Zeljko M. Prijovich[1,2]
Pierre-Alain Burnouf[1]
Steve R. Roffler[1]

[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
[2]Faculty of Medicine, University of Patras, Rio, Greece

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Research Article

Versatile online SPE–HPLC method for the analysis of Irinotecan and its clinically relevant metabolites in biomaterials

Monitoring levels of Irinotecan and its metabolites during cancer therapy could help link broad interpatient variations in antitumor activity and toxicity to the patient's metabolic status. We have developed and validated a versatile and highly sensitive method for the simultaneous determination of Irinotecan and its clinically relevant metabolites 7-ethyl-10-hydroxy-camptothecin (SN-38) and SN-38 glucuronide. Sample clean-up involves precipitation by acetone/methanol/0.5 M trichloroacetic acid at 4:4:2 v/v followed by extraction of the metabolites on an SPE column by 20% methanol in 25 mM KH_2PO_4 pH 2.9. Online transfer to an analytical μBondapak C18 column, elution with 24% acetonitrile (ACN) in 0.1 M KH_2PO_4 pH 2.9 and fluorescence detection with excitation at 375 nm and emission at 430 nm for SN-38 glucuronide and Irinotecan or 540 nm for SN-38 results in high sensitivity (1–2 pg) and short (~10 min) run times. The method was used to determine the degree of SN-38 glucuronidation in mice after Irinotecan administration and in cultured cancer cells exposed to SN-38. The method may be used to better understand Irinotecan metabolism, personalize therapy, and develop Irinotecan-based tumor targeting therapies.

Keywords: Biomaterials / Glucuronide / Irinotecan / Metabolites
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1 Introduction

The clinically important camptothecin derivative Irinotecan [Camptosar®, Irinotecan, (*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1*H*-pyrano [3′,4′:6,7]-indolizino [1,2-b]quinolin-9-yl-[1,4′bipiperidine]-1′-carboxylate (CPT-11)] is a prodrug with minimal antitumor activity, requiring conversion by carboxylesterases CE1 and CE2 in the liver to the active but exceptionally toxic topoisomerase I inhibitor 7-ethyl-10-hydroxy-camptothecin (SN-38) [1–3]. Consequent rapid conjugation of SN-38 by UDP-glucuronosyltransferase in the liver [4] and to some extent in tumors [5–7] generates the inactive SN-38 glucuronide (SN-38G) (Fig. 1A). The concentration of SN-38G in the circulation typically exceeds the concentration of SN-38 by 5–25-fold [8–10]). Large amounts of SN-38G that are excreted from the liver by bile to the intestine, can be hydrolyzed back to SN-38 by β-glucuronidase in normal gut microflora, possibly contributing to antitumor activity [1, 11, 12] but also damaging the intestinal walls and causing severe and dose-limiting diarrhea [13–15]. SN-38G also may be converted back to SN-38 in tumors that have high levels of β-glucuronidase, either naturally or enriched by antibody (ADEPT) or gene (GDEPT) targeting approaches [16,17]. The contribution of other CPT-11 metabolites to antitumor activity appears to be minor [9, 18–20].

The extremely complex metabolism of CPT-11 [21–23] leads to broad interpatient variations in both antitumor activity and toxicity [22, 24]. Commonly used doses of 200 mg/m^2 CPT-11 resulted in CPT-11 and SN-38 plasma peaks at ~2.5 μg/mL and 250 ng/mL, respectively, declining 1000-fold over 50 h to ~1–25 ng/mL with kinetics described by a two- or three-phase decay, with broad interpatient variability [22]. The antitumor activity and toxicity of camptothecin drugs mostly depend on prolonged exposure to even low levels of the drugs, which are highly variable. Recent investigations of CPT-11 are oriented toward discovering the correlations between each patient's status and toxicity and therapeutic response [25], paving the road for personalized CPT-11 therapy. An analytical method capable of determining even low amounts of CPT-11 and its metabolites in liquid and solid biological materials would be of great help.

Camptothecins exist in a pH-dependent equilibrium of lactone and carboxy forms (Fig. 1B) so either each form or

Correspondence: Dr. Steve Roffler, IBMS, Academia Sinica, Academia Road, Section 2, No. 128, Taipei 11529, Taiwan
E-mail: sroff@ibms.sinica.edu.tw
Fax: 886-22-782-9142

Abbreviations: ADS, alkyl-diol silica; **CPT-11**, Irinotecan, (*S*)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo 1*H*-pyrano[3′,4′:6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4′bipiperidine]-1′-carboxylate; **SN-38**, 7-Ethyl-10-hydroxy-camptothecin; **SN-38G**, 7-Ethyl-10-hydroxy-camptothecin glucuronide; **TCA**, trichloroacetic acid

