Journal of Chromatography B, 877 (2009) 3038–3044

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Quantitation of Irinotecan and its two major metabolites using a liquid chromatography–electrospray ionization tandem mass spectrometric

Wei Zhang[a,b], Ginger E. Dutschman[a,b], Xin Li[b], Min Ye[a,b], Yung-Chi Cheng[a,b,*]

[a] Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA
[b] Yale Cancer Center, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 13 May 2009
Accepted 15 July 2009
Available online 24 July 2009

Keywords:
Irinotecan
LC–MS-MS
Metabolites
Pharmacokinetics

ABSTRACT

A sensitive and selective liquid chromatographic–tandem mass spectrometric (LC–MS/MS) method for the determination of Irinotecan (CPT-11) and its metabolites in human plasma has been developed. Samples were prepared after protein precipitation and analyzed on a C_{18} column interfaced with a Q-Trap tandem mass spectrometer. Positive electrospray ionization was employed as the ionization source. The mobile phase consisted of acetonitrile–water (0.05% formic acid), using gradient procedure. The analytes and internal standard camptothecin were both detected by use of multiple reaction monitoring mode. The method was linear in the concentration range of 10.0–2000.0 ng/ml for CPT-11 and 0.5–200.0 ng/ml for 7-ethyl-10-hydroxycamptothecin (SN-38), respectively. The lower limit of quantification (LLOQ) was 10 ng/ml for CPT-11 and 0.5 ng/ml for SN-38. The intra- and inter-day relative standard deviation across three validation runs over the entire concentration range was less than 10.6%. The accuracy determined at three concentrations was within ±11.4% in terms of relative error. Due to the unavailability of standard for 7-ethyl-10-*O*-glucuronyl-camptothecin (SN-38G) and the importance of knowing the concentration of this metabolite, we developed a method for analysis SN-38G by taking advantage of the quantitive conversion of SN-38G to SN-38 using glucuronidase. This enzymatic method of identification and quantitation of gluconated compound can be widely used when the standard for phase II glucuronide metabolites are not available.

1. Introduction

Irinotecan or 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin (CPT-11), an anticancer prodrug, is a water-soluble derivative of camptothecin, a plant alkaloid isolated from the Chinese tree, *Camptotheca acuminuta*. CPT-11 is currently used for the treatment of colon cancer, lung cancer, as well as other types of cancers [1,2]. CPT-11 is converted *in vivo* by carboxylesterase enzymes into 7-ethyl-10-hydroxycamptothecin (SN-38), a potent inhibitor of *Topoisomerase* I, which plays a critical role in DNA replication and transcription [3,4]. The active metabolite SN-38 is in turn excreted intact, or as a glucuronide metabolite (SN-38G) [5]. Other metabolic pathways for CPT-11 are oxidation reactions mediated by cytochrome P-450 (CYP) 3A. Major oxidation products catalyzed by CYP3A4 are 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin (NPC), both of which are weak inhibitors of Topoisomerase I. APC is not a substrate for carboxylesterase in the human, whereas NPC is converted to SN-38 by human carboxylesterase in the liver or plasma [6]. The structures of CPT-11 and its metabolites are shown in Fig. 1.

The wide inter-patient variability observed in pharmacokinetic and pharmacodynamic properties of CPT-11 has increased the difficulty of predicting CPT-11-induced toxicity [7]. The rate at which SN-38 undergoes biotransformation to SN-38G has been shown to directly influence the amount of the active metabolite SN-38 being formed. For a better usage of CPT-11, it is important to learn the pharmacokinetics of CPT-11 and its metabolites. These considerations underscore the potential utility of a rapid and convenient assay for the measurement of CPT-11 and its metabolites.

A sensitive and user friendly analytical method to determine the concentration of CPT-11 and its metabolites in biological fluids is necessary for the timely and accurate translation of *in vitro* findings to the clinical setting. Several high-performance liquid chromatography methods have been developed for the

* Corresponding author at: Department of Pharmacology, Yale University School of Medicine, SHM B-250, 333 Cedar Street, New Haven, CT 06520, USA.
E-mail address: yccheng@yale.edu (Y.-C. Cheng).

