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A simple and sensitive LC/MS/MS assay for 7-ethyl-10-hydroxycamptothecin (SN-38) in mouse plasma and tissues: application to pharmacokinetic study of liposome entrapped SN-38 (LE-SN38)

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Abstract

An LC/MS/MS method to quantify SN-38 in mouse plasma and tissue homogenates containing liposome entrapped SN-38 (LE-SN38) was developed. Camptothecin (CPT) was used as the internal standard (IS). Sample preparation consisted of simple protein precipitation by acetonitrile containing 0.5% acetic acid. SN-38 and IS were separated by a C18 HPLC column and detected using a mass spectrometer operating in the multiple reaction monitoring (MRM) mode. The peak area of the *m/z* 393.3 → 349.1 transition of SN-38 and that of the *m/z* 349.1 → 305.2 transition of the IS were measured and a standard curve was generated from their ratios. The method had a LLOQ of 0.5 ng/mL in mouse plasma, which corresponds to 2.5 pg for the 5 μL injection volume. The linear range was 0.5–1000 ng/mL of SN-38 in plasma sample spiked with LE-SN38. The LLOQ in tissue homogenates (5%, w/v) quantitation was 1 ng/mL (20 ng/g tissue) of SN-38 in kidney, liver, lung, and spleen homogenates, and 2 ng/mL (40 ng/g tissue) in heart homogenate containing LE-SN38. The assay was linear up to 400 ng/mL of SN-38 in tissue homogenates, and may be extended to 120 μg/mL by proper dilution of samples over the upper limit of quantitation. Acceptable precision and accuracy were obtained for concentrations over the entire standard curve range, both between-run and within-run for plasma and tissue homogenates. The method was successfully used to quantify SN-38 in plasma and tissues samples for pharmacokinetic and tissue distribution studies of LE-SN38 in mice.

Keywords: Camptothecin; SN-38; Liposome; LC/MS/MS; Pharmacokinetics; Mice

1. Introduction

SN-38 (7-ethyl-10-hydroxycamptothecin) is the active metabolite of CPT-11 (Irinotecan, 7-ethyl-10- [4-(1-piperodino)] carbonyloxy-camptothecin), an anti-cancer drug against many malignancies including colorectal carcinoma [1,2]. It has been shown that SN-38 is 100- to 1000-fold more active in vitro than its parent drug, CPT-11 [3]. Despite its promising anti-cancer potential, SN-38 has not been used as an anti-cancer drug due to its poor solubility in any pharmaceutical solvents. To surmount this solubility obstacle, we have developed a liposome entrapped SN-38 (LE-SN38) formulation. LE-SN38 demonstrated an enhanced efficacy toward various xenograft models when compared with CPT-11 [4,5] and had better preclinical safety [6,7].

In recent years, various methods have been reported for quantification of SN-38 and CPT-11 in biological samples using HPLC with fluorescence or mass spectrometer detection (LC/MS) to support preclinical and clinical studies of CPT-11. Most reported approaches involve long and tedious sample preparation procedures, either solid phase extraction [8,9] or liquid–liquid extraction [10–12]. Some methods use protein precipitation as a sample preparation technique, but

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often involve multiple steps [13,14]. Here we describe a sensitive LC/MS/MS method to quantify SN-38 in mouse plasma and tissue homogenates in the presence of LE-SN38. The sample preparation is simple and requires only a one-step protein precipitation. In the present study, between-run and within-run precision and accuracy of the assay were evaluated, as were the effects of plasma freeze-thaw cycles and dilution. Short-term room temperature stability and long-term storage stability were also studied. The method was used to quantify SN-38 in plasma and tissue samples to support a pharmacokinetics and tissue distribution study of LE-SN38 in mice.

2. Experimental

2.1. Chemicals and reagents

Camptothecin (CPT) (Fig. 1), glacial acetic acid, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO). SN-38 (Fig. 1) was obtained from Qventas (Newark, DE). Acetonitrile, methanol and isopropanol (HPLC grade) were purchased from EM Science (Gibbstown, NJ). Ammonium acetate was obtained from Fisher Scientific (Hampton, NH). High purity water was obtained from an in-house Milli-Q purification system (Millipore, Bedford, MA). Mouse plasma was obtained from Bioreclamation (Bioreclamation, Inc., Hicksville, NY). Normal mouse tissues were collected from CD2F1 mice obtained from Harlen Sprague–Dawly (Indianapolis, IN).