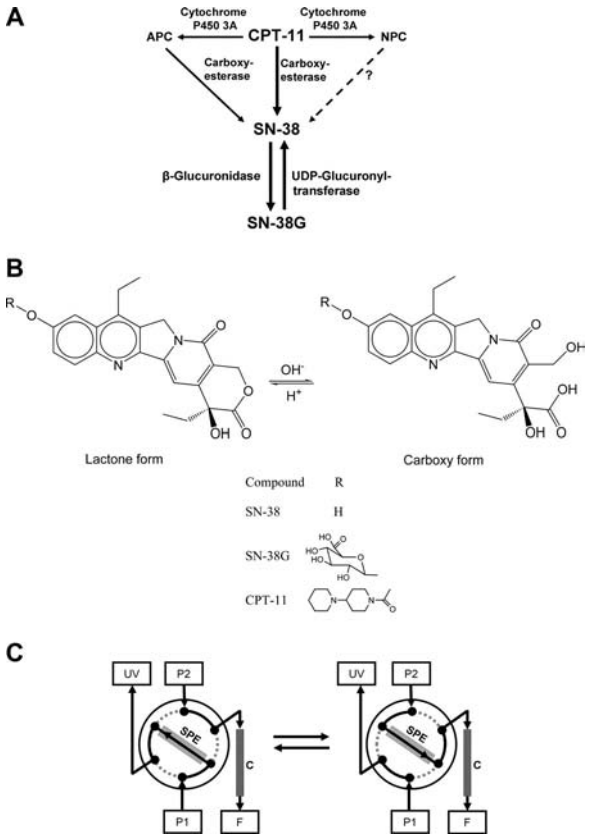

Figure 1. Structure of analytes and online SPE setup. (A) Metabolic fate of CPT-11 in humans. (B) Lactone–carboxy equilibrium of camptothecins. (C) Online SPE setup. Extraction, equilibration (left), transfer (right). P1, SPE pump; P2, analytical pumps; UV, UV detector; F, fluorescence detector; SPE, SPE column; C, analytical column.

total levels of the drugs can be determined. A number of methods have been developed for the determination of CPT-11 alone or with its active form SN-38 in serum, plasma, and mouse tissue homogenates (for a review see Ref. [26]), some with excellent sensitivity [27–29]. Determination of SN-38G is made indirectly after enzymatic conversion to SN-38 [30] or by separate methods [31]. Published methods for the direct determination of all three metabolites suffer from laborious sample preparation, complicated mobile phase compositions involving gradients and the use of ion-pairing agents, require an expensive PROSPECT system and long run times [9, 18, 20, 32]. Here we present the development and validation of a versatile method for the analysis of CPT-11 and its metabolites SN-38 and SN-38G in a broad range of biological materials.

2 Materials and methods

2.1 Reagents and material

MiliQ purified water was used for all preparations (Millipore, Billerica, MA). HPLC-grade acetonitrile and methanol were from J. T. Baker (Phillipsburg, NJ, USA). DMSO, coarse LiChroprep C_{18} (40–60 μm particle size) packing material and an alkyl-diol silica (ADS) restricted-access SPE (25 × 4 mm, 25 μm particle size) column were from Merck (Darmstadt, Germany). A μBondapak C_{18} (300 × 3.9 mm, 10 μm particle size) column and a μBondapack Guard-pak precolumn insert were from Waters (Milford, MA, USA). CPT-11 was from Sigma-Aldrich (St. Louis, MO, USA), while SN-38 was from Scino-Pharm (Tainan, Taiwan). SN-38G was produced from the urine of SCID/Beige female mice intravenous(ly) (i.v.) injected with 50 mg/kg of CPT-11 by an original procedure (Supporting Information). All other chemicals used were of highest purity available.

Stock solutions in DMSO of 1 mg/mL for CPT-11 and SN-38 and 0.1 mg/mL for SN-38G were stored at −80°C. Standard drug solutions were prepared by diluting stock solutions to 10 μg/mL in DMSO followed by dilution by the mobile phase to generate solutions containing 1 μg/mL to 100 pg/mL of the analyte.

LS174T human colon carcinoma cells were obtained from the American type culture collection (ATCC), while LS-174T βG-KD cells with reduced β-glucuronidase expression (<5% of parental cells) were generated by the lentiviral knockdown of β-glucuronidase by shRNA as previously described [33]. The cells were cultivated in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% bovine calf serum (BCS), penicillin, and streptomycin in a 5% CO_2 atmosphere at 37°C.

All experiments involving animals were conducted according to the UK Coordinating Committee on Cancer Research (UKCCCR) [34] and local animal committee guidelines. BALB/c mice were kept in standard laboratory cages with free access to food and water and fed a standard dry diet. Blood was collected from the heart before the organs of interest were harvested. Human blood from informed healthy volunteers was obtained after written agreement. The serum and plasma were obtained by routine methods.

2.2 HPLC procedure

Solid tissues were accurately weighed, immersed in 0.2 M trichloroacetic acid (TCA) at a 1:1 m/v ratio followed by the addition of two volumes of ice-cold homogenization buffer containing ACN, methanol, and 0.5 M TCA at 4:4:2 v/v ratio and homogenized on an Ultraturax four times for 20 s each time on ice. Liquid samples (plasma, serum, full or hemolyzed blood, cell culture media or cells homogenates) were diluted 1:1 with 0.2 M TCA followed by two volumes of homogenization buffer and vortex mixed for 10 s. Urine or bile were diluted tenfold with blank plasma and processed as plasma samples. The homogenized samples were centrifuged 5 min at 15 000 × *g* at 4°C and the clear supernatants were further diluted fourfold with 10 mM KH_2PO_4, pH 2.9 so as to maintain the final concentration of organic solvent <20%, optionally filtered with a 0.45 μm HPLC syringe filter and 20 μL to 2 mL injected by an autosampler equipped with a 200 μL or 2 mL loop.

Quality control samples, prepared by spiking human plasma with known amounts of the three metabolites and processing the samples as described above, were stored at −80°C and injected in duplicate before and after daily runs.