1570-0232/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2009.07.025

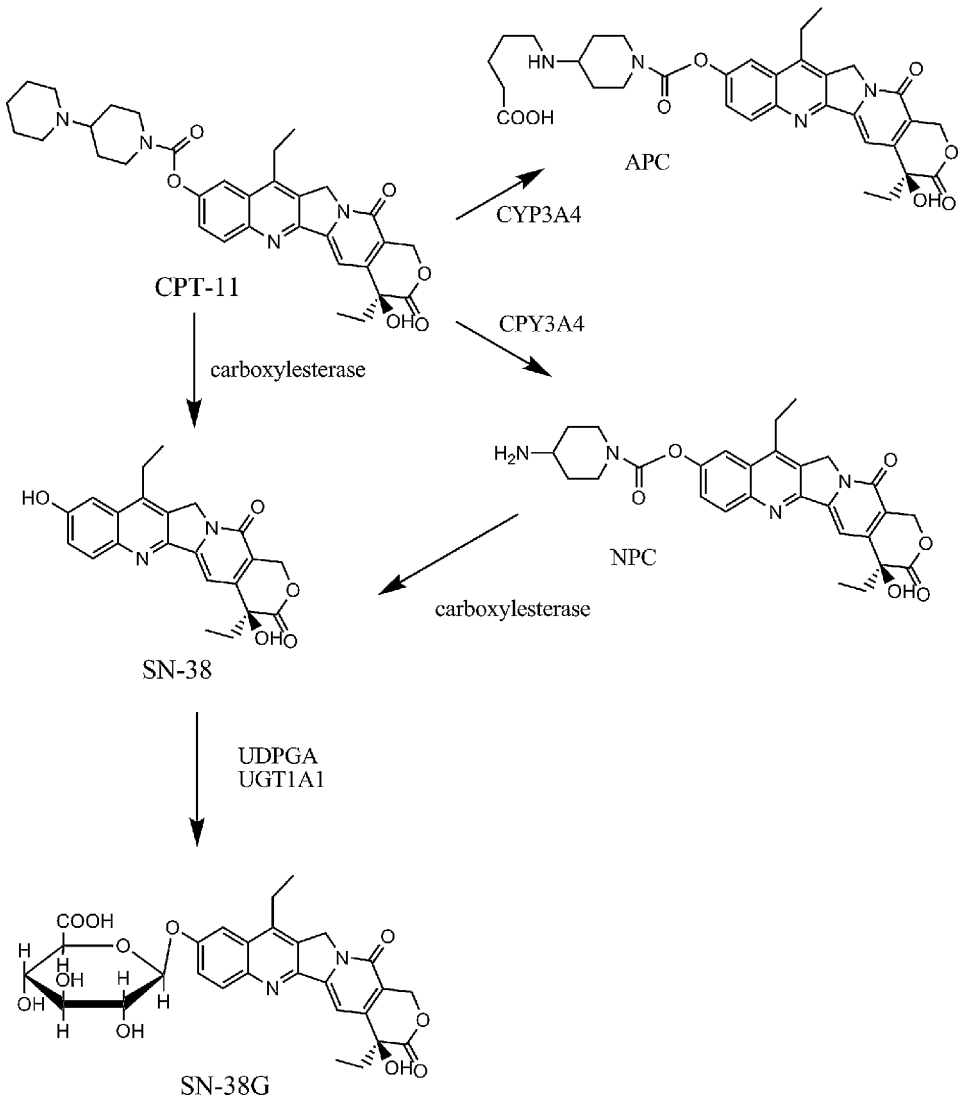

Fig. 1. The structures of CPT-11 and its metabolites.

determination of CPT-11 and its metabolites in biological fluids [8–10]. For example, de Jong et al. determined the CPT-11 and SN-38 in human whole blood and red blood cells by liquid chromatography with fluorescence detection [11]. The more sensitive liquid chromatography–mass spectrometry methods were developed to assay CPT-11 and its metabolite [6,12,13].

Preclinical studies determining the *in vitro* cytotoxicity after 24 h of drug incubation in a variety of human tumor cell lines showed that SN-38 had the highest cytotoxicity with a concentration inhibiting cell growth by 50% of 1.5–15 ng/ml [14]. Even though Sai et al. [13] reported a method for analysis of CPT-11, SN-38 and SN-38G in human plasma using HPLC with MS detection, the method was not sensitive enough to investigate the cytotoxicity of SN-38 since the detection level for SN-38 i was above 2 ng/ml. Q TRAP™ System, which combines a linear ion trap with the third quadrupole, retained the excellent quantitative capabilities of the triple quadrupole with the addition of a broad range of informative fragment ions. Thus both quantitative and qualitative information could be concomitantly obtained. In this paper, we described a simpler, selective and highly sensitive HPLC–MS/MS method using small volumes of plasma from humans, which allow the quantification of CPT-11, SN-38 and SN-38G even without the standard of SN-38G. This method is fully validated for clinical studies and therefore could be the basis for further clinical studies with CPT-11.

2. Experimental

2.1. Materials

CPT-11, SN-38 and Camptothecin (internal standard, I.S.) were purchased from Sigma, (Louis, MO, USA). HPLC–mass grade methanol, acetonitrile, water and 0.05% formic acid were obtained from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Instrumentation

The chromatographic system used consisted of an Agilent 1200 HPLC series, including a binary pump (Model G1312B), a vacuum degasser (Model G1379B), an autosampler (Model G1367C) and a column oven (Model G1316B). The mass spectrometer was an Applied Biosystems Sciex 4000 Q-trap® mass spectrometer (Applied Biosystems Sciex, Foster, CA,USA). Data acquisition was carried out by Analyst 1.4.2® software on a DELL computer.