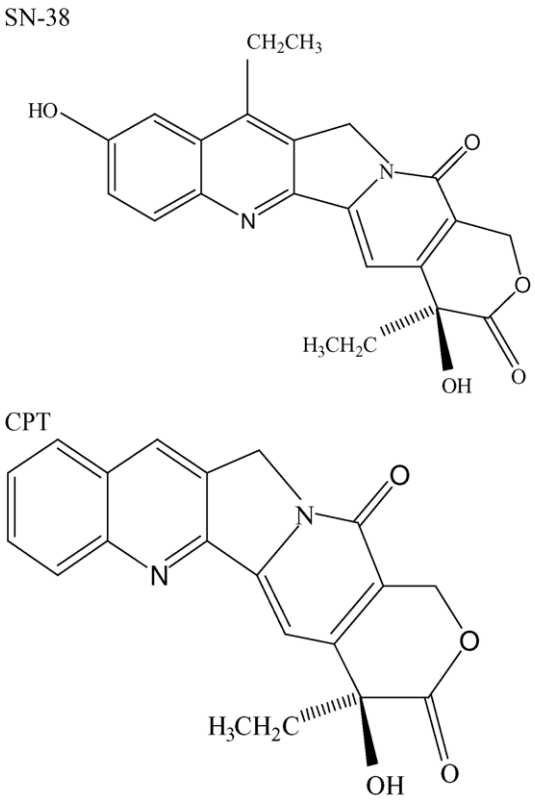

Fig. 1. Chemical structures of SN-38 and camptothecin (CPT), the IS.

2.2. Preparation of the liposome entrapped formulation of SN-38 (LE-SN38)

LE-SN38 contains 2 mg/mL of SN-38, dioleoyl phosphatidylcholine (DOPC), cholesterol, and cardiolipin. The drug to lipid ratio and DOPC:choleterol:cardiolipin mole percent ratios are approximately 1:18 and 50:40:10, respectively. Sucrose and α-tocopherol were added to the formulation as stabilizers. Blank liposomes were prepared by using same methodology without SN-38. Prior to use, LE-SN38 was reconstituted with the lactate buffer and further dilutions were made with normal saline.

2.3. Standard and quality control solutions

CPT and SN-38 were dissolved in methanol: dimethylsulfoxide (50:50, v/v) at concentrations of 0.5 and 1 mg/mL, respectively. SN-38 was then further diluted with acetonitrile:20 mM ammonium acetate, pH 3.5 (20:80, v/v) to make a set of standards ranging from 0.5 to 1000 ng/mL. These standards were used to determine the concentration of total SN-38 in the LE-SN38 stock solution.

2.3.1. Plasma

Two separate vials of LE-SN38 were used for preparation of SN-38 stock solutions in plasma. One of the LE-SN38 vials was used to prepare a standard stock solution at 10,000 ng/mL. This was further diluted in plasma to make a set of standards from 0.5 to 1000 ng/mL. The second LE-SN38 vial was used to make 80,000 ng/mL quality control (QC) samples stock solution, which was further diluted in plasma to 0.5, 400, 800, 16,000 and 80,000 ng/mL to prepare a set of QC samples.

2.3.2. Tissue homogenates

Standards and QC stock solutions of LE-SN38 were prepared by dilution of the stock solutions in tissue homogenate to achieve standard concentrations of 1, 2, 5, 50, 100, 300 and 400 ng/mL and QC samples of 3, 15, 150 and 350 ng/mL, respectively.

2.4. Sample preparation

2.4.1. Plasma

A single-step protein precipitation technique was used for sample preparation. Briefly, 200 μL of acetonitrile with 0.5% acetic acid (containing 25 ng/mL of IS) were added to 100 μL of a plasma sample. The mixture was vortexed and centrifuged at 13,000 rpm for 10 min. A 270 μL aliquot of the supernatant was transferred to a clean microtube and evaporated to dryness under vacuum at 50 °C. The dry extracts were

redissolved in 100 μL of a mixture of acetonitrile:20 mM ammonium acetate, pH 3.5 (20:80, v/v), of which 5 μL was injected onto the HPLC system.