The HPLC setup consisted of two pumps with Waters 600 controllers which delivered helium-degassed mobile phases through a Waters 717 autosampler and an optional Rheodyne manual injector to a Rheodyne six-port valve, controlled by a Gilson 817 valve actuator, accommodating the SPE and analytical columns, as depicted in Fig. 1C. The SPE column was either a commercial restricted access ADS or a hand-packed LiChroprep C_{18} column, while the analytical column was a Waters μBondapak with a precolumn insert. The outlet of the SPE column was connected to a Jasco UV975 UV/Vis detector set at 280 nm, while the outlet of the analytical column was connected to a Jasco FP-2020 plus fluorescence detector set to 375 nm excitation and programed to switch to either 430 or 540 nm emission at predefined times. The signals were acquired and analyzed on a PC with Beckman Gold 6 software.

Analysis was performed by injecting 20 μL to 2 mL samples into the SPE column and washing away proteins and contaminants with washing mobile phase containing 20% methanol, 10 mM KH_2PO_4, pH 2.9 at 1.0 to 2.5 mL/min until the UV reading dropped to baseline (1.0–2.5 min). The six-port valve was then switched so that the separation mobile phase (24% ACN, 0.1 M KH_2PO_4, pH 2.9) flows in the reverse direction through the SPE column at 2 mL/min, transferring the captured metabolites to the analytical column. The valve was switched back after 2 min to prepare the SPE column and accommodate the next sample injection. Larger sample volumes were accommodated by performing repeated injections onto the SPE column before switching for the analysis.

The efficacy of the SPE column for capturing metabolites was determined by comparing peak areas after injecting 10 pg to 200 ng of CPT-11, SN-38G, and SN-38 directly into the analytical column versus injecting the samples into the SPE column followed by assay on the analytical column. The influence of the biological matrix on SPE extraction was determined by comparison of recoveries of 1 ng/mL CPT-11, SN-38G, and SN-38 prepared in phosphate-buffered saline and plasma.

Method validation was made by analyzing human plasma samples spiked with known amounts of the drugs. Intraday and interday precision and accuracy were determined by repeated analysis of all three metabolites at concentrations ranging from 10 pg to 10 ng. The LOQ and LOD were determined as described [35] by the formulas $LOD = 3 \times SD \times C/C_t$ and $LOQ = 10 \times SD \times C/C_t$, where C is the average determined concentration and C_t is the theoretical concentration. The linearity of the assay was determined by calculating the regression line of the area under the peak (AUP) versus concentration using the least-squares method. Student's *t*-test was used for statistical analysis. All calculations were made on a PC using the Excel and GraphPad Prism software packages.

Pharmacokinetics and tissue distribution of CPT-11 and metabolites were determined after injection of 10 mg/kg CPT-11 i.v. in the lateral tail vein of mice. Blood samples were periodically collected in heparinized haematocrit capillaries and sealed. After centrifugation to separate cells, the plasma was processed as described above and 20–200 μL was injected for analysis.

The generation of SN-38G from SN-38 by cancer cells in culture was demonstrated in cells with natural or down-regulated expression of human β-glucuronidase seeded in triplicate in 1.5 mL of medium containing 5×10^6 cells per well in six-well plates and incubated at 37°C overnight. The medium was replaced with fresh medium containing 10 nM SN-38 and harvested at predetermined times, immediately mixed with TCA followed by sample processing as described above. Samples of 500 μL were analyzed by SPE–HPLC as described above.

3 Results and discussion

The analytical method proposed here was developed and optimized bearing in mind its practical application. Although camptothecins exist in a pH-dependent lactone–carboxy equilibrium (Fig. 1B) [36–38], the total drug levels frequently better correlate with anticancer activity [32, 39]. Separation was therefore carried out at acidic pH, which favors the lactone form and protonated carboxy group of SN-38G, promoting its retention and allowing measurement of all three analytes in one run.

3.1 Sample preparation

Most procedures for the analysis of biomaterials containing CPT-11 and the metabolites involve deproteinization and extraction by acid and/or cold organic solvents [13, 40] followed by injection in an analytical column. These procedures, however, significantly dilute the samples while failing to remove contaminants and limit the amount of sample that can be analyzed.

A mixture of ACN/methanol/0.5 M TCA at 4:4:2 v/v ratio was chosen as the most effective to inhibit the enzymes that otherwise could hydrolyze CPT-11 or SN-38G, precipitate proteins and extract analytes at the same time facilitating their conversion to the lactone forms. The extraction efficiency of the metabolites was reproducible from different biomaterials, with average recoveries of 92.6 ± 6.9, 99.4 ± 1.8, and 87.8 ± 5.1% for SN-38G, CPT-11, and SN-38, respectively, from mouse organs spiked with 1 ng/mL of the metabolites (Table 1). Prepared extracts were stable for 12 h at room temperature or even longer if kept cold without noticeable degradation (data not shown).

Clear plasma rather than urine or bile was chosen as a common diluent for urine and bile samples to avoid unnecessary background increase and the matrix effect variations yet complying with the FDA recommendations and accepted practice [18].