2.3. LC–MS conditions

The chromatographic separation was achieved on a ZORBAX SB-C_{18} column (100 mm × 2.1 mm i.d., 3.5 μm, Agilent, Palo Alto, CA, USA). Mobile phase A was water with 0.05% formic acid, mobile

Table 1
Gradient procedure of CPT-11 and SN-38.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.01	88	12
2.5	80	20
12	72	28
18	64	36
18.1	20	80
20	20	80
20.1	88	12
30	88	12

phase B was acetonitrile. The gradient procedure was shown in Table 1. The liquid flow-rate was set at 0.3 ml/min, and the column temperature was maintained at 30 °C.

After chromatographic separation, the mobile phase was directly introduced into the mass spectrometer via an electrospray ionization (ESI) source operating in the positive mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 587.3 → m/z 167.1 for CPT-11, m/z 393.2 → m/z 349.3 for SN-38, m/z 569.0 → m/z 393.2 for SN-38G, m/z 349.1 → m/z 305.1 for Camptothecin (internal standard, I.S.), respectively, with a dwell time of 150 ms.

In order to optimize all the MS parameters, a standard solution (0.1 μg/ml) of the analyte and I.S. was infused into the mass spectrometer. Some mass spectrometer parameters were identical for all analyte. The curtain gas reached 35 psi. The ionspray voltage was set at 4500 V and the temperature at 550 °C. The nebulizer gas (GS1) and turbo gas (GS2) were 55 and 45 psi. The declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) were optimized for each analyte. The declustering potential were set at 141, 91 and 86 V for CPT-11, SN-38 and I.S., respectively. The values of the collision energy were 57, 38 and 35 V for CPT-11, SN-38 and I.S., respectively. The collision cell exit potentials were 8, 8 and 6 V for CPT-11, SN-38 and I.S., respectively.

2.4. Sample preparation

The plasma was prepared by removing protein through a precipitation method. In a 1.5 ml centrifuge tube an aliquot 100 μl of human plasma was spiked with 10 μl of camptothecin solution (internal standard, 0.5 μg/ml). After vortexing, 400 μl of extraction solvent (acetonitrile:methanol = 2:1) was added to the tubes and the tubes were vortex mixed for 1 min. After centrifugation at 14,000 rpm for 10 min in a Microfuge@18 Centrifuge (Beckman Coulter, Inc. Alto, CA, USA) in the cold room (4 °C), the clear supernatant fluid was then transferred into another centrifuge tube and evaporated to dryness in Speed Vac apparatus (Speed Vac SC 110, savant Instruments; Inc Farmingdale, NY). The dry residue was dissolved in 100 μl solution (acetonitrile:water = 12:88) with vortex-mixing for 0.5 min. The 70 μl reconstituted plasma extract was taken and centrifuged at 14,000 rpm for 10 min and 10 μl of supernatant fluid was injected into the HPLC–MS/MS system for SN-38G. The remaining 30 μl reconstituted plasma extract was mixed with 30 μl methanol. After centrifuged at 14,000 rpm for 10 min, 10 μl of supernatant fluid was injected into the HPLC–MS /MS system for CPT-11 and SN-38. The prepared samples were kept in an autosampler at 4 °C until injection.

2.5. Preparation of standard and quality control samples

Stock solutions of CPT-11, SN-38 were prepared in methanol at the concentration of 1,000 μg/ml. Stock solution of I.S. was prepared in methanol at the concentration of 100 μg/ml and diluted to 0.5 μg/ml with methanol. Calibration curves were prepared by spiking the appropriate standard solution in 0.1 ml of blank plasma. Effective concentrations in plasma samples were 10, 20, 50, 100, 200, 500, 1000, 2000 ng/ml for CPT-11 and 0.5, 1.0, 2.0, 5.0, 10, 20, 100, 200 ng/ml for SN-38. The quality control (QC) samples were separately prepared in blank plasma at the concentrations of 20, 100 and 1000 ng/ml for CPT-11 and 1.0, 5.0 and 100.0 ng/ml for SN-38, respectively. The spiked plasma samples (standards and quality controls) were then treated following Section 2.4 procedure on each analytical batch along with the unknown samples.

2.6. Method validation

Plasma samples were quantified using the ratio of the peak area of each analytes to that of I.S. as the assay parameter. Peak area ratios were plotted against analytes concentrations and standard curves were in the form of $y = A + Bx$.

To evaluate linearity, plasma calibration curves were prepared and assayed in duplicate on three separate days. The accuracy and precision were also assessed by determining QC samples at three concentration levels on three different validation days. The accuracy was expressed by (mean observed concentration − theoretical concentration)/(theoretical concentration) × 100% and the precision by relative standard deviation (RSD%).

Absolute recoveries of CPT-11 and SN-38 at three QC levels were determined by assaying the samples as described above and comparing the peak areas of CPT-11, SN-38 and I.S. with those obtained from direct injection of the compounds dissolved in the supernatant of the processed blank plasma.