2.4.2. Tissues

Tissues (heart, kidney, liver, lung, and spleen) were collected from normal CD2F1 mice, weighed accurately and homogenized in water (5%, w/v). A 600 μL of acetonitrile with 0.5% acetic acid (containing 50 ng/mL of IS) was added to 200 μL of tissue homogenate. The mixture was vortexed for 1 min, kept on ice for 10 min, then centrifuged at 13,000 rpm for 10 min. A 700 μL aliquot of the supernatant was transferred to a clean microtube and evaporated to dryness under vacuum at 50 °C. The dry extracts were reconstituted in 100 μL of a mixture of acetonitrile:20 mM ammonium acetate, pH 3.5 (20:80, v/v) by vortex-mixing for 30 s. The samples were centrifuged once more for 5 min at 10,000 rpm and 20 μL of the clear supernatant was injected into the HPLC system.

2.5. Chromatographic conditions

An Agilent 1100 (Agilent Technologies, Palo Alto, CA) chromatographic system consisting of a binary pump, degasser, and thermostated autosampler was used. Chromatographic separation was achieved by using a Zorbax SB-C18 (5 μm, 50 mm × 2.1 mm) analytical column (Agilent Technologies, Palo Alto, CA) protected by a C18 guard cartridge. For plasma, the mobile phase was 20 mM ammonium acetate (pH 3.5):acetonitrile, 65:35 (v/v) with a 0.2 mL/min flow rate. The retention times for SN-38 and CPT were 1.3 and 1.5 min, respectively, with a total run time of 3 min. For tissue samples, the binary pump was set to deliver (60:40) solvent A (20 mM ammonium acetate (pH 3.5):methanol, 80:20, v/v) and solvent B (acetonitrile:isopropanol, 75:25, v/v) isocratically, and the flow rate was 0.1 mL/min with total run time of 5 min. The retention times for SN-38 and CPT were 2.7 and 3.2 min, respectively. Separate chromatographic conditions for plasma versus tissue homogenate samples were used to achieve better separation and quantification of SN-38 in the different matrices.

2.6. Mass spectrometric conditions

An Applied Biosystems-Sciex API 4000 (Applied Biosystems, Foster City, CA) triple quadrupole mass spectrometer equipped with electrospray ionization interface was used. Samples were analyzed in the Multiple Reaction Monitoring (MRM), positive mode. Other details for quantification of selected ions of SN-38 and CPT (IS) in plasma and tissue homogenate samples are described in Table 1. The Analyst 1.2 software (Applied Biosystems, Foster City, CA) was used to control the LC/MS/MS system and to perform sample and data analyses. The peak area ratio of SN-38 to IS was used to construct a linear calibration curve using weighted ($1/\text{concentration}^2$) regression analysis. The SN-38 concentrations in plasma and tissue homogenate samples were determined by interpolation from the calibration curve.

Table 1
Ionization mode and transition for SN-38 and camptothecin (IS)

Parameter	Plasma	Tissue homogenate
Probe temperature (°C)	400	350
Ionization voltage (V)	4500	5500
Nebulizing gas (psi)	15	15
Auxiliary gas (psi)	15	15
Curtain gas (psi)	10	10
CAD[a] gas (psi)	6	6
Collision energy (eV)	40	35
SN-38 transition	393.3 → 349.1	
CPT (IS) transition	349.1 → 305.2	

[a] Collision-activated dissociation.

2.7. Assay validation

2.7.1. Plasma

Linearity, limit of quantitation, between-run and within-run precision and accuracy, dilution, and stability for SN-38 in mouse plasma spiked with LE-SN38 were evaluated during the method validation. A total of four runs were conducted. Each validation run included calibration curve with nine non-zero standards and QC samples in duplicates except the within-run, where six replicates of QC samples were analyzed.