Table 1. Recoveries of CPT-11, SN-38, and SN-38G from different mouse tissues. Recoveries were determined by analysis of tissue homogenates spiked with 1 ng/ml of CPT-11, SN-38, and SN-38G

Organ	Recovery (%)		
	SN-38G	CPT-11	SN-38
Plasma	80.2	99.1	88.1
Liver	96.5	99.8	96.4
Spleen	88.8	96.1	81.1
Kidney	96.2	99.7	85.6
Lungs	97.4	101.5	89.5
Heart	96.9	100.2	85.8
Average	92.6 ± 6.9	99.4 ± 1.8	87.8 ± 5.1

3.2 SPE

The use of online SPE allows remaining biocontaminants to be more completely removed while analytes are concentrated in a single step [41,42]. Both the commercially available ADS column [41] or a hand-packed Lichroprep C_{18} matrix as the SPE column produced satisfactory retention of all the metabolites. The washing mobile phase containing 20% methanol in 10 mM KH_2PO_4, pH 2.9 provided excellent retention for even the most polar analyte (SN-38G) yet effectively removed the protein contaminants at a flow rate of 1 mL/min over 1 min in most applications. Injecting increased volumes of samples containing 100 pg/mL of analytes from 20 μL to as large as 4 mL resulted in linear responses with correlation coefficients >0.999 for all three analytes. Likewise, injecting 200 pg of the analytes in 20 μL to 2 mL of sample gave the same peak areas, demonstrating a clear advantage of SPE over direct injection where a limited volume of sample may be injected (Fig. 2A). Extending the washing time to as long as 10 min and a flow rate to 2.5 mL/min did not cause detectable loss of the most weakly bound analyte SN-38G. The most practical timing consists of 2.5 min for SPE column loading and washing, followed by 2.5 min for transfer to the analytical column, and 5 min equilibration/analysis before the next sample was loaded. New samples were thus injected every 10 min. For larger sample volumes (1 mL) the time was adjusted to 2.5 min and as long as 5 min for the largest volumes (2 mL or more) of high-protein samples.

The large particle sized LiChroprep C_{18} RP matrix strongly retained the analytes but did not interact with proteins, being able to process over 1000 samples before repacking (Supporting Information), serving as a cost effective alternative to expensive RA columns or disposable cartridges.

3.3 The analytical procedure

Mobile phases based either on methanol or ACN have been used for separation of CPT-11 and its metabolites. Methanol-based mobile phases, which typically require complicated gradients and ion-pairing agents, result in long retention times and poor separation of SN-38G from background interference peaks and poor peak shapes, especially for CPT-11. The use of ACN-based mobile phases, either alone or with 1–2% tetrahydrofuran (THF) as a modifier, improves the peak shape remarkably, but still requires ion-pairing agents such as alkyl sulfonic acids for CPT-11 and SN-38 or tetrabutylammonium ions for SN-38G.

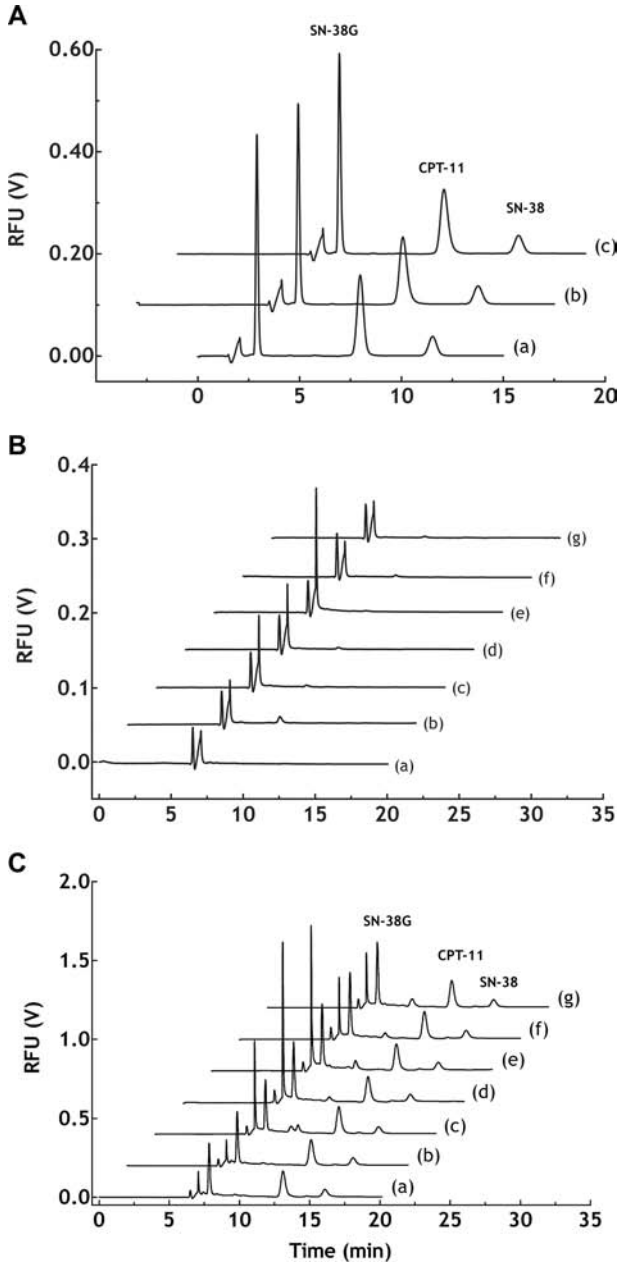

Figure 2. Chromatograms of CPT-11 and metabolites. (A) Chromatograms of CPT-11 and metabolites obtained by direct versus SPE–HPLC analysis. Injection of 200 pg of the sample either in 200 μL volume directly into the analytical column (a) or into the SPE column in 20 μL (b) or 2 ml (c) sample volumes. (B) Chromatograms of blank tissue samples analyzed by SPE–HPLC. (C) Samples spiked with 200 pg of CPT-11, SN-38, and SN-38G. The samples were human plasma (a), mouse plasma (b), liver (c), spleen (d), kidney (e), lung (f), and heart (g).