2.7. Identification of SN-38G by Information-Dependent Acquisition (IDA)

To identify SN-38G in patient plasma by LC–MS/MS, an IDA method consisting of multiple reaction monitoring (MRM) and enhanced product ion (EPI) scan mode was developed. The MRM mode used transitions from m/z 393.2 to 349.3 and from 569.0 to 393.2 with the collision energy of 57, 30 V, declustering potential (DP) 140,100 and collision cell exit potential (CXP) of 8 V. For EPI scans, a scans rate of 4000 amu/s, collision energy of 50 V, declustering potential (DP) of 100 and dynamic fill time were used. All IDA experiments were performed in the range of m/z 50 to m/z 600 with an Q3 entry barrier of 8 V.

2.8. Cloning and purification of Escherichia coli β-glucuronidase

The gene encoding the *Escherichia coli* β-glucuronidase was amplified by PCR. The PCR product was ligated to pET21b(+) vector and obtain the expression construct. The expression construct was introduced into *E. coli* BL21(DE3) (Invireogen) and a transformant was used to inoculate Luria–Bertani (LB) broth supplemented with ampicillin at 37 °C. When the culture density reached an optical density of 0.5 at 600 nm wavelength, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM to induce expression. After 3 h, the bacterial cells were harvested, lysed, and centrifuged to collect the soluble recombinant β-glucuronidase. The enzyme was purified by applying the clarified lysate to a HisTrap HP column (GE Healthcare, Piscataway, NJ, USA) and eluted with an imidazole gradient (20 mM to 200 M) in buffer (50 mM Tris (pH 7.4), 500 mM sodium chloride, 10% glycerol, 10 mM imidazole, 0.05% triton 100).

2.9. Enzymatic deconjugation of SN-38G in patient plasma

100 μl of patient plasma was buffered with Tris–HCl (100 μl, 0.1 M, pH 6.8). After glucuronidase (protein amount 2 mg/ml, 5 μl, cloned and purified by our laboratory) was added, the sample was

sealed in a microfuge tube and gently mixed [15]. Hydrolysis of SN-38G in plasma by glucuronidase was accomplished by incubating at 37 °C for 1 h. The sample was prepared as Section 2.4 procedure.

2.10. Analysis of patient samples

Blood samples were obtained from a patient with metastatic colorectal cancer after intravenous injection 180 mg/m^2 CPT-11 and centrifuged for 15 min at 2000 × *g*. The resulting plasma was transferred to polypropylene tubes and stored at −80 °C until analysis. The patients who provided samples was enrolled in a protocol approved by the Yale human investigation committee and given written, informed consent before their participation in this study.

3. Results and discussion

3.1. Mass spectrometry

Because CPT-11, SN-38 and I.S. have numerous basic functional groups (Fig. 1), the positive ionization mode was initially chosen. The Q1 full scan spectra of CPT-11, SN-38 and I.S. were dominated by protonated molecules $[M+H]^+$ and no significant solvent adduct ions and fragments ions were observed. In the product spectra of $[M+H]^+$ ions for CPT-11, SN-38 and I.S., when the CID energy was increased more fragment ions were observed, while the response of $[M+H]^+$ lowered significantly. When the CID energy was set at 57, 38 and 35 eV, respectively, the main fragment ion from CPT-11, SN-38 and I.S. showed the highest MS response. Since the standard of SN-38G was unavailable in this experiment, the parameters of SN-38G was selected according to the parameters of SN-38 and previous reference [12,13].

3.2. Identification and quantitation of SN-38G

Since SN-38G standard was unavailability, confirmation of the identity of SN-38G was required. Therefore, plasma samples from patient given CPT-11 were also analyzed by a LC–MS/MS QTrap instrument using the IDA scan method to confirm the presence of SN-38G. The linear ion trap mass spectrometer combined with the software tool IDA allows mass scans including a MRM for both CPT-11 and SN-38 to select the analytes, an enhanced product ion scan to record a mass spectrum of each analyte recognized by the MRM mode. These mass spectral data are obtained in a single separation.

In Fig. 2(B), the characteristic EPI mass spectrum of SN-38 is presented, and Fig. 2 (A) shows the spectrum of SN-38G obtained by IDA analysis in a patient plasma sample collected 2.5 h after infusion of CPT11. From the typical fragment *m*/*z* 393 and *m*/*z* 349, the compound in Fig. 2(A) was tentatively identified as the SN-38G.

From Fig. 3(A) and (B), it is obvious that the peak of SN-38 increases and the peak of SN-38G disappears after treatment. The combination of both LC–MS/MS methods permitted a conclusive identification of SN-38G even in low concentrations. These results are more reliable as compared with methods that only use a single chromatographic trace and retention time. In addition the quantitative SN-38G was obtained through the increase of free SN-38 after treatment.

