BRIEF REPORT

Determination of irinotecan, SN-38 and SN-38 glucuronide using HPLC/MS/MS: Application in a clinical pharmacokinetic and personalized medicine in colorectal cancer patients

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Background: Irinotecan (CPT-11) is chemotherapy used mainly in the metastatic colorectal cancer. The purpose of this study was to develop and validate the LC-MS/MS for the simultaneous determination of CPT-11, SN-38, and SN-38G.

Methods: A 100 μ L of plasma was prepared after protein precipitation and analyzed on a C18 column using 0.1% acetic acid in water and 0.1% acetic acid in acetonitrile as mobile phases. The mass spectrometer worked with multiple reaction monitoring (MRM) in positive scan mode. The standard curves were linear on a concentration range of 5–10 000 ng/mL for CPT-11, 5–1000 ng/mL for SN-38, and 8–1000 ng/mL for SN-38G.

Results: In this assay, the intra and interday precision consisted of $\leq 9.11\%$ and $\leq 11.29\%$ for CPT-11, $\leq 8.70\%$ and 8.31% for SN-38, and $\leq 9.90\%$ and 9.64% for SN-38G.

Conclusion: This method was successfully used to quantify CPT-11, SN-38, and SN-38G and applied to a pharmacokinetic study.

KEYWORDS

colorectal cancer, CPT-11, LC-MS/MS, SN-38, SN-38G

1 | INTRODUCTION

Irinotecan (CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin), a prodrug topoisomerase I inhibitor, is used in colorectal, gastric, and lung cancers.¹ CPT-11 is metabolized by carboxylesterase to active metabolite SN-38 (7-ethyl-10-hydrox-

ycamptothecin), which is 100–1000 times more cytotoxic than the parent drug.² SN-38 is metabolized to the inactive metabolite 7-ethyl-10-[3,4,5-trihydroxy-pyran-2-carboxylic acid]-camptothecin (SN-38G) by uridine diphosphate glucuronosyltransferase (UGT1A1).^{3,4} However, CPT-11 has clinical adverse effects, including neutropenia and diarrhea.⁵

Several studies described the relationship between CPT-11 pharmacokinetic and clinical toxicity, including severe diarrhea and neutropenia.^{6,7} The *UGT1A1**28 polymorphism, the most common allele, is associated with increase toxicity and leads to higher or more prolonged exposure of SN-38.⁸⁻¹⁰ Furthermore, studies have shown that the area under curve (AUC) ratio SN-38G/SN-38 is clinically influencing factors in CPT-11-induced toxicity.^{11,12} Therapeutic drug monitoring (TDM) of CPT-11 and its metabolites are necessary to study with the interindividual variability in PK of CPT-11.¹³ The purpose of this study was to develop and validate the LC-MS/MS for the simultaneous determination of CPT-11, SN-38, and SN-38G. This develop method was applied to study in pharmacokinetic parameters of CPT-11 and its metabolites in human plasma.

2 | MATERIALS AND METHODS

CPT-11, SN-38, and camptothecin (Internal standard, IS) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). SN-38G was provided by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The drug-free EDTA plasma was obtained from Hematology Division, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

The liquid chromatographic system was an Agilent 1260 HPLC system. The analytical column was a Kinetex C18, 1.7 μ m, 50 $\times$ 2.1 mm (Phenomenex, Torrance, CA, USA). Mobile phase A (0.1% acetic acid in water [v/v], MP A) and mobile phase B (0.1% acetic acid in acetonitrile [v/v], MP B) were operated with a gradient elution at 0.3 mL/min as follows: 95% MP A (0-1 minute), 95% MP A to 30% (1-6.5 minutes), 30% MP A (6.5-8 minutes), 30% MP A to 10% (8-8.5 minutes), 10% MP A (8.5-10 minutes), 10% MP A to 95% (10-11 minutes), 95% MP A (11-18 minutes).

The mass spectrometer was an API 3200 (Applied Biosystems Sciex, Foster City, CA, USA). The system was operated in positive ion mode and multiple reactions monitoring (MRM) scan mode. The mass spectrometer parameters were TurbolonSpray source operated at 550°C and with electrospray ionization voltage of 5500 V. MRM quantification transitions were as follows: irinotecan *m/z* 587.3 $\rightarrow$ 167.0; SN-38 *m/z* 392.9 $\rightarrow$ 349.1; SN-38G *m/z* 569.0 $\rightarrow$ 393.3; CPT (IS) *m/z* 349.1 $\rightarrow$ 305.1. The data were performed with analyst 1.5.2 software version (SCIEX, Framingham, MA, USA).