Between-run accuracy and precision were assessed by analyzing QC samples at four concentrations (1.5, 6, 400, and 800 ng/mL) in duplicates over four different runs. Within-run accuracy and precision were determined by assaying six replicates of four concentrations in a single run. Assay precision was calculated and was reported as percent coefficient of variation (% CV). Accuracy was determined as the agreement between the interpolated concentration and the nominal concentration and was reported as percent analytical recovery (% AR). Between-run and within-run experiments should have precision (% CV) of ≤15% as well as accuracy (% deviation) of ≤15% from the nominal concentrations, except at the lowest limit of quantification (LLOQ) where ≤20% is acceptable for both parameters [15].

The LLOQ is defined as the lowest concentration on the standard curve that can be measured with acceptable accuracy and precision. To be acceptable, six replicates of LLOQ samples prepared independently of standards should have a percent analytical recovery of 100 ± 20% (accuracy) and % CV of ≤20% (precision).

Dilution effect was investigated to ensure that samples could be diluted with blank matrix and be accurately quantified. LE-SN38 spiked plasma samples prepared at three concentrations: 400, 16,000, 80,000 ng/mL of SN-38 were diluted at 2-, 20-, and 100-folds with blank plasma in six replicates and analyzed to determine the precision and accuracy.

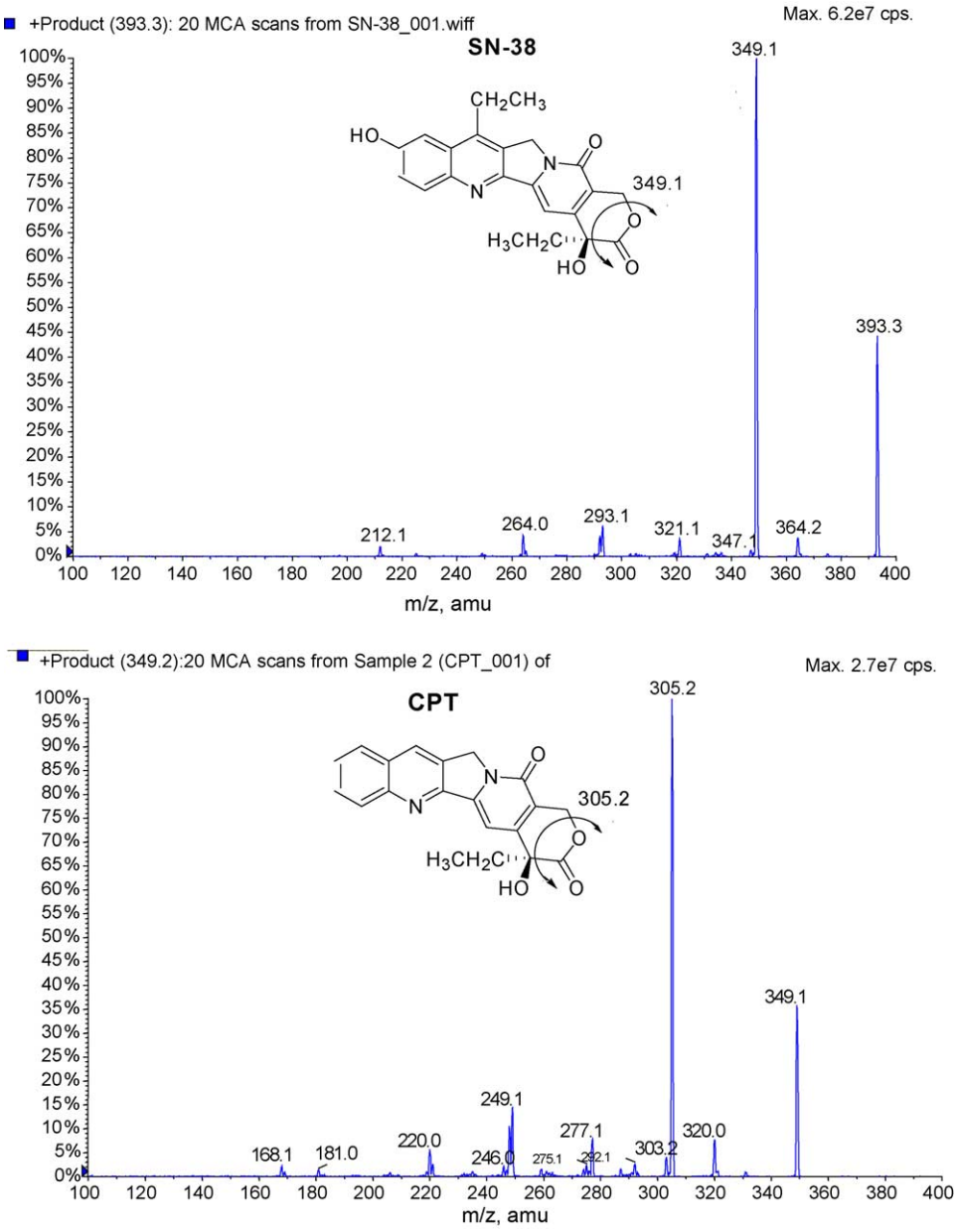

Fig. 2. Mass spectra of product ion scans for ion *m*/*z* 393.3 of SN-38 and *m*/*z* 349.1 for IS.