3.3. Preparation of plasma samples

Sample preparation is a critical step for accurate and reliable LC–MS/MS assays. The most widely employed biological sample preparation methodologies currently are liquid–liquid extraction (LLE), protein precipitation (PPT) and solid-phase extraction (SPE). The PPT is a simpler and time saving method. In order to avoid reducing the sensitivity, the supernatant fluid was dried in this experiment. Two kinds of solvent system were chosen because the polar character is significantly different between CPT-11, SN-38 and SN-38G, which could cause a problem of solubility. The accuracy immediately determined at high concentrations CPT-11 and SN-38 reconstituted in solution (acetonitrile:water = 12:88) was more than 85%. However when the sample was put in the autosampler (at 4 °C) for 8 h, the high concentration CPT-11 will have the problem of solubility because the ratio of CPT-11 area to that of I.S. decrease significantly while the ratio of low concentration and the area of I.S. were stable. At least two kinds of solvent was separately

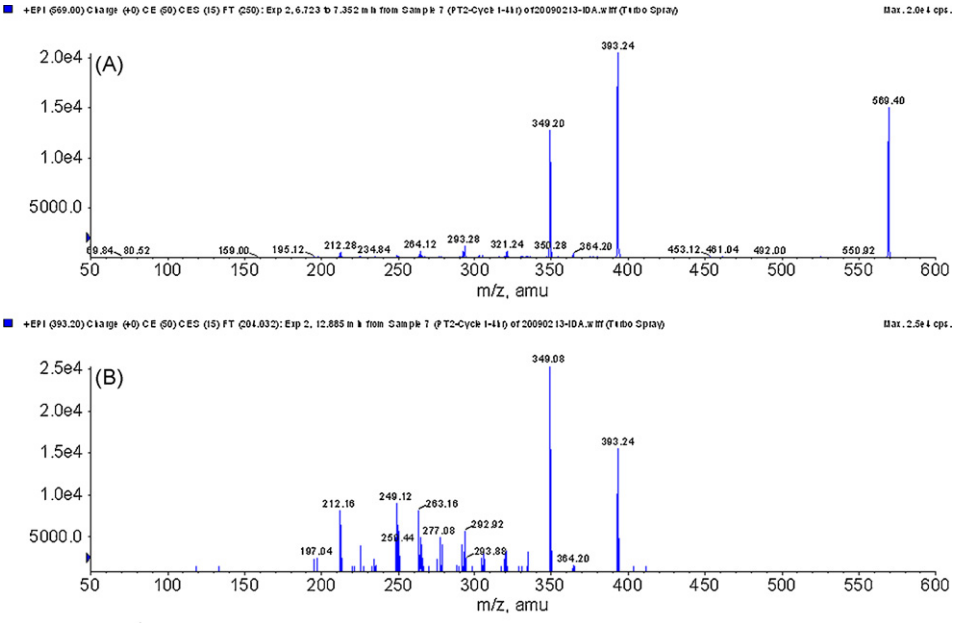

Fig. 2. EPI mass spectrum of the patient plasma sample collected 2.5 h after infusion of CPT11 (A) IDA-EPI of SN-38G; (B) IDA-EPI of SN-38.

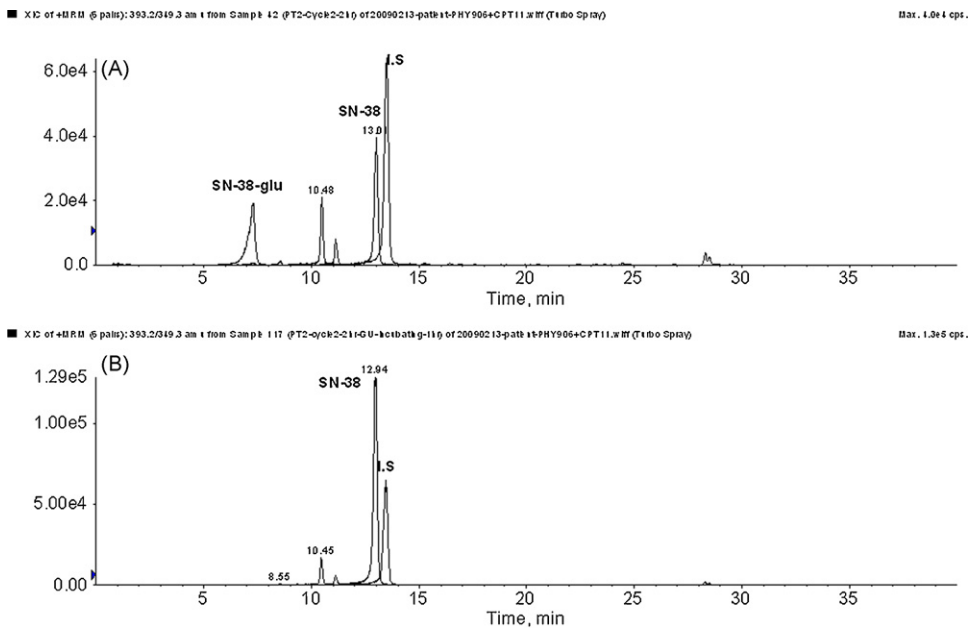

Fig. 3. Chromatogram of the SN-38 and SN-38-glu in a patient plasma sample collected 0.5 h after infusion of CPT11. (A) Before treatment of β-glucuronidase (B) after treatment of β-glucuronidase.

used for CPT-11, SN-38 and SN-38G because of their high extraction efficiency and stability.

