was filtered and degassed before use, and was freshly prepared for each run.

### 2.4. Calibration

Separate standard stock solutions of CPT-11 and metabolites were prepared at 1.0 mg/ml in dimethyl sulfoxide (DMSO) and were stored in polypropylene at −80°C. A mixture of all five compounds as the free lactone forms was obtained in methanol–0.01 *M* hydrochloric acid (40:60, v/v) by dilution of the standard solutions in DMSO, yielding a final concentration of 20 or 1000 μg/ml for each standard. This solution was subsequently diluted serially with an appropriate volume of methanol–0.01 *M* hydrochloric acid (40:60, v/v) to obtain 1-ml aliquots. Plasma, urine and feces homogenate standards were prepared on the day of sample analysis by transferring 25 μl of the diluted stock mixtures into 975 μl of the biological matrix (i.e. plasma, urine or feces homogenate) to prepare a series of standards ranging

from 2 to 200 ng/ml in plasma, or 100 to 5000 ng/ml in urine and feces homogenate. Acquisition and integration of HPLC data was performed with the Chrom-Card data analysis system (Fisons, Milan, Italy), running on an ICW chromatographic workstation. Calibration curves were made by linear least-squares regression analysis of peak areas versus $1/(\text{nominal concentration})^2$ using the LOTUS v2.4 software package (Lotus Development Corporations, New York, NY, USA).

### 2.5. Method validation

Method validation was performed according to the guidelines recorded in the conference report [25], with minor modifications as described previously [22]. All validation runs were performed on three (urine and feces) or four (plasma) consecutive days, and included a calibration curve processed in duplicate, and sets of quality control (QC) samples in quintuplicate analyzed with repeated freezing and thawing. QC samples were prepared in all biological matrices at concentrations which fell within all four quartiles of the corresponding standard curve range. The accuracy (or percent deviation from the nominal concentration) and intra- and inter-assay (or within-day and between-day) precision were calculated by one-way analysis of variance (ANOVA) using the NUMBER CRUNCHER STATISTICAL SYSTEM v5.0 package (Dr J.L. Hinze, East Kaysville, USA).

### 2.6. Patient samples

The patient studied participated in a clinical Phase I and pharmacokinetic study of CPT-11 in combination with the anticancer drug cisplatin, in various nonhematological malignancies [24]. The CPT-11 dose of 300 $mg/m^2$ was delivered as a 90-min intravenous infusion, followed by a 3-h intravenous infusion of cisplatin at 80 $mg/m^2$, with treatment cycles repeated every 3 weeks. The protocol was approved by the Institutional Review Board of the Rotterdam Cancer Institute (Rotterdam, The Netherlands) and written informed consent was obtained prior to treatment.

Blood samples were collected in lithium heparin containing glass tubes before and during the 90-min CPT-11 infusion (0.5 h, 1 h and 1 h 25 min after initiation), and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 11, 24, 32 and 48 h after the end of the CPT-11 infusion. The samples were centrifuged instantaneously for 5 min at 3000 *g* at ambient temperature, and the plasma supernatant was snap-frozen at −80°C. The renal and hepato-biliary excretion of CPT-11 and metabolites was studied by obtaining complete collections of urine and stools for the duration of the study (48 h). Urine samples were diluted immediately with one volume of drug-free human plasma, and 1.0-ml aliquots were kept frozen in polypropylene microtubes (Eppendorf). After collection, feces specimens were stored instantly at −80°C until later analysis.

## 3. Results and discussion

### 3.1. Analytical procedure

The reversed-phase HPLC method in this study had been developed initially for the quantitative determination of CPT-11 and SN-38 in human plasma samples [22]. Our data from stability studies indicated that lactone forms of CPT-11 and SN-38 were unstable after storage at room temperature or at 37°C [22], necessitating rapid freezing of clinical samples after blood collection to prevent continued hydrolysis to the carboxylate forms. In view of the limited clinical applicability of methods based on estimation of lactone levels only, total (i.e. lactone plus carboxylate forms) concentrations of the drug and its metabolites were measured in the present study instead.