The freeze-thaw stability of SN-38 in plasma was investigated by using QC samples at 6, 400, and 800 ng/mL. These samples were stored at $-70 \pm 10\,^{\circ}C$ for over 12 h, and then thawed unassisted at room temperature. This procedure was repeated a total of three times. Samples that did not undergo freeze-thaw cycles served as references. Samples were analyzed in six replicates at each concentration. To be considered stable, the relative recovery (defined as the agreement between three freeze-thaw cycles and reference samples) should be within $100 \pm 15\%$. Short-term stability up to 4 h at room temperature as well as long-term stability at $-70 \pm 10\,^{\circ}C$ up to 31 days was also studied.

2.7.2. Tissue homogenates

The linearity, precision and accuracy were evaluated during the method validation for SN-38 in mouse tissue homogenates spiked with LE-SN38.

2.8. Application

Female CD2F1 mice weighing 18–23 g (11–12 weeks of age) were purchased from Harlen Sprague–Dawly (Indianapolis, IN). The animals were used in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council, housed under standard conditions, and had ad libitum access to water and to a standard

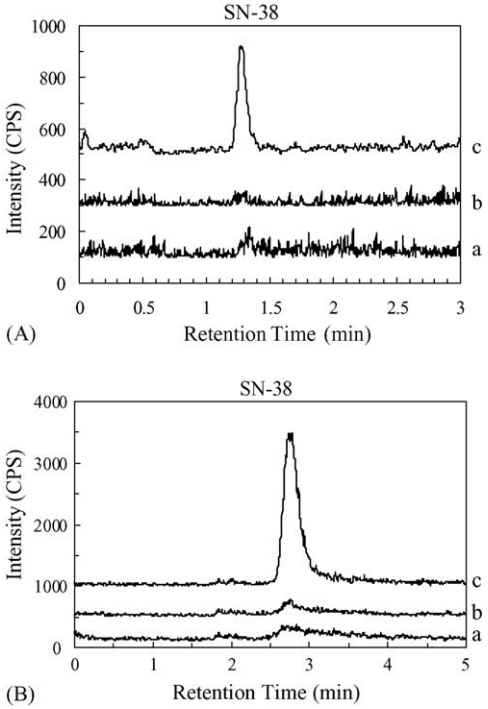

Fig. 3. (A) Representative MRM chromatograms from the SN-38 (*m/z* 393.3 → 349.3) channel of extracts from (a) blank control plasma without IS, (b) blank control plasma with 50 ng/mL IS, and (c) blank control plasma with 0.5 ng SN-38/mL (LLOQ) and 50 ng/mL IS. (B) Representative MRM chromatograms from the SN-38 (*m/z* 393.3 → 349.3) channel of extracts from (a) blank control liver homogenate without IS, (b) blank control liver homogenate with 50 ng/mL IS, and (c) blank control liver homogenate with 1.0 ng SN-38/mL (LLOQ) and 50 ng/mL IS.