3.4. Method validation

3.4.1. Specificity

The specificity tested the ability of the method to differentiate and quantitate the analyte in the presence of other endogenous constituents in the sample and to detect potential interferences. The chromatographs of human blank plasma and the same plasma spiked at the LLOQ of CPT-11 and SN-38 are presented in Fig. 4. Fig. 5 shows the typical chromatogram of a plasma sample from a patient 4 h after an intravenous administration of Irinotecan. There was no significant interference or ion suppression from endogenous substances observed at the retention times of the analytes. Typical retention times for CPT-11, SN-38 and SN-38G were about 11.2, 13.0 and 7.3 min, respectively.

3.4.2. Linearity of calibration curves and lower limits of quantification

Standard curves were performed in triplicate for each analyte in plasma. In all cases the regression coefficient was >0.99. CPT11 and SN-38 curves were linear over a range of 10–2000 ng/ml and 0.5–200 ng/ml, respectively, with a weighting on $1/x^2$.

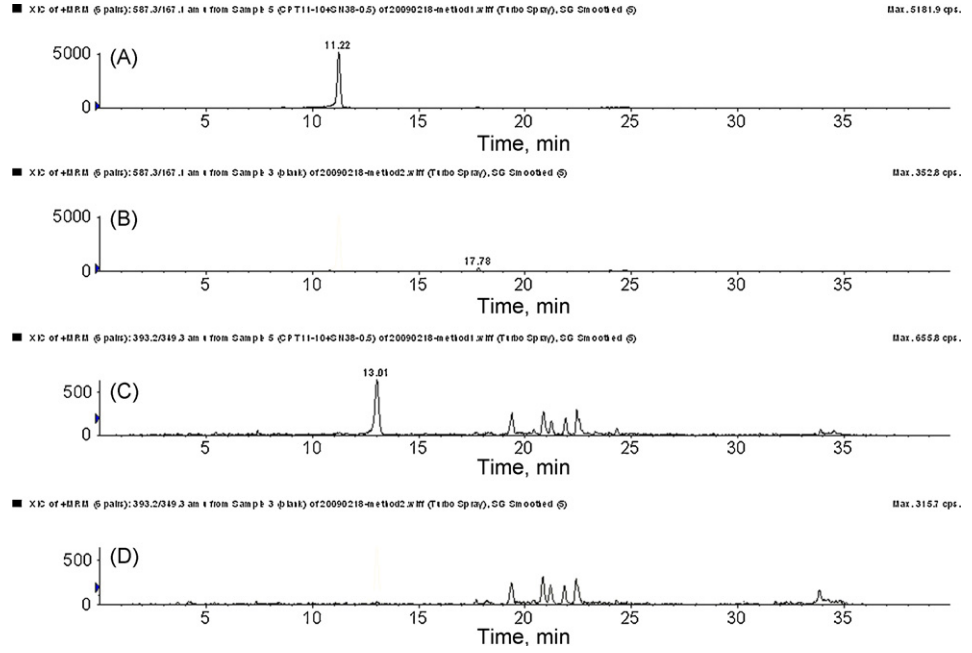

Fig. 4. Representative chromatogram of a blank human plasma and spiked plasma sample at LLQ: (A) a blank plasma sample spiked with CPT-11 at 10 ng/ml; (B) a blank plasma sample at m/z 587.3 → m/z 167.1; (C) a blank plasma sample spiked with SN-38 at 0.5 ng/ml; (D) a blank plasma sample at m/z 393.2 → m/z 349.3.

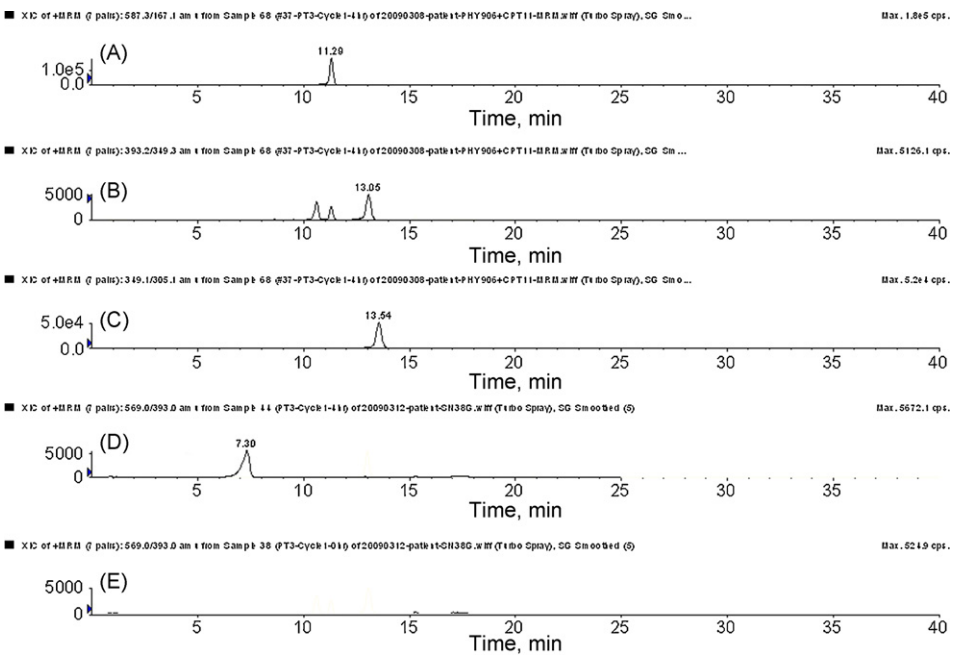

Fig. 5. Representative chromatogram of a plasma sample from a patient 4.0 h after administration of CPT11 (A) CPT-11; (B) SN-38; (C) Camptothecin (internal standard); (D) SN-38G; (E) representative chromatogram of a plasma sample from a patient 0 h at m/z 569.0 → m/z 393.2.

