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Short communication

Quantification of sunitinib in mouse plasma, brain tumor and normal brain using liquid chromatography–electrospray ionization-tandem mass spectrometry and pharmacokinetic application

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ABSTRACT

The primary challenge associated with the development of an assay method for the determination of drug concentrations in relatively small amount of mouse plasma and tissue samples is to improve extraction efficiency and detection sensitivity. In this work, a liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based method combined with protein precipitation, liquid–liquid extraction and solid-phase extraction techniques was developed for the determination of sunitinib in mouse plasma, brain tumor and normal brain tissue, respectively. The instrument was operated under the multiple reaction monitoring (MRM) mode using electrospray ionization (ESI) in the positive ion mode. A good linear relationship with coefficients of determination ≥0.99 was achieved over the concentration ranges of 1.37–1000 ng/mL for plasma and 4.12–1000 ng/g for the normal brain and brain tumor. The limits of quantification (LOQs) for sunitinib in mouse plasma, brain tumor and normal brain tissue are 1.37 ng/mL, 4.12 ng/g and 4.12 ng/g, respectively. The reproducibility of the LC–MS/MS method is reliable, with the intra- and inter-day precision being less than 15% and accuracy within ±15%. The established method was successfully applied to the characterization of sunitinib disposition in the brain and brain tumor as well as its systemic pharmacokinetics in a murine orthotopic glioma model.

1. Introduction

Characterization of drug disposition at the site of action can provide enhanced insight into pertinent pharmacokinetic–pharmacodynamic relationships, thus providing a quantitative basis to design and adjust therapies. In the context of optimizing chemotherapeutic dosing regimens for the treatment of cancer, measurements of drug concentrations in tumor would be of great value to the translation of preclinical data to the clinic.

Sunitinib (*N*-(2-diethylaminoethyl)-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide) is an oral multitargeted tyrosine kinase inhibitor with antiangiogenic and antitumor activities attributable to the inhibition of several related tyrosine kinase receptors, including vascular endothelial growth factor receptors (VEGFRs) types 1 and 2 (FLT1 and FLK1/KDR), platelet-derived growth factor receptors (PDGFR-α and PDGFR-β), stem cell factor receptor (c-KIT), and FMS-related tyrosine kinase 3 (FLT-3), which are implicated in tumor proliferation, angiogenesis, and metastasis [1–3]. The systemic pharmacokinetics of sunitinib has been characterized in human [4,5] and in animals [6,7]. However, little is known about the kinetics of sunitinib distribution in tissues.

Liquid chromatography with tandem mass spectrometry (LC–MS/MS) based on atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) is one of the commonly used tools to quantify drugs and their metabolites in biological matrices to support the pharmacokinetic characterization of drugs due to its specificity, selectivity and sensitivity. The determination of sunitinib in human plasma [8,9] and monkey tissues [10] by LC–MS/MS with liquid–liquid extraction has been reported. Barattè et al. [10] for the first time developed the method for determining the levels of sunitinib and its metabolite in several monkey tissues including liver, kidney, brain and white fat homogenates using LC–MS/MS following semi-automated liquid–liquid extraction. This assay employed a commercially unavailable reagent, the stable labeled sunitinib, as the internal standard. Minkin et al. [8]

Abbreviations: LC–MS/MS, liquid chromatography–tandem mass spectrometry; MRM, multiple reaction monitoring; LOQ, limit of quantification; ESI, electrospray ionization; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; c-KIT, stem cell factor receptor; APCI, atmospheric pressure chemical ionization; MTBE, methyl tert-butyl ether; DMSO, dimethyl sulfoxide; PK, pharmacokinetic; IS, internal standard; QC, quality control; EP, entrance potential; DP, declustering potential; CE, collision energy; CXP, collision-cell exit potential.

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reported a LC–MS/MS assay method for determination of sunitinib in human plasma, in which the sample preparation involved a liquid–liquid extraction by the addition of 0.2 mL of plasma with 4.0 mL methyl tert-butyl ether (MTBE) extraction solution. For our planned mouse pharmacokinetic studies, an LC–MS/MS assay that could be applied to small sample volumes and to both plasma and tissue samples was required.

Targeting tumor vessels is thought to be an attractive strategy for the treatment of glioblastomas, given the characteristic high degree of endothelial cell proliferation, vascular permeability and pro-angiogenic growth factor expression in the malignant brain tumors. Sunitinib has demonstrated potential activity against glioblastomas in preclinical studies when used as a single agent or in combination with cytotoxic drugs [6,11,12]. However, there is no information concerning the disposition of sunitinib in the brain and brain tumor in relative to its systemic pharmacokinetics. In support of our preclinical evaluation of the pharmacokinetic (PK) characteristics of sunitinib in a murine orthotopic glioma model, an LC–MS/MS assay method using relatively small amount of biological samples was developed and validated for the determination of sunitinib concentrations in mouse plasma, normal brain and brain tumor.