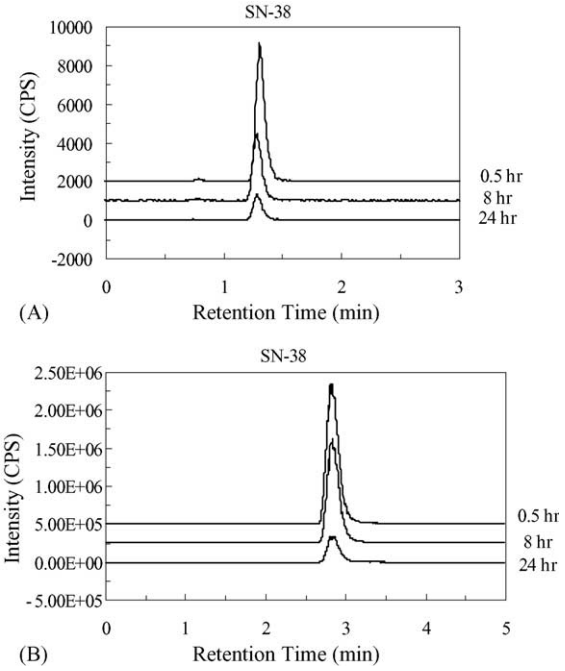

Fig. 4. Representative of MRM chromatogram of plasma (A) and liver homogenate (B) samples collected from mice at 0.5, 8, and 24 h after a single intravenous dose (10 mg/kg) of liposome entrapped SN-38 (LE-SN38). (Plasma samples were diluted 25- and 2-folds at 0.5 and 8 h, respectively, and liver homogenate samples were diluted 10-folds).

laboratory rodent diet. Mice were randomized according to body weight, grouped three per time point, and administered a single 10 mg/kg i.v. dose of LE-SN38 via the tail vein. At predefined time points, blood samples were collected by terminal retro-orbital bleeding under CO_2 anesthesia into pre-labeled, chilled micro tubes containing heparin as an anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Immediately after collection, each blood sample was gently inverted several times to ensure complete mixing with the anticoagulant, and placed on ice. The blood samples were centrifuged for 10 min at 4 °C and 3000 × *g* to separate plasma, and the latter was transferred to cryotubes. Heart, kidneys, liver, lungs, and spleen were rapidly excised following blood collection, and quickly placed on dry ice. All samples were stored at −70 ± 10 °C until analysis.

3. Results and discussion

3.1. Assay development

SN-38 and CPT (IS) were first characterized by MS and MS/MS, respectively, through infusion to ascertain their precursor ions and to select product ions for use in multiple reactions monitoring (MRM) mode. Both SN-38 and IS were found to give the most intense deprotonated molecular ions under positive ionization. Full scan spectra produced a predominant peak for $(MH)^+$ at *m/z* 393.3 for SN-38. Collision

Table 2
Validation standards of SN-38 in mouse plasma spiked with LE-SN38

Nominal concentration[a] (ng/mL)	Observed mean concentration (ng/mL)	Coefficient of variation (% CV)	Analytical recovery (% AR)
0.500	0.509	12.1	102
2.00	1.99	9.17	99.4
5.00	5.02	10.7	100
10.0	9.70	12.7	97.0
50.0	49.9	8.97	100
100	101	10.5	101
200	201	7.98	101
500	512	8.06	102
1000	997	8.98	99.7

[a] Minimum of six replicates at each concentration.

Table 3
Between-run and within-run results of SN-38 in mouse plasma QC samples spiked with LE-SN38

	Nominal concentration[a] (ng/mL)	Observed mean concentration (ng/mL)	Coefficient of variation (% CV)	Analytical recovery (% AR)
Between-run	1.50	1.62	12.2	108
	6.00	6.44	11.3	107
	400	420	9.88	105
	800	827	10.2	103
Within-run	1.50	1.53	14.7	102
	6.00	2.77	6.67	92.4
	400	461	6.79	115
	800	893	11.4	112

[a] Minimum of six replicates at each concentration.

Table 4
Dilution effect for SN-38 in mouse plasma spiked with LE-SN38

Nominal concentration[a] (ng/mL)	Dilution factor	Observed mean concentration (ng/mL)	Coefficient of variation (% CV)	Analytical recovery (% AR)
400	2	431	7.40	108
16000	20	17183	6.43	107
80000	100	87933	4.90	110

[a] Minimum of six replicates at each concentration.

induced dissociation of the $(MH)^+$ ion produced a major fragment at *m/z* 349.1, as shown in Fig. 2. Therefore, the transition pair 393.3 → 349.1 was optimized for SN-38. Using similar procedures, the precursor ion of IS was determined to be the base peak $(MH)^+$ ion at *m/z* 349.1, and the MRM transition of IS was determined to be *m/z* 349.1 → 305.2. The fragments used for SN-38 and IS in MRM transition mode resulted due to the loss of CO_2 (−44 U) from the respective protonated precursor ions (Fig. 2).