The stock solutions of CPT-11, SN-38, and SN-38G were prepared in DMSO at a concentration of 1 mg/mL for CPT-11 and SN-38, 0.5 mg/mL for SN-38G. The stock solution of IS was prepared at 1 mg/mL in methanol: DMSO (50:50, [v/v]). All the stock solutions were stored at -20°C. The working solution was prepared in MP A: MP B (50:50, [v/v]). Final concentrations of calibration standards were 5, 50, 100, 500, 1000, 4000, 7000, 10 000 ng/mL for CPT-11; 5, 20, 40, 120, 240, 560, 720, 1000 ng/mL for SN-38; and 8, 20, 40, 120, 240, 560, 720, 1000 ng/mL for SN-38G. QC samples were prepared at the following concentrations: 15, 2500, 8000 ng/mL for CPT-11; 15, 400, 800 ng/mL for SN-38; and 24, 400, 800 ng/mL for SN-38G.

The plasma samples were prepared by protein precipitation extraction. An aliquot 100 μ L of EDTA plasma sample was spiked with 10 μ L of IS working solution (50 ng/mL) and vortex-mixed. The solution was extracted with 400 μ L acetonitrile-methanol (2:1, [v/v]) solution and vortex-mixed. After centrifugation at 19 955 g for 10 minutes, the supernatant fluid was transferred into another polypropylene tube and evaporated to dryness in a speed vacuum system. The residue was reconstituted with 100 μ L solution (acetonitrile: water, 12:88), vortex-mixed and centrifuged again at 14 000 rpm for 10 minutes. A volume of 5 μ L was injected to LC-MS/MS system for analysis. This study was conducted in accordance with the US Food and Drug Administration guidance for bioanalytical method validation.¹⁴

The method was used to determine CPT-11 and its metabolites in plasma samples from patients with colorectal cancer at first line of treatment by FOLFIRI regimen (180 mg/m² of CPT-11 in combination with 5-fluorouracil and leucovorin). The samples were taken at 0 and 85 minutes during infusion, 10, 30 minutes and 1, 2, 4, 8, 24, 32, and 48 hours after the end of the infusion. The pharmacokinetic parameters of CPT-11 and its metabolites were calculated by noncompartmental mode using 5.1 Thermo Scientific Kinetica program (Thermo Fisher Scientific, Waltham, MA, USA). The study was approved by the Ethics Committee, Ramathibodi Hospital, Mahidol University, Thailand and all patients signed written informed consent.

3 | RESULTS

Method validation was performed using blank EDTA plasma. The eight-point standard curves were linear in the concentration range of 5-10 000 ng/mL for CPT-11, 5-1000 ng/mL for SN-38, and 8-1000 ng/mL for SN-38G. The average correlations of calibration curve were >0.9900. Quadratic regression with 1/x weighted concentrations was used to achieve homogeneity of variance. The signal to noise (S/N) was $\geq$ 5. The acceptable LLOQ of CPT-11, SN-38, and SN-38G was determined to be 5 ng/mL, 5 ng/mL, and 8 ng/mL, respectively and was validated through analysis of six replicates.

Accuracy, intraday and interday precision were determined by running six replicates of LLOQ and QC (QCL, QCM, QCH) samples on three different days. For US FDA guidance, these results demonstrated that the precision and accuracy values were in the acceptance range (<15%). In this assay, the intra and interday precision consisted of $\leq$ 9.11% and $\leq$ 11.29% for CPT-11, $\leq$ 8.70% and 8.31% for SN-38, and $\leq$ 9.90 and 9.64% for SN-38G.

The mean extraction recoveries were determined in six replicates at three QC concentrations. The recovery was in range 100.63-110.63% (CV $\leq$ 9.69%) for CPT-11, 86.21-109.03% (CV $\leq$ 7.05%) for SN-38, and 85.41-96.51% (CV $\leq$ 8.71%) for SN-38G. The stabilities of CPT-11, SN-38, and SN-38G in plasma were determined by analyzing six replicates of QCL and QCH levels. The analytes were found to be stable for 24 hours in the autosampler. CPT-11 and its metabolites were stable for up to at least 6 months when stored at -80°C. Analytes in plasma were also found to be stable after three freeze-thaw cycles from either -20°C or -80°C.