Using our previous HPLC method, several major chromatographic peaks in addition to CPT-11 and SN-38 were detected in processed samples obtained from patients after CPT-11 treatment that were not present in preinfusion specimens. One of these peaks was determined to be a β-glucuronide conjugate of SN-38 (SN-38G), a known metabolite of CPT-11 [14,15], based on treatment of the sample with β-glucuronidase. In the present study, we observed that the remaining unknown peak in our clinical plasma samples could be identified with the aid of pure reference standards as 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin

(RPR 121056A; also referred to as APC [19,23]; Fig. 1).

Sample pretreatment of total drug forms in plasma was initially performed by protein precipitation using a mixture of 5% (w/v) perchloric acid–methanol (1:1, v/v), with direct injection of the supernatant into the HPLC system. This sample handling, however, resulted in an unusual chromatographic behavior of compounds RPR 121056A and SN-38G, with separation into two peaks of comparable peak-area proportion (data not shown). As suggested by Rivory and Robert, this artefact may relate to self-association of the carboxylate forms of the drugs with dimerization due to the formation of hydrogen bonds between carboxyl functions in organic solvents [26]. Peak separation of compounds RPR 121056A and SN-38G was efficiently removed by a 2-fold dilution of the plasma extract prior to chromatography in a mixture of methanol–0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (30:70, v/v), pH 5.3 (hydrochloric acid). This sample handling was also chosen for its optimal elimination of endogenous interference, while maintaining a high extraction efficiency for all compounds.

In order to ensure sufficient selectivity and analyte separation in our new assay, we have slightly modified the mobile phase composition as compared to the earlier method by decreasing the organic modifier content from 35 to 30%. This change in mobile phase, however, resulted in poor accuracy and precision for the two late-eluting compounds (CPT-11 and SN-38), due to severe tailing bands (asymmetry factor ($A_s$)$\geq$2.0), arising from secondary retention effects on the reversed-phase column. This chromatographic distortion was particularly evident at the low end of plasma-calibration curves (i.e. below 100 ng/ml). Although this problem could be overcome in part by the use of a (less retentive) gradient elution, we noticed the occurrence of severe baseline drift during the chromatographic run. Therefore, concentrations of CPT-11 and SN-38 in plasma