Typical standard curves were $f = 46.69C_i - 0.089$ for SN-38 and $f = 0.0043C_i + 0.0042$ for CPT-11. Where f represents the ratios of analyte peak area to that of I.S. and C_i represents the plasma concentrations of analyte.

The LLOQ was defined as the lowest concentration on the calibration curve for which an acceptable accuracy of ±15% and a precision below 15% were obtained. The present LC–MS/MS method offered an LLOQ of 0.5 ng/ml for SN-38 and 10 ng/ml for CPT-11 in 0.1 ml plasma sample. This is sensitive enough to investigate the pharmacokinetic behaviors of CPT-11, to establish the relationship between dose and pharmacological effect in humans.

3.4.3. Precision and accuracy

Table 2 summarizes the intra- and inter-day precision and accuracy for CPT-11 and SN38 evaluated by assaying the QC samples. The precision was calculated by using one-way ANOVA. In this assay, the intra-run precision was 7.8% or less, and the inter-run precision was 10.6% or less for each QC level of CPT-11 and SN-38. The accuracy was within ±11.4.0%. The results above demonstrated that the values were within the acceptable range and the method was accurate and precise.

3.4.4. Matrix effects

To evaluate the absolute matrix effect (ME), i.e., the potential ion suppression or enhancement due to co-eluting matrix components, five different batches of blank plasma were extracted and then spiked with the analyte at three QC concentrations. The corresponding peak areas of the analyte in spiked plasma post-extraction (A) were then compared to those of the aqueous standards in mobile phase (B) at equivalent concentrations. The ratio (A/B × 100) is defined as the ME. A ME value of 100% indicates that the responses for CPT-11 in the solution (acetonitrile:methanol:water = 25:25:50) and in the plasma extracts were the same and that no absolute ME was observed. A value of >100% indicates ionization enhancement and a value of<100% indicates ionization suppression. The assessment of the relative ME was made by a direct comparison of the analyte peak area values between different lots (sources) of plasma. The variability in the values, expressed as RSD (%), is a measure of the relative ME for the target analyte. The ME data at three QC concentrations of CPT-11 in five different lots of human plasma are presented in Table 3.

Despite some ionization suppression (>15%) was found in SN-38 (1 ng/ml), the sensitivity of the assay was still sufficient to detect the CPT-11 and SN-38 in the human plasma. Indeed, in bioanalytical mass spectrometry, absolute matrix effects are almost inescapable. However, much more important is the demonstrable lack of relative matrix effects, i.e., variance between patient results brought about by the inter-individual differences in the constitution of their plasma. The relative matrix effect confirms that the matrix effect has practically no effect on the quantification of CPT-11 and SN-38. In addition, the accuracy and precision results showed that matrix does not affect the precision of determination of the CPT-11 and SN-38. The accuracy for the CPT-11 and SN-38 were achieved in the range of 95.0–100.1% and 96.7–111.4% in plasma.

Table 2
Accuracy and precision for the analysis of CPT11 and SN38 in human plasma (in pre-study validation, $n = 3$ days, five replicates per day).

	Added C (ng/ml)	Found C (ng/ml)	Intra-run RSD (%)	Inter-run RSD (%)	Relative error (%)
CPT11	20.0	22.3	7.8	7.4	11.4
	100.0	96.7	2.7	4.3	−3.3
	1000.0	1043.0	2.8	3.9	4.3
SN38	1.0	1.1	3.6	10.6	5.0
	5.0	4.8	2.6	5.8	−5.0
	100.0	100.1	3.5	4.1	0.1

Table 3
Recovery and matrix effect data for CPT-11 and SN-38 in five different lots of human plasma ($n = 5$).

	Concentration (ng/ml)	Absolute ME	Relative ME RSD (%)	Recovery	RSD (%)
CPT-11	20.0	88.6	4.4	80.6	8.9
	100.0	92.2	7.6	93.1	3.5
	1000.0	86.2	4.1	94.9	1.3
SN-38	1.0	84.8	2.8	93.9	11.6
	5.0	90.8	1.6	94.0	2.9
	100.0	97.9	6.6	89.2	3.8

Table 4
Plasma concentration of CPT-11, SN-38 and SN-38G after intravenous injection CPT-11.