3.2. Assay validation

3.2.1. Plasma

The standard curve was established by plotting the ratio of the peak area of SN-38 to that of the IS with linear-regression correlation coefficients of ≥0.99 in all validation runs. The concentration of each standard calculated from the standard curve showed minimum deviation from its corresponding nominal concentration. Standard curves generated acceptable data over the concentration range of 0.5–1000 ng/mL plasma spiked with LE-SN38, as shown in Table 2. The % CV (precision) ranged from 8 to 12%, whereas the accuracy (% AR) ranged from 97.0 to 102%.

The LLOQ, determined to be 0.5 ng/mL in plasma, met the acceptance criteria with a % CV (precision) of 18.7% and % AR (accuracy) of 86.2%. Fig. 3A illustrates representative MRM chromatograms of blank plasma without or with IS, and 0.5 ng/mL SN-38 in plasma with IS. No interference was observed at or near the retention time of SN-38 when the selected MRM transition pair was monitored.

A summary of the between-run and within-run precision and accuracy data generated for the assay validation is presented in Table 3. The method was shown to be accurate, with an analytical recovery at the four tested concentrations within 100 ± 15%, and precise with a % CV within 15%.

Six replicates of three concentrations representing 2-, 20-, and 100-fold dilutions were analyzed. The results show that samples with concentrations up to 80,000 ng/mL can be analyzed by proper dilution with acceptable precision and accuracy (Table 4).

SN-38 was shown to be stable in LE-SN38 spiked plasma through three freeze-thaw cycles. It was also demonstrated

Table 5
Stabilities of SN-38 in mouse plasma spiked with LE-SN38

	Nominal concentration[a] (ng/mL)	Observed mean concentration (ng/mL)	Coefficient of variation (% CV)	Analytical recovery (% AR)
Three freeze-thaw cycles	6.00	5.89	14.7	98.1
	400	412	12.9	103
	800	854	10.8	107
4 h Room temperature storage	6.00	6.47	11.1	108
	400	425	6.66	106
	800	855	8.62	107
31 Days at −70 ± 10 °C	6.00	5.71	6.44	95.2
	400	408	2.65	102
	800	826	3.24	103

[a] Minimum of six replicates at each concentration.

Table 6
Precision and accuracy for SN-38 standards in mouse tissue homogenates spiked with LE-SN38

Nominal concentration[a] (ng/mL)	1	2	5	50	100	200	300	400
Kidney								
Observed concentration (ng/mL)	1.03	1.93	5.04	45.1	98.6	205	302	391
Coefficient of variation (% CV)	8.54	5.20	12.1	7.07	6.59	5.61	7.63	2.98
Analytical recovery (% AR)	103	96.5	101	90.2	98.6	103	101	97.7
Liver								
Observed concentration (ng/mL)	1.04	2.03	4.98	45.1	101	215	282	381
Coefficient of variation (% CV)	13.4	6.25	6.39	7.07	3.81	5.80	7.95	3.67
Analytical recovery (% AR)	104	101	99.6	90.2	101	108	94.0	95.2
Spleen								
Observed concentration (ng/mL)	1.01	2.02	4.82	48.8	94.9	206	296	384
Coefficient of variation (% CV)	6.68	1.97	6.06	4.39	6.00	4.29	4.64	5.33
Analytical recovery (% AR)	101	101	96.4	97.6	94.9	103	98.6	96.0
Lung								
Observed concentration (ng/mL)	0.990	1.95	4.83	48.8	101	216	295	376
Coefficient of variation (% CV)	11.3	8.86	8.38	4.39	4.73	5.30	5.83	4.94
Analytical recovery (% AR)	99.0	97.5	96.6	97.6	101	108	98.3	94.0
Heart								
Observed concentration (ng/mL)	NA	1.99	5.12	52.3	101	206	297	387
Coefficient of variation (% CV)	NA	6.83	9.21	8.27	7.37	5.61	7.27	11.1
Analytical recovery (% AR)	NA	99.5	103	105	101	103	99.0	96.7

ND: not applicable.
[a] Minimum of six replicates at each concentration.

that SN-38 is stable in mouse plasma for at least 4 h at room temperature, and for at least 31 days at $-70 \pm 10\,^{\circ}C$ (Table 5).