FIGURE 1 The plasma concentration time curves of CPT-11, SN-38, and SN-38G observed in patients (N=4) receiving 180 mg/m² of CPT-11 in 90 minutes

TABLE 1 Pharmacokinetic parameters of CPT-11, SN-38, and SN-38G (n=4)

Pharmacokinetic parameter	CPT-11 (mean±SD)	SN-38 (mean±SD)	SN-38G (mean±SD)
C _{max} (ng/mL)	2161.25±39.24	22.88±7.12	88.01±58.15
AUC _{0-48 h} (ng·h/mL)	15 673.35±3278.81	331.67±143.07	1992.93±1495.04
t _{1/2} (h)	9.97±1.56	11.93±5.13	13.90±2.37
t _{max} (h)	1.5±0.12	1.75±0.3	2.25±0.29
Vd _{ss} (L/m ²)	130.81±26.75	—	—
CL (L/h/m ²)	9.14±1.61	—	—

C_{max}, maximum observed concentration; AUC, area under the curve; t_{1/2}, terminal half-life; t_{max}, time of maximum observed concentration; Vd_{ss}, volume of distribution at steady state; CL, clearance.

The validated LC-MS/MS method was applied to a pharmacokinetic study of CPT-11, SN-38, and SN-38G from four patients with colorectal cancer receiving 180 mg/m² of CPT-11 as a 90 minutes continuous intravenous infusion. The plasma concentration time curves of CPT-11 and its metabolites versus time are shown in Figure 1, during the first cycle of treatment. The C_{max} and AUC of CPT-11, SN-38, and SN-38G were 2161.25±39.24 ng/mL and 15 673.35±3278.81 ng·h/mL for CPT-11, 22.88±7.12 ng/mL and 331.67±143.07 ng·h/mL for SN-38, and 88.01±58.15 ng/mL and 1992.93±1495.04 ng·h/mL for SN-38G; whereas the terminal elimination half-life of CPT-11, SN-38, and SN-38G was 9.97±1.56, 11.93±5.13, and 13.90±2.37 hours, respectively (Table 1).

4 | DISCUSSION

In this study, the LC-MS/MS method was successfully used to determine the CPT-11 and its metabolites in human plasma with a high accuracy and high precision. A 100-μL aliquot of the plasma samples, small-volume plasma, was performed by protein precipitation.¹⁵ The process was used dry and reconstitute method to eliminate the organic solvent in protein precipitation. This LC-MS/MS method had a gradient process within 18 minutes that was faster than the method

from Zhang et al.¹⁶ with a gradient process in 30 minutes. The pharmacokinetic parameters of CPT-11 and its metabolites in this study had similar results to previous study.¹⁷ The plasma AUC of SN-38 was 47-fold lower than CPT-11, whereas the AUC of SN-38G was sixfold higher than SN-38. The terminal elimination half-life of CPT-11 was 9.97±1.56 hours with a rapid initial elimination rate and an extended terminal elimination. SN-38 and SN-38G showed an elimination half-life with 11.93±5.13 and 13.90±2.37 hours, respectively. Previous studies reported that the plasma SN-38G/SN-38 ratio might be used as indicators to predict CPT-11-induced neutropenia and adjustment of the optimal dose.^{11,18,19} Wang et al.²⁰ demonstrated that high AUC of CPT-11 and SN-38, but low AUC ratio of SN-38G/SN-38 in patients with UGT1A1*28. Thus, this method could be applied to study the relationship between the plasma SN-38G/SN-38 ratio and clinical toxicity in patient receiving CPT-11. Furthermore, the UGT1A1 polymorphisms testing should be determined to correlate with pharmacokinetic of CPT-11, SN-38, and SN-38G.

5 | CONCLUSION

In this study, we developed and validated acceptable accuracy, precision, and extraction recovery with a good linearity according to the

US FDA guidance on bioanalytical method validation. The method was successfully applied to a pharmacokinetic study of CPT-11 and its metabolites in human plasma. The further study, a pharmacokinetic study of CPT-11 and its metabolites should be studied in large sample size in Thai population with the consideration of *UGT1A1* polymorphisms testing.

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