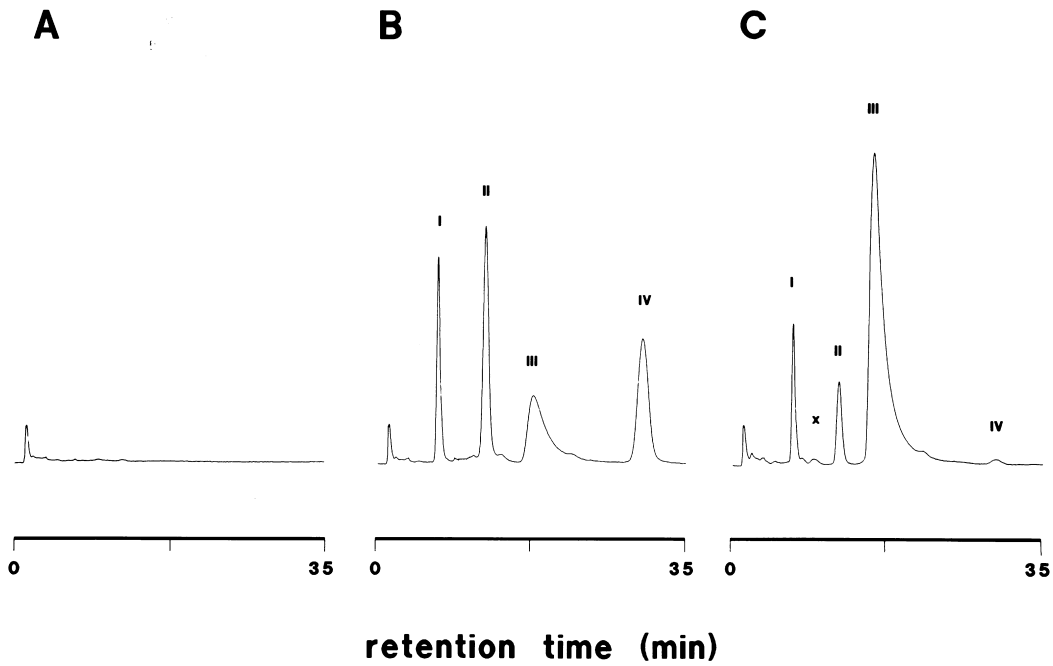


Fig. 2. HPLC chromatograms of (A) a blank human plasma sample (B) a spiked human plasma sample (200 ng/ml of each compound) and (C) a patient plasma sample taken 30 min after i.v. infusion of CPT-11 at a dose level of 300 mg/m$^2$. Based on chromatographic behavior and spectrometric properties relative to pure reference standards, peaks labeled I, II, III, and IV were identified as SN-38G ($t_R$=7.08 min), RPR 121056A ($t_R$=12.7 min), CPT-11 ($t_R$=17.9 min) and SN-38 ($t_R$=29.8 min), respectively. The peak labeled x ($t_R$=9.52 min) has not yet been formally identified and characterized.

were quantitated after reinjection of the sample using the previously developed mobile phase [22].

Modification of our plasma assay for urine samples was easily achieved by addition of the unknown specimens to drug-free human plasma and further processing as if they were human plasma samples. In order to allow convenient sample-handling of fecal specimens, several homogenization and extraction procedures were evaluated. Our preliminary data indicated that upon standing at room temperature, SN-38G was highly unstable in feces homogenated in (weakly acidic) aqueous media. The disappearance of SN-38G in such matrices was completed within 1 h (data not shown), and was accompanied by a concomitant increase in the SN-38 peak area, suggesting sustained activity by β-glucuronidase from the intestinal microflora present in feces. The procedure reported by Li and Zhang [27] for measurement of 10-hydroxycamptothecin in feces, based on EDTA and proteinase K treatment of the sample at 37°C, also proved unfavorable. Among various other procedures tested, homogenization and simultaneous extraction with 5% (w/v) perchloric acid was shown to result in the optimal combination of sufficient sample stability and acceptable analyte recovery (see below).

Criteria for selection of the reversed-phase analytical column and the fluorescence-detection wavelength couple were as described previously [22].

### *3.2. Method validation*

Chromatograms of blank and spiked human plasma, urine and feces homogenate samples are shown in Figs. 2–4. The selectivity for the analytes is shown by the sharp and symmetrical resolution of the peaks, with no significant interfering peaks for all compounds in drug-free specimens, obtained from five different individuals. The retention times for SN-38G, RPR 121056A, CPT-11 and SN-38 were 6.95±0.21, 11.8±1.27, 16.1±2.55 and 28.6±1.77 min, respectively, with the overall chromatographic

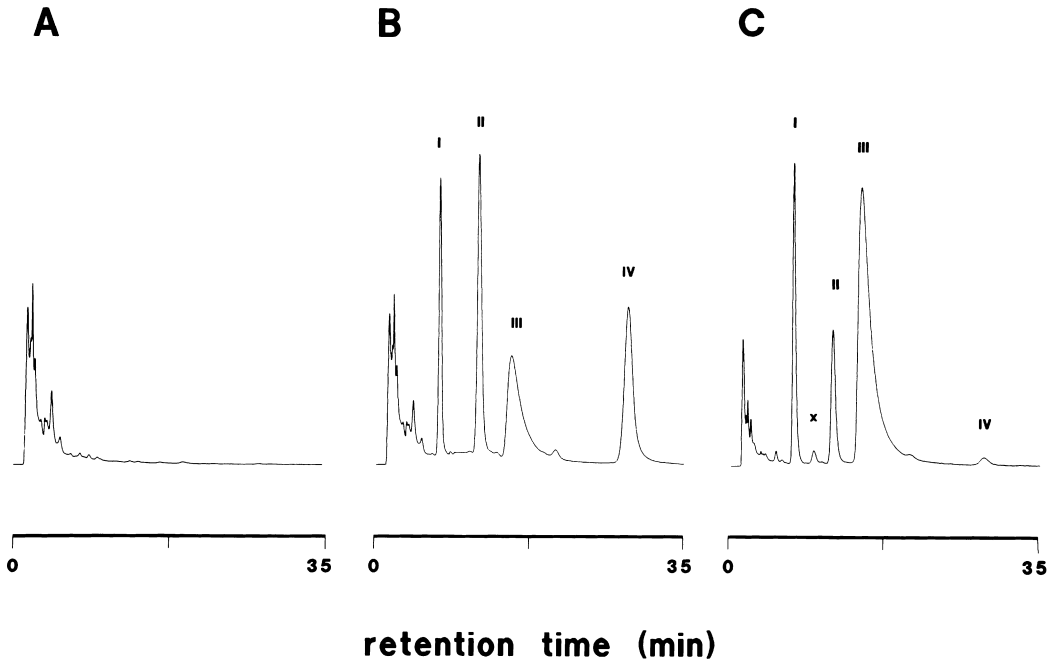


Fig. 3. HPLC chromatograms of (A) a blank human urine sample (B) a spiked human urine sample (1000 ng/ml of each compound) and (C) a patient urine sample collected during the first 0–5 h after i.v. infusion of CPT-11 at a dose level of 300 mg/m$^2$. Based on chromatographic behavior and spectrometric properties relative to pure reference standards, peaks labeled I, II, III, and IV were identified as SN-38G ($t_R$=6.83 min), RPR 121056A ($t_R$=10.9 min), CPT-11 ($t_R$=14.3 min) and SN-38 ($t_R$=27.3 min), respectively. The peak labeled x ($t_R$=8.82 min) has not yet been formally identified and characterized.