We found that relatively high concentrations of either ammonium or phosphate ions in the ACN-based mobile phase combined with low pH can protonate SN-38G to provide good retention with satisfactory peak shapes. Taking into consideration the pH dependence of the analytes' fluorescent properties (Supporting Information), 24% ACN, 0.1 M KH_2PO_4, pH 2.9 was employed as a mobile phase at 2 mL/min flow.

μBondapack, a moderately polar analytical column with larger particle size, resulted in satisfactory retention times and peak shapes for all the analytes with good tolerance of trace biomaterial carryover. Other columns of similar packing were able to separate CPT-11 and SN-38, but the retention of SN-38G, which was critical for the analysis, was evasive without relying on gradients or ion-pairing agents.

Direct injection into the analytical column of 0.2 ng of the analytes in 200 μL samples produced retention times and peak symmetry factors of 3.0 min and 1.30 for SN-38G, 8.0 min and 1.15 for CPT-11 and 11.6 min and 1.05 for SN-38, respectively (Fig. 2A). For the analysis of cleaner biomaterials (i.e. plasma), the ACN concentration can be increased to 26% to shorten the run time to <10 min with excellent separation.

SPE analysis of the same sample using 5 min for loading and washing resulted in retention times and symmetries of 8.0 min and 1.41 for SN-38G, 13.0 min, and 1.35 for CPT-11 and 16.7 min and 1.12 for SN-38, respectively. Apparently, when the analytical step was used in combination with SPE, the retention times were prolonged by the loading and washing periods, but peak symmetries remained good. However, using time overlapping during SPE analysis allowed new sample injections every 10 min or less. Online SPE sample precleaning also improved the sensitivity by allowing application of much larger sample volumes. Retention times and peak shapes obtained after SPE analysis of 0.2 ng of the analytes either in 20 μL of sample (b in Fig. 2A) or in 2 mL sample (c in Fig. 2A) were similar to those obtained by direct injection. Since the injected sample volume is scalable, the assay is limited by the amount of the sample available rather than the concentration of the analyte.

3.4 Standards and the method validation

Standard curves were constructed by recording the area under the peak versus concentration for 3–5 replicate injections of the analyte either directly into the analytical column or by SPE–HPLC. Excellent linear responses as calculated by least squares regression for all three analytes were obtained over concentrations ranging from 2.0 pg to 200 ng, spanning five orders of magnitude. The equations for the analytes as determined by SPE were as follows: $y = 0.089 + 66.954 \times X$, $r = 0.9999$ for SN-38, $y = -0.117 + 310.89 \times X$, $r = 0.9999$ for CPT-11 and $y = 0.21484 + 288.47 \times X$, $r = 0.9999$ for SN-38G. SDs ranged from 0.1 to 10% for CPT-11, 0.09–6% for SN-38G and <1% for SN-38 for the highest and lowest concentrations, respectively.

The system successfully detected all three analytes in plasma at amounts of 0.5 pg for CPT-11 and SN-38G to 2.0 pg for SN-38 and quantified them at 1.5 pg for CPT-11 and SN-38G to 6.5 pg for SN-38. The lowest levels measured in the present study were more than an order of magnitude better than in most published methods, without the need to determine SN-38G separately or rely on its enzymatic conversion to SN-38.

Table 2. Precision and accuracy for determination of CPT-11, SN-38, and SN-38G

Compound	*n*	Nominal (ng)	Found (ng)	Precision (%)	Accuracy (%)
SN-38G	6	20.0	19.1	8.2	−4.6
	6	2.00	2.09	2.0	4.5
	6	0.200	0.216	1.7	8.2
	6	0.020	0.023	1.2	14.0
	6	0.010	0.011	6.4	14.7
CPT-11	6	20.0	19.6	0.6	−2.1
	6	2.00	2.02	0.5	1.3
	6	0.200	0.211	1.9	5.6
	6	0.020	0.026	4.6	17.9
SN-38	5	10.0	9.5	1.5	−5.4
	5	2.00	1.99	5.3	−0.4
	5	0.200	0.200	3.2	0.0
	5	0.050	0.052	7.4	3.6
	5	0.020	0.022	13.7	8.5

Five to six repetitive injections of four different amounts of CPT-11 and five different amounts of SN-38G and SN-38, covering the range of 10 pg to 20 ng were analyzed. Average values, deviation from nominal concentration (accuracy) and RSD (precision) are shown.

Accuracy and precision were determined by six repetitive injections of four different amounts of CPT-11 and five different amounts of SN-38G and SN-38, covering the expected range of analytes in biomaterials (Table 2). Accuracy ranged from −4 to 15% for SN-38G, from −2 to 15% for CPT-11, and from −5 to 8% for SN-38. Intra and interday precision and long-term reproducibility were determined by six repeated injections of 0.1 ng of the analytes at four different days within a month. The intraday precision was 0.5% for SN-38G, 1.8% for CPT-11 and 0.2% for SN-38, while interday precision was 0.5% for SN-38G, 1.3% for CPT-11, and 0.3% for SN-38.