2. Material and methods

2.1. Chemicals and solvents

Sunitinib was supplied by Dr. M.V. Reddy (Fels Institute for Cancer Research, Temple University, Philadelphia, PA, USA). Ammonium hydroxide (~5N), ammonium acetate, acetic acid, camptothecin and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). HPLC-grade acetonitrile, methanol and MTBE were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water (~18 MΩ) (Nanopure deionization system, Barnstead/Thermolyne, Dubuque, IA, USA) was used for all aqueous solutions.

2.2. Preparation of stock solution, calibration standards and quality control samples

Stock solutions of sunitinib and camptothecin (the internal standard (IS)) were prepared separately in DMSO at a target concentration of 1 mg/mL as free base and diluted in methanol to create stock working solutions of sunitinib at a concentration of 0.4 mg/mL and the IS at concentrations of 20 and 1000 ng/mL. The stock working solution of sunitinib was then used to prepare calibration standards and quality control (QC) samples in individual biological matrices.

Blank plasma, normal brain and brain tumor samples were obtained from untreated nude mice bearing intracerebral U87 human glioma xenografts. To each gram of normal brain and brain tumor tissue was added 3 and 5 mL of deionized water, respectively. Tissue homogenization was carried out using a Polytron PT2100 homogenizer. The same matrix from all untreated animals was pooled and used as the control matrix for preparation of standard curves and QCs.

Calibration standards were prepared by spiking mouse plasma, normal brain and brain tumor homogenate with the stock standard working solution, which was further diluted with the matched-matrix to give seven calibration standards in the concentration range of 1.37–1000 ng/mL for plasma, and six calibration standards in the range of 4.12–1000 ng/g for the normal brain and brain tumor. Similar to calibration standards, QC samples were prepared in replicates ($n = 3$ and 5 for the intra-day and inter-day validation, respectively) at three concentration levels representing the entire range of concentrations (1.37, 111.1 and 1000 ng/mL for plasma 4.12, 111.1 and 1000 ng/g for normal brain and brain tumor).

2.3. Extraction procedure

2.3.1. Plasma

Sunitinib and the IS were isolated from plasma using protein precipitation. To 10 μL of plasma sample aliquots were added 10 μL of the IS solution (20 ng/mL of camptothecin in methanol) and 20 μL of methanol containing 0.1% acetic acid. The samples were vortex-mixed for approximately 15 s and centrifuged for 10 min at 22,000 × *g*. An aliquot of the clear supernatant (~30 μL) was transferred to an amber glass autosampler vial with a 250-μL plastic insert and subject to the LC–MS/MS assay. The injection volume was 10 μL.

2.3.2. Brain tumor

To 0.2 mL of the brain tumor homogenate were added 2 μL of the IS solution (1000 ng/mL of camptothecin in methanol) and 1 mL of MTBE. The mixture was vortex-mixed for 5 min. After centrifugation for 5 min at 22,000 × *g*, the supernatant organic layer was transferred into a clean micro-centrifuge tube. This solvent extraction procedure was repeated once more by adding 50 μL of 0.05N NH_4OH and 1 mL of MTBE to the aqueous layer. The two organic layer fractions were combined and evaporated to dryness under a gentle stream of nitrogen at 45 °C. The dried residue was then reconstituted with 50 μL of the mobile phase (see Section 2.4) and 10 μL was injected into the LC–MS/MS system.

2.3.3. Normal brain

To 0.2 mL of the normal brain homogenate were added 4 μL of the IS solution (1000 ng/mL of camptothecin in methanol) and 0.2 mL of methanol. The mixture was vortex-mixed for 5 min and centrifuged at 22,000 × *g* for 5 min. The supernatant was subject to the solid-phase extraction (SPE). The analytes were extracted from the normal brain homogenate using SPE cartridges with C8 sorbent (50 mg/1 mL Bond Elut C8; Varian Inc., Lake Forest, CA, USA). Sorbent was conditioned with 2.0 mL of methanol and equilibrated with 2.0 mL of water. The cartridge was washed with 1.0 mL of water followed by 1.0 mL of 20% methanol. The analytes were eluted with 0.4 mL of methanol containing 0.1% acetic acid. The eluent was evaporated to dryness with nitrogen gas at 45 °C and the sample reconstituted with 50 μL of mobile phase and 10 μL was injected into the LC–MS/MS system.