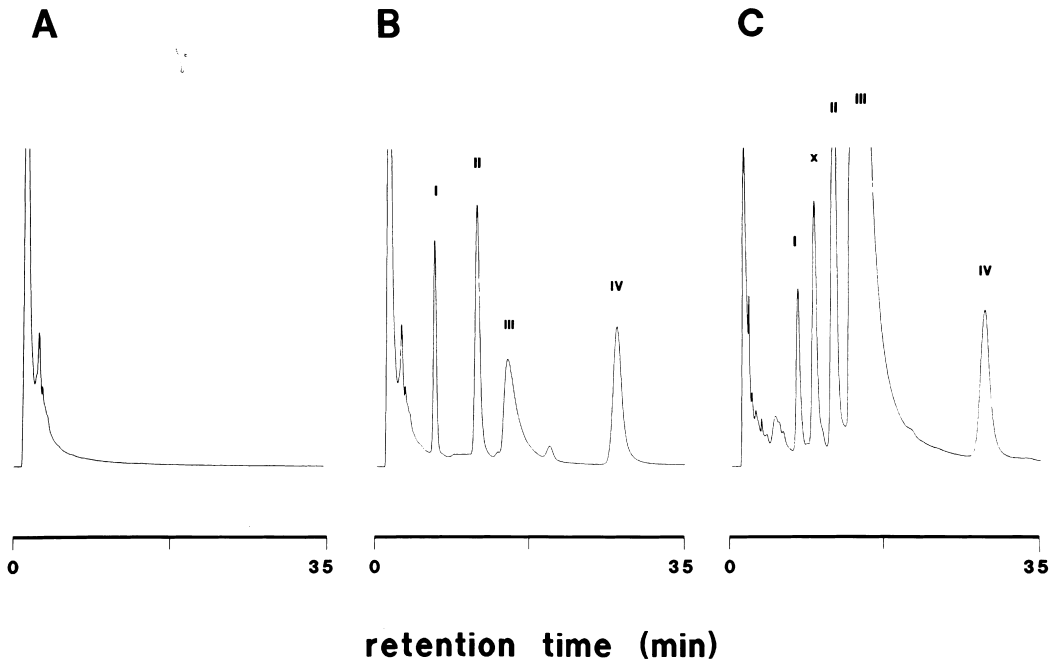


Fig. 4. HPLC chromatograms of (A) a blank human feces sample (B) a spiked human feces sample (5000 ng/ml of each compound) and (C) a patient feces sample collected during the first 0–5 h after i.v. infusion of CPT-11 at a dose level of 300 mg/m$^2$. Based on chromatographic behavior and spectrometric properties relative to pure reference standards, peaks labeled I, II, III, and IV were identified as SN-38G ($t_R$=6.92 min), RPR 121056A ($t_R$=11.1 min), CPT-11 ($t_R$=14.4 min) and SN-38 ($t_R$=26.9 min), respectively. The peak labeled x ($t_R$=9.08 min) has not yet been formally identified and characterized.

run time established at 35 min. Applying the peak height in combination with a weight factor of $1/x^2$, linear calibration curves were obtained over the concentration ranges tested for all compounds (except CPT-11 and SN-38 in plasma), i.e. 10-400 ng/ml for plasma ($r>0.998$), and 100–5000 ng/ml for urine ($r>0.998$) and feces homogenate ($r>0.9991$).

Validation data of the analytical methods in terms of accuracy (percent deviation) and precision for spiked plasma, urine and feces samples are shown in Tables 1–3, respectively. The accuracy for all analytes showed values ranging within ±12.2% (plasma), ±16.9% (urine) and ±15.1% (feces) of the nominal values. The intra- and inter-assay variability as assessed by one-way ANOVA varied up to 14.3% and 7.98%, respectively, in the various biological matrices (Tables 1–3).

The extraction recovery for each compound was determined at six different concentrations in three (urine and feces) or four (plasma) analytical runs by comparing peak areas of samples prepared in the appropriate biological matrix with those for non-processed samples prepared in the mobile phase. The mean overall extraction efficiency for CPT-11 were 85.3±5.3% in plasma, 89.2±12.4% in urine and 101.5±9.3% in feces homogenate. Recoveries observed for the metabolites ranged between 82.6 and 113.6% (Table 4), and were not significantly different from CPT-11 (Student's *t*-test, $P>0.05$), suggesting that concentrations of these compounds might be estimated using the CPT-11 calibration curve in the absence of pure reference compounds, after correcting for differences in the molar extinction coefficient.

### *3.3. Pharmacokinetic studies*

The suitability of the developed methods for clinical use was demonstrated by the determination of CPT-11 and its metabolites in biological specimens obtained from a 58-year-old male with ad-

Table 1
HPLC validation characteristics of CPT-11 and three major metabolites in human plasma samples

| Compound | Nominal (ng/ml) | $n$[a] | Observed (ng/ml) | Deviation (%) | Precision (%) | |
|---|---|---|---|---|---|---|
| | | | | | Within-day | Between-day |
| SN-38G | 10 | 20 | 10.16 | +1.55 | 8.60 | [b] |
| | 25 | 20 | 25.98 | +3.93 | 1.69 | 1.75 |
| | 150 | 20 | 150.0 | ±0.00 | 1.47 | 2.53 |
| | 300 | 20 | 295.5 | −1.49 | 4.76 | [b] |
| RPR 121056A | 10 | 20 | 10.16 | +1.57 | 5.89 | 4.88 |
| | 25 | 20 | 26.86 | +7.45 | 1.65 | 6.27 |
| | 150 | 20 | 156.9 | +4.58 | 1.64 | 6.48 |
| | 300 | 20 | 312.9 | +4.29 | 2.57 | 6.14 |
| CPT-11[c] | 2 | 20 | 2.135 | +6.75 | 14.3 | 4.25 |
| | 10 | 20 | 9.213 | −7.87 | 3.38 | 3.08 |
| | 75 | 19 | 73.01 | −2.60 | 2.27 | 2.35 |
| | 150 | 20 | 155.4 | +2.93 | 1.75 | 2.46 |
| | 750 | 20 | 752.1 | +0.28 | 1.75 | 2.22 |
| SN-38[c] | 2 | 20 | 2.006 | +0.32 | 12.4 | [b] |
| | 10 | 20 | 10.96 | +9.57 | 2.52 | 2.89 |
| | 75 | 19 | 82.43 | +9.90 | 2.56 | 2.62 |
| | 150 | 20 | 168.3 | +12.2 | 2.08 | 2.79 |
| | 750 | 20 | 809.9 | +7.98 | 1.58 | 2.78 |