Time (h)	CPT-11 (ng/ml)	SN-38 (ng/ml)	SN-38G (ng/ml)
0.5	1288	19.4	43.7
2.5	515	3.8	20.3
4.5	396	2.9	16.9

3.4.5. Recovery and stability

The recovery of CPT-11 and SN-38, determined at three concentrations (20, 100, 1000 ng/ml), and (1, 5, 100 ng/ml) were $80.6 \pm 8.9\%$, $93.1 \pm 3.5\%$, $94.9 \pm 1.3\%$ and $93.9 \pm 11.6\%$, $94.0 \pm 2.9\%$, $89.2 \pm 3.8\%$ ($n = 5$), respectively.

The stabilities of QC samples at three different concentrations (20.0, 100.0, 1000.0 ng/ml), and (1.0, 5.0, 100.0 ng/ml) respectively, in the whole blood prepared according to the above-mentioned method were tested by short-term stability (4 °C) assays. CPT-11 and its metabolites were stable for 24 h at different concentrations ranging from 95.2% to 110.4%.

3.5. Analysis of patient samples

When applied to clinical samples, the assay proved suitable for quantitating the concentrations of CPT-11 and both metabolites in the plasma of a patient treated with his intravenous dose of CPT-11 (Table 4). The detailed parameters of the pharmacokinetics will be published in a future paper. Of note, at 4.5 h after ingestion of CPT-11, plasma concentrations of CPT-11 and SN-38 were still in excess of the LLOQ for each of these analytes.

4. Conclusions

The proposed method of analysis provided a sensitive and specific assay for determination of CPT-11 and its metabolites in human plasma. The Simple protein precipitation procedure and short LC–MS/MS run time can allow a large number of samples to be analyzed. The method of identification and quantitation of SN-38G could be also used for analyzing the phase II glucuronide metabolites of other drugs when the standards for the glucuronide metabolites are unavailable. It was shown that this method is suitable for the analysis of CPT-11 and its metabolites in human plasma samples collected for pharmacokinetic, bioavailability or bioequivalence studies in humans and mice.

Acknowledgements

We wish to thank Dr. Lam Wing and Guan Fulan for glucuronide enzyme preparation. And we wish to thank Dr M. Wasif Saif and his colleagues and staff at the Yale Cancer Center for collecting the plasma of cancer patients. This work is supported by NIH grants RO1CA63477 and Yale comprehensive cancer center grants #3P30CA16359. Y-C. Cheng is a fellow of the National Foundation for Cancer Research.

References

[1] R. Philippe, B. Roland, Semin. Oncol. 23 (1996) 34.
[2] S. Negoro, M. Fukuka, N. Masuda, M. Takada, Y. Kusunoki, K. Matsui, N. takifuji, S. Kudoh, H. Niitani, T. Taguchi, J. Natl. Cancer Inst. 83 (1991) 1164.
[3] M.C. Haaz, L.P. Rivory, C. Riché, J. Robert, Naunyn Schmiedebergs Arch. Pharmacol. 356 (1997) 257.
[4] J. Slatter, L. Schaaf, J. Sams, K. Feenstra, M. Johnson, P. Bombardt, K. Cathcart, M. Verburg, L. Pearson, L. Compton, L. Miller, D. Baker, C. Pesheck, R. Lord, Drug Metab. Dispos. 28 (2000) 423.
[5] E. Gupta, T.M. Lestingi, R. Mick, J. Ramirez, E.E. Vokes, M.J. Ratain, Cancer Res. 54 (1994) 3723.
[6] K. Sai, N. Kaniwa, S. Ozawa, J.I. Sawada, Drug Metab. Dispos. 29 (2001) 1505.
[7] R.H.J. Mathijssen, R.J. van Alphen, J. Verweij, W.J. Loos, K. Nooter, G. Stoter, A. Sparreboom, Clin. Cancer Res. 7 (2001) 2182.
[8] Z.P. Hu, X.X. Yang, X. Chen, E. Chan, W. Duan, S.F. Zhou, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 850 (2007) 575.
[9] L. Zufia, A. Aldaz, J. Giraldez, J. Chromatogr. B Biomed. Sci. Appl. 764 (2001) 141.
[10] M. Palumbo, C. Sissi, B. Gatto, S. Moro, G. Zagotto, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 764 (2001) 121.
[11] F.A. de Jong, R.H. Mathijssen, P. de Bruijn, W.J. Loos, J. Verweij, A. Sparreboom, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 795 (2003) 38.
[12] F. D'Esposito, B.N. Tattam, I. Ramzan, M. Murray, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 875 (2008) 522.
[13] K. Sai, N. Kaniwa, S. Ozawa, J. Sawada, Biomed. Chromatogr. 16 (2002) 209.
[14] S.A. Veltkamp, E.O. Witteveen, A. Capriati, A. Crea, F. Animati, M. Voogel-Fuchs, I.J. van den Heuvel, J.H. Beijnen, E.E. Voest, J.H. Schellens, Clin. Cancer Res. 14 (2008) 7535.
[15] K. Inoue, M. Kawaguchi, Y. Funakoshi, H. Nakazawa, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 789 (2003) 17.