3.2.2. Tissues

The linearity range for SN-38 was 1–400 ng/mL in liver, kidney, spleen, and lung homogenates and 2–400 ng/mL in heart homogenate with correlation coefficients >0.99. Fig. 3B illustrates representative MRM chromatograms of control liver homogenate without or with IS, and 1 ng/mL SN-38 in control liver homogenate spiked with LE-SN38 and IS. No interference was observed at or near the retention time of SN-38 when the selected MRM transition pair was monitored.

Table 7
Precision and accuracy for SN-38 quality control samples in mouse tissue homogenates spiked with LE-SN38

Nominal concentration[a] (ng/mL)	3	15	150	350
Kidney				
Observed concentration (ng/mL)	2.97	13.7	143	340
Coefficient of variation (% CV)	2.83	2.97	5.32	7.02
Analytical recovery (% AR)	99.0	91.3	95.3	97.1
Liver				
Observed concentration (ng/mL)	3.17	15.3	154	366
Coefficient of variation (% CV)	5.52	3.71	9.14	10.4
Analytical recovery (% AR)	106	102	103	105
Spleen				
Observed concentration (ng/mL)	3.15	14.4	145	347
Coefficient of variation (% CV)	5.45	9.14	9.53	1.18
Analytical recovery (% AR)	105	96.0	96.7	99.1
Lung				
Observed concentration (ng/mL)	3.11	15.9	149	322
Coefficient of variation (% CV)	6.18	3.88	6.44	2.84
Analytical recovery (% AR)	104	106	99.3	92.0
Heart				
Observed concentration (ng/mL)	2.78	13.3	137	326
Coefficient of variation (% CV)	6.37	3.65	6.12	7.94
Analytical recovery (% AR)	92.7	88.7	91.3	93.1

[a] Minimum of six replicates at each concentration.

Standard curves generated acceptable data in mouse tissue homogenates spiked with LE-SN38 as shown in Table 6. An acceptable precision and accuracy for quality control samples in mouse tissue homogenates are presented in Table 7.

SN-38 was shown to be stable in tissue homogenate through three freeze-thaw cycles, at least 4 h at room temperature, and at least 31 days at $-70 \pm 10\,^{\circ}C$ (data not shown).

3.3. Application

The LC/MS/MS method described here was used to quantify SN-38 in plasma and tissue samples obtained from a pharmacokinetic and tissue distribution study of LE-SN38 in mice. Fig. 4A and B show MRM chromatograms for SN-38 acquired from plasma and liver samples, respectively, obtained at 0.5, 8 and 24 h after intravenous administration of 10 mg/kg LE-SN38 to mice. The mean SN-38 concentrations were 1150, 32.5, and 5.07 ng/mL, respectively, in plasma samples obtained at 0.5, 8 and 24 h after LE-SN38 administration. Similarly, the mean SN-38 concentrations were 2195, 975 and 338 ng/mL liver homogenate corresponding to 43.9, 19.5, and 6.76 μg/g tissue in liver samples obtained at 0.5, 8 and 24 h after LE-SN38 administration.

4. Conclusion

The LC/MS/MS method presented here for SN-38 quantification in mouse plasma and tissue homogenates is the first procedure for quantitative determination of total SN-38 in the presence of LE-SN38. In plasma, the LLOQ is 0.5 ng/mL and assay is linear over a concentration range of 0.5–1000 ng/mL. Additionally, the method requires only 100 μL plasma for SN-38 quantification. The sample preparation is rapid and involves one-step protein precipitation compared to other tedious methods employing liquid–liquid or solid phase extraction. This procedure allows for timely analysis of large numbers of samples. The sample preparation procedure can also be applied to tissue homogenates. Overall, the LC/MS/MS method is simple, sensitive, precise, and accurate to quantitate SN-38 in mouse plasma and tissue samples in the presence of LE-SN38. The method was used to support a LE-SN38 pharmacokinetic and tissue distribution study in mice.

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