[a] Number of replicate observations of each concentration in four separate validation runs.
[b] No additional variation was observed as a result of performing the assay in different runs.
[c] Data from De Bruijn et al. [22]; mobile phase composition: methanol–0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (35:65, v/v).

Table 2
HPLC validation characteristics of CPT-11 and three major metabolites in human urine samples

| Compound | Nominal (ng/ml) | $n$[a] | Observed (ng/ml) | Deviation (%) | Precision (%) | |
|---|---|---|---|---|---|---|
| | | | | | Within-day | Between-day |
| SN-38G | 100 | 14 | 99.61 | −0.39 | 13.6 | [b] |
| | 400 | 15 | 404.9 | +1.22 | 2.10 | 3.47 |
| | 2500 | 15 | 2520 | +0.79 | 1.40 | 1.18 |
| | 4500 | 15 | 4444 | −1.25 | 4.83 | 1.53 |
| | 4500[c] | 15 | 4358 | −3.16 | 5.08 | 2.39 |
| RPR121056A | 200 | 14 | 204.7 | +2.37 | 4.51 | 4.17 |
| | 400 | 15 | 401.4 | +0.35 | 0.94 | 2.50 |
| | 2500 | 15 | 2533 | +1.31 | 1.34 | 2.60 |
| | 4500 | 15 | 4597 | +2.17 | 4.64 | 2.29 |
| | 4500[c] | 15 | 4313 | −4.16 | 1.98 | 3.14 |
| CPT−11 | 200 | 14 | 200.1 | +0.06 | 6.82 | 6.91 |
| | 400 | 15 | 379.8 | −5.06 | 4.78 | 4.12 |
| | 2500 | 15 | 2543 | +1.74 | 1.82 | 2.11 |
| | 4500 | 15 | 4819 | +7.09 | 4.34 | 2.78 |
| | 4500[c] | 15 | 4141 | −7.97 | 2.98 | 2.57 |
| SN-38 | 100 | 14 | 98.76 | −1.24 | 6.08 | 7.38 |
| | 400 | 15 | 418.5 | +4.61 | 1.32 | 7.98 |
| | 2500 | 15 | 2497 | −0.11 | 1.08 | 0.99 |
| | 4500 | 15 | 4514 | +0.31 | 0.90 | 2.12 |
| | 4500[c] | 15 | 4238 | −5.82 | 5.76 | 9.70 |

[a] Number of replicate observations of each concentration in three separate validation runs.
[b] No additional variation was observed as a result of performing the assay in different runs.
[c] Quality control sample diluted 10-fold prior to chromatography.

Table 3
HPLC validation characteristics of CPT-11 and three major metabolites in homogenized human feces samples

| Compound | Nominal (ng/ml) | $n$[a] | Observed (ng/ml) | Deviation (%) | Precision (%) | |
|---|---|---|---|---|---|---|
| | | | | | Within-day | Between-day |
| SN-38G | 100 | 15 | 99.19 | −0.81 | 6.36 | 5.49 |
| | 400 | 15 | 392.1 | −1.97 | 1.24 | 1.58 |
| | 2500 | 15 | 2557 | +2.27 | 1.50 | 2.07 |
| | 4500 | 15 | 4637 | +3.05 | 1.36 | 2.00 |
| | 4500[c] | 15 | 4568 | +1.51 | 1.32 | 0.98 |
| RPR121056A | 200 | 15 | 202.1 | +1.04 | 1.73 | 2.20 |
| | 400 | 15 | 403.8 | +0.95 | 0.97 | 0.46 |
| | 2500 | 15 | 2604 | +4.14 | 1.27 | 1.41 |
| | 4500 | 15 | 4796 | +6.59 | 1.02 | 1.93 |
| | 4500[c] | 15 | 4341 | −3.54 | 1.17 | 1.92 |
| CPT-11 | 200 | 15 | 200.4 | +0.22 | 5.81 | 4.20 |
| | 400 | 15 | 408.3 | +2.08 | 2.59 | 1.96 |
| | 2500 | 15 | 2732 | +9.26 | 1.76 | 0.99 |
| | 4500 | 15 | 5179 | +15.1[b] | 1.28 | 2.37 |
| | 4500[c] | 15 | 3953 | −12.2 | 4.48 | 3.51 |
| SN-38 | 100 | 15 | 105.3 | +5.31 | 6.37 | 2.23 |
| | 400 | 15 | 416.8 | +4.20 | 1.93 | 0.99 |
| | 2500 | 15 | 2654 | +6.15 | 0.96 | 2.80 |
| | 4500 | 15 | 4805 | +6.78 | 1.11 | 2.45 |
| | 4500[c] | 15 | 4300 | −4.45 | 2.09 | 1.23 |

[a] Number of replicate observations of each concentration in three separate validation runs.
[b] Percent deviation outside the acceptable 85–115% range for accuracy [25].
[c] Quality control sample diluted 10-fold prior to chromatography.

vanced gastric adenocarcinoma, treated with CPT-11 at a dose level of 300 mg/m$^2$. Preliminary analyses were carried out in duplicate in the presence of both blank and spiked plasma, urine and feces samples. Examples of the patient's sample trace are shown in Fig. 2C (plasma), Fig. 3C (urine) and Fig. 4C (feces). Distinct peaks were obtained for CPT-11 and the three metabolites in all matrices, with a minor unknown metabolite (designated x) detectable in urine, feces and eight out of the seventeen plasma samples assayed. The plasma concentration versus time profiles for the various compounds are shown in Fig. 5. All pharmacokinetic curves could be fitted to a triexponential equation using the SIPHAR v4.0 software package (Simed, Creteil, France), assuming a three-compartment model for the distribution and elimination processes. The area under the plasma concentration–time curve (AUC), calculated up to the last sampling point with detectable levels with extrapolation to infinity, for unchanged CPT-11 was 43.7 $\mu M \cdot$h, whereas that of total metabolites was 31.8 $\mu M \cdot$h, indicating an apparent predominance of

Table 4
Mean recovery of CPT-11 and three major metabolites in plasma, urine and homogenized human feces extracts

| Compound | Recovery (%) | | | | | |
|---|---|---|---|---|---|---|
| | Plasma | $n$[a] | Urine | $n$[a] | Feces | $n$[a] |
| SN-38G | 95.0±4.3 | 48 | 98.9±11.2 | 35 | 110.5±8.3 | 36 |
| RPR121056A | 82.6±4.2 | 48 | 90.7±4.6 | 36 | 100.5±5.1 | 36 |
| CPT-11 | 85.3±5.3[b] | 47 | 89.2±12.4 | 35 | 101.5±9.3 | 36 |
| SN-38 | 99.3±9.2[b] | 47 | 98.1±6.5 | 35 | 113.6±4.7 | 36 |

[a] Total number of replicate observations in three or four separate validation runs.
[b] Data from De Bruijn et al. [22]; mobile phase composition: methanol–0.1 *M* ammonium acetate containing 10 m*M* tetrabutylammonium sulphate (35:65, v/v).