Effectiveness of the extraction from biomaterials was confirmed by analyzing samples of human and mouse origin spiked with 100 pg/mL or 1 ng/mL of the three analytes. Selectivity was assured by recording blanks for biomaterials obtained from six subjects (Fig. 2B). One visible peak at ~10 min was present in an otherwise clear background, but is well separated from the analyte peaks (Fig. 2C). Because of high levels of the analytes in urine and bile, small sample volumes were adequate, resulting in very low background.

3.5 Analysis of biomaterials

The usefulness and versatility of the method was demonstrated by measuring CPT-11, SN-38, and SN-38G in plasma

of mice treated with CPT-11 (Fig. 3A) as well as in tissue culture cells and media (Fig. 3B and C). The pharmacokinetics in mice plasma after i.v. injection of 10 mg/mL CPT-11 was described by a two-phase decay with C_{max} of 2.15 μg/mL and area under curve (AUC) of 1108 ng·h/mL. The metabolite SN-38 has a C_{max} of 278 ng/mL at 10 min followed by two-phase decay and AUC of 304 ng·h/mL, while SN-38G concentration increases to C_{max} of 339.4 ng/mL at 20 min followed by a two-phase decay and AUC of 1037 ng·h/mL. The extent of SN-38 glucuronidation in mice was reflected by the three times higher AUC of SN-38G than SN-38, <5–25-fold difference found in humans, where it may be used as the internally generated prodrug for targeted cancer therapies [16, 17].

The capability of cells to conjugate SN-38 to SN-38G by UDPGT is of increased interest as a potential drug resistance mechanism. Measurement of the extent of SN-38 conversion to SN-38G in human LS174T and LS-174T βG-KD colon adenocarcinoma cells exposed to SN-38 found that over 10% of SN-38 was converted to SN-38G after 25 h at 37°C, which was relatively independent of the expression level of β-glucuronidase (Fig. 3B, C). The concentration of SN-38 decreased at a relatively constant rate for both LS174T and LS174T βG-KD cells over a period of 25 h. The consumption of SN-38, however, was faster for LS174T βG-KD cells, and the excretion of SN-38G was also greater. This behavior was not observed in CL1-5 lung cancer cells, suggesting that this phenomenon is cell-type dependent. Colon cells are naturally exposed to toxic metabolites and might acquire this ability as a defense mechanism, while in tumors it may serve as a drug resistance mechanism [43–46].

4 Concluding remarks

Irinotecan was originally developed as a drug for the treatment of colon cancer but recently has proven useful in several other malignancies. The clinical applications of CPT-11 and increased interest in broadening its use have been hampered by its complicated metabolic fate. The antitumor effects and toxicity of CPT-11 result from complex processes that are difficult to predict and optimize. A more personalized approach may require follow-up of the levels of the metabolites in broad types of samples during therapy.

The analytical method presented here employs a simple isocratic mobile phase without ion-pairing agents, affordable LiChroprep instead of costly RA matrix for SPE and is probably the fastest and most sensitive method for analysis of CPT-11 and associated metabolites. This method may be used in research laboratories to better understand CPT-11 pharmacokinetics and pharmacodynamics, develop CPT-11 based tumor targeting strategies as well as in clinical laboratories to personalize CPT-11 therapy. We successfully adapted the method for analysis of other camptothecins by simply changing the percentage of ACN in the separation phase, making it a versatile platform for analysis of camptothecins in biomaterials.