2.4. LC–MS/MS conditions

The LC–MS/MS configuration was analogous for plasma, normal brain and brain tumor samples. The LC–MS/MS assay was carried out using an Agilent series 1100 high-performance liquid chromatography system equipped with a binary pump, autosampler and degasser coupled to an API 4000 triple-quadrupole tandem mass spectrometer from Applied Biosystems/MDS SCIEX with ESI source operated in the positive ion mode. Analyst software version 1.4.2 (Applied Biosystems/MDS SCIEX) was used for instrument control, data acquisition and data processing for both chromatography and mass spectrometry.

Separation was achieved on a 50 mm × 2.0 mm Luna 3 μm C8 column (Phenomenex Inc., Torrance, CA, USA) with a pre-column of the same material. The sample solutions (10 μL) were injected and the analytes were eluted using acetonitrile/1 mM ammonium acetate containing 0.1% acetic acid (28:72, v/v) at a flow rate fixed at 0.3 mL/min. The isocratic separation run was completed within 3.2 min at 30 °C.

The ESI instrumental settings were optimized for the analysis and the appropriate MRM transitions and MS/MS parameters were

Table 1
Optimized ESI–MS/MS operating, MRM and MS/MS parameters for sunitinib and the internal standard.

Operating parameters	Setting	
Collision gas (psi)	5	
Curtain gas (psi)	40	
Ion source gas 1 (psi)	55	
Ion source gas 2 (psi)	55	
Ion spray voltage (V)	5500	
Temperature (°C)	450	
EP (V)	10	
Run duration (min)	3	
	Sunitinib	Camptothecin (IS)
Precursor ion (*m*/*z*)	399	349
Product ion (*m*/*z*)	326	305
Dwell time (ms)	400	400
DP (V)	86	81
CE (V)	31	31
CXP (V)	10	8

determined for individual compounds by direct infusion into the mass spectrometer. The optimized tandem mass spectrometry conditions are summarized in Table 1. Nitrogen was used as the curtain, collision and ion source gas.

2.5. Validation study

The validation study was carried out for sunitinib in three biological matrices of mouse: plasma, normal brain and brain tumor. Linearity of the method was evaluated in five sets of matrix-matched calibration standards. It was considered satisfactory when coefficients of determination (R^2) were higher than 0.99.

The extraction efficiency of the analyte was determined in triplicate at three concentration levels for each biological matrix by comparing the peak areas of the extracted QC samples ($N = 20$ for each concentration in each biomatrix) with those spiked in the reconstituted blank extracts after extraction.

Intra-day accuracy and precision were determined in sextuplicate by analyzing QC samples at low, medium and high concentrations across the linear range. Inter-day accuracy and precision were evaluated on five separate days. Precision was expressed as the relative standard deviation of the determined concentrations. Accuracy was calculated using the following equation: [(mean measured concentration − nominal concentration)/nominal concentration] × 100. Precision less than 15% and accuracy within ±15% were accepted.

The stability of sunitinib in spiked mouse plasma, normal brain and brain tumor homogenate after freeze/thaw cycles from −80 °C to ambient temperature was assessed in triplicates by comparing the freshly prepared QC samples with those being frozen and thawed three times with each freeze cycle lasting at least 24 h.

2.6. Sunitinib treatment and sample collection

Male NIH Swiss nude mice (nu/nu, 8–10 weeks old) were purchased from Taconic Farms (Germantown, NY, USA). All animal experiments were approved by the Institutional Animal Care and Use Committee and performed according to the guidelines of the National Institutes of Health. The orthotopic murine model of human glioma was established by stereotactically injecting U87MG human glioma cells into the left caudate putamen as previously described [12]. A single dose of 20 mg/kg sunitinib was given as a 20-min IV infusion to the tumor-bearing animals exhibiting a total weight loss of 2 g over two consecutive days. Serial blood samples were taken from the cannulated carotid artery prior to infusion and at 5, 10 and 30 min, and 1, 2, 4, 6, 8 and 12 h after end of infusion. Blood samples were immediately separated by centrifugation and the plasma was stored at −80 °C until the LC–MS/MS analysis. Immediately after the last time point of blood sampling, animals were anesthetized and sacrificed by terminal blood sampling from the vena cava. Brain tumor and normal brain tissues were snap frozen on dry ice and stored at −80 °C until the LC–MS/MS analysis. PK data analysis was performed using the software package WinNonlin Version 5.1.2 (Pharsight, Mountain View, CA). Systemic pharmacokinetics of sunitinib were analyzed by standard non-compartmental methods that yielded individual animal estimates of total clearance, the volume of distribution at steady-state, the elimination half-life, as well as the area under the sunitinib plasma concentration–time curves from time 0 to infinity ($AUC_{0-\infty}$).

Table 2
Extraction efficiency for sunitinib in mouse plasma, normal brain and brain tumor at three quality control concentrations.

Nominal concentration (ng/mL[a], ng/g[b])	Extraction efficiency (%, $N = 20$)					
	Plasma		Normal brain		