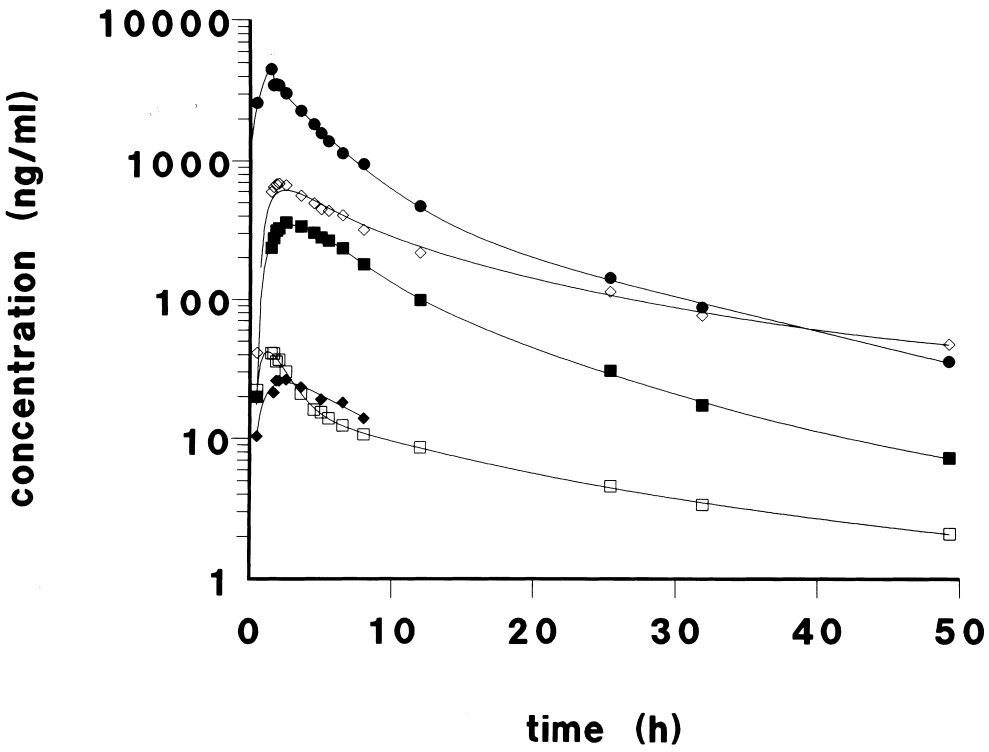


Fig. 5. Plasma concentration versus time profile of CPT-11 (●) and its metabolites SN-38G (◊), peak x (♦), RPR 121056A (■) and SN-38 (□) in a single patient given CPT-11 by a 90-min i.v. infusion at a dose level of 300 mg/m$^2$. Concentrations of peak x were estimated using the CPT-11 calibration curve, assuming similar extinction coefficients for both compounds.

the parent drug. Disappearance of CPT-11 from the central plasma compartment was characterized by a terminal elimination half life of approximately 12 h, which is within the same range as described for this compound previously [5]. The elimination phases of SN-38 and SN-38G showed a parallel decline with a prolonged half life as compared to CPT-11 and the cytochrome *P* 450-mediated metabolite, which may reflect, among others, differences in binding affinity for plasma proteins. The time course of the cumulative urinary and fecal elimination demonstrated that the excretion pattern was virtually completed within the first 15 h (data not shown). The total cumulative urinary excretion was approximately 40%, with unchanged CPT-11 and SN-38G as the main excretion products. Fecal excretion of unchanged CPT-11 also constituted a major route of elimination, with unconjugated SN-38 being the main metabolite.

In conclusion, we have developed and evaluated liquid chromatographic methods for measuring CPT-11 and three major metabolites in human plasma, urine and feces. The methods were shown to meet the current requirements as to validation of bioanalytical methodologies, providing good accuracy and precision. The described methods permit the analysis of patient samples, and will be implemented in future studies to further investigate the metabolic fate and disposition of CPT-11 in cancer patients.

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