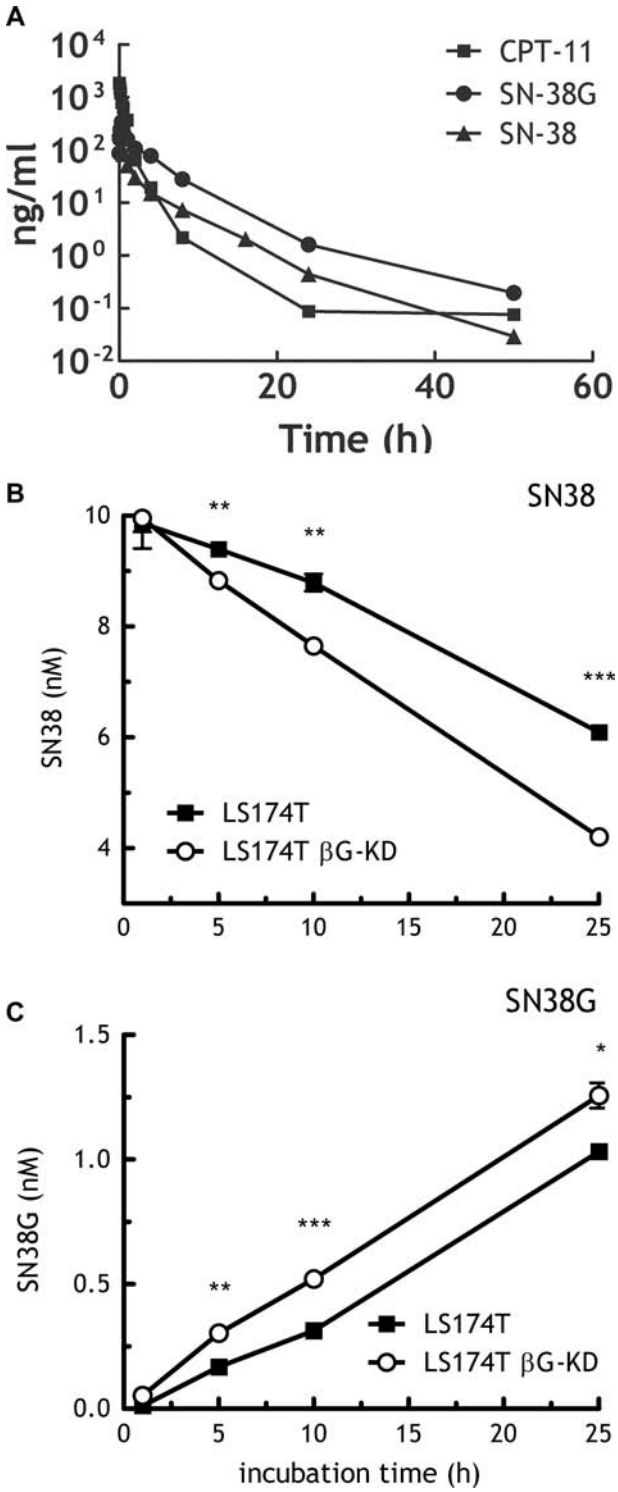

Figure 3. Analysis of CPT-11 and its metabolites in biological materials. (A) Pharmacokinetic profile of CPT-11 and its metabolites SN-38 and SN-38G in plasma after 10 mg/kg i.v. CPT-11 administration. (B) Metabolism of SN-38 and (C) generation of SN-38G by LS174T and LS174T KD β-glucuronidase suppressed colon cancer cells exposed to SN-38. Stars indicate significance $P <$ 0.05 (*), 0.005 (**), and 0.0005 (***).

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The authors have declared no conflict of interest.

5 References

[1] Kaneda, N., Nagata, H., Furuta, T., Yokokura, T., *Cancer Res.* 1990, *50*, 1715–1720.

[2] Kawato, Y., Aonuma, M., Hirota, Y., Kuga, H., Sato, K., *Cancer Res.* 1991, *51*, 4187–4191.

[3] Rivory, L. P., Riou, J. F., Haaz, M. C., Sable, S., Vuilhorgne, M., Commercon, A., Pond, S. M., Robert, J., *Cancer Res.* 1996, *56*, 3689–3694.

[4] Iyer, L., King, C. D., Whitington, P. F., Green, M. D., Roy, S. K., Tephly, T. R., Coffman, B. L., Ratain, M. J., *J. Clin. Invest.* 2004.

[5] Jinno, H., Tanaka-Kagawa, T., Hanioka, N., Saeki, M., Ishida, S., Nishimura, T., Ando, M., Saito, Y., Ozawa, S., Sawada, J.-I., *Drug Metab. Dispos.* 2003, *31*, 108–113.

[6] Tobin, P., Clarke, S., Seale, J. P., Lee, S., Solomon, M., Aulds, S., Crawford, M., Gallagher, J., Eyers, T., Rivory, L., *Br. J. Clin. Pharmacol.* 2006, *62*, 122–129.

[7] Tobin, P. J., Dodds, H. M., Clarke, S., Schnitzler, M., Rivory, L. P., *Oncol. Rep.* 2003, *10*, 1977–1979.

[8] Escoriaza, J., Aldaz, A., Castellanos, C., Calvo, E., Girõldez, J., *J. Chromatogr. B*, 2000, *740*, 159–168.

[9] Poujol, S., Pinguet, F., Malosse, F., Astre, C., Ychou, M., Culine, S., Bressolle, F., *Clin. Chem.* 2003, *49*, 1900–1908.

[10] Sparreboom, A., de Jonge, M. J., de Bruijn, P., Brouwer, E., Nooter, K., Loos, W. J., van Alphen, R. J., Mathijssen, R. H., Stoter, G., Verweij, J., *Clin. Cancer Res.* 1998, *4*, 2747–2754.

[11] Kaneda, N., Kurita, A., Hosokawa, Y., Yokokura, T., Awazu, S., *Cancer Res.* 1997, *57*, 5305–5308.

[12] Rivory, L. P., Robert, J., *Cancer Chemother. Pharmacol.* 1995, *36*, 176–179.

[13] Araki, E., Ishikawa, M., Iigo, M., Koide, T., Itabashi, M., Hoshi, A., *Jpn. J. Cancer Res.* 1993, *84*, 697–702.

[14] Gupta, E., Lestingi, T. M., Mick, R., Ramirez, J., Vokes, E. E., Ratain, M. J., *Cancer Res.* 1994, *54*, 3723–3725.

[15] Takasuna, K., Hagiwara, T., Hirohashi, M., Kato, M., Nomura, M., Nagai, E., Yokoi, T., Kamataki, T., *Cancer Res.* 1996, *56*, 3752–3757.

[16] Huang, P. T., Chen, K. C., Prijovich, Z. M., Cheng, T. L., Leu, Y. L., Roffler, S. R., *Cancer Gene Ther.* 2011, *18*, 381–389.

[17] Prijovich, Z. M., Chen, K.-C., Roffler, S. R., *Mol. Cancer Ther.* 2009, *8*, 940–946.

[18] Sparreboom, A., de Bruijn, P., de Jonge, M. J. A., Loos, W. J., Stoter, G., Verweij, J., Nooter, K., *J. Chromatogr. B.* 1998, *712*, 225–235.

[19] Ma, M. K., Zamboni, W. C., Radomski, K. M., Furman, W. L., Santana, V. M., Houghton, P. J., Hanna, S. K., Smith, A. K., Stewart, C. F., *Clin. Cancer Res.* 2000, *6*, 813–819.

[20] Owens, T. S., Dodds, H., Fricke, K., Hanna, S. K., Crews, K. R., *J. Chromatogr. B.* 2003, *788*, 65–74.

[21] Rosner, G. L., Panetta, J. C., Innocenti, F., Ratain, M. J., *Clin. Pharmacol. Ther.* 2008, *84*, 393–402.

[22] Mathijssen, R. H., van Alphen, R. J., Verweij, J., Loos, W. J., Nooter, K., Stoter, G., Sparreboom, A., *Clin. Cancer Res.* 2001, *7*, 2182–2194.

[23] Mathijssen, R. H. J., Loos, W. J., Verweij, J., Sparreboom, A., *Curr. Cancer Drug Targets* 2002, *2*, 103–123.

[24] Canal, P., Gay, C., Dezeuze, A., Douillard, J. Y., Bugat, R., Brunet, R., Adenis, A., Herait, P., Lokiec, F., Mathieu-Boue, A., *J. Clin. Oncol.* 1996, *14*, 2688–2695.

[25] Innocenti, F., Undevia, S. D., Iyer, L., Chen, P. X., Das, S., Kocherginsky, M., Karrison, T., Janisch, L., Ramírez, J., Rudin, C. M., Vokes, E. E., Ratain, M. J., *J. Clin. Oncol.* 2004, *22*, 1382–1388.

[26] Loos, W. J., de Bruijn, P., Verweij, J., Sparreboom, A., *Anti-Cancer Drugs* 2000, *11*, 315–324.

[27] de Bruijn, P., de Jonge, M. J. A., Verweij, J., Loos, W. J., Nooter, K., Stoter, G., Sparreboom, A., *Anal. Biochem.* 1999, *269*, 174–178.

[28] Rivory, L. P., Findlay, M., Clarke, S., Bishop, J., *J. Chromatogr. B.* 1998, *714*, 355–359.

[29] Bardin, S., Guo, W., Johnson, J. L., Khan, S., Ahmad, A., Duggan, J. X., Ayoub, J., Ahmad, I., *J. Chromatogr. A.* 2005, *1073*, 249–255.

[30] Gupta, E., Mick, R., Ramirez, J., Wang, X., Lestingi, T. M., Vokes, E. E., Ratain, M. J., *J. Clin. Oncol.* 1997, *15*, 1502–1510.

[31] Hanioka, N., Jinno, H., Nishimura, T., Ando, M., Ozawa, S., Sawada, J., *Biomed. Chromatogr.* 2001, *15*, 328–333.

[32] Kurita, A., Kaneda, N., *J. Chromatogr. B.* 1999, *724*, 335–344.

[33] Chen, K.-C., Schmuck, K., Tietze, L. F., Roffler, S. R., *Mol. Pharmaceutics* 2013.

[34] Workman, P., Twentyman, P., Balkwill, F., Balmain, A., Chaplin, D., Double, J., Embleton, J., Newell, D., Raymond, R., Stables, J., Stephens, T., Wallace, J., *Br. J. Cancer* 1998, *77*, 1–10.

[35] González-Ruiz, V., Mussardo, P., Corda, E., Girotti, S., Olives, A. I., Martín, M. A., *J. Sep. Sci.* 2010, *33*, 2086–2093.

[36] Kaneda, N., Hosokawa, Y., Yokokura, T., Awazu, S., *Biol. Pharm. Bull.* 1997, *20*, 992–996.

[37] Prijovich, Z. M., Leu, Y. L., Roffler, S. R., *Biochem. Pharmacol.* 2003, *66*, 1181–1187.

[38] Prijovich, Z. M., Leu, Y.-L., Roffler, S. R., *Cancer Chemother. Pharmacol.* 2007, *60*, 7–17.

[39] de Vries, N. A., Ouwehand, M., Buckle, T., Beijnen, J. H., van Tellingen, O., *Biomed. Chromatogr.* 2007, *21*, 1191–1200.

[40] Boyd, G., Smyth, J. F., Jodrell, D. I., Cummings, J., *Anal. Biochem.* 2001, *297*, 15–24.

[41] Schäfer, C., Lubda, D., *J. Chromatogr. A.* 2001, *909*, 73–78.

[42] Boos, K. S., Grimm, C. H., *TrAC Trends Anal. Chem.* 1999, *18*, 175–180.

[43] Cummings, J., Boyd, G., Ethell, B. T., Macpherson, J. S., Burchell, B., Smyth, J. F., Jodrell, D. I., *Biochem. Pharmacol.* 2002, *63*, 607–613.

[44] Cummings, J., Boyd, G., Macpherson, J. S., Wolf, H., Smith, G., Smyth, J. F., Jodrell, D. I., *Cancer Chemother. Pharmacol.* 2002, *49*, 194–200.

[45] Cummings, J., Ethell, B. T., Jardine, L., Boyd, G., Macpherson, J. S., Burchell, B., Smyth, J. F., Jodrell, D. I., *Cancer Res.* 2003, *63*, 8443–8450.

[46] Cummings, J., Zelcer, N., Allen, J. D., Yao, D., Boyd, G., Maliepaard, M., Friedberg, T. H., Smyth, J. F., Jodrell, D. I., *Biochem. Pharmacol.* 2004, *67*, 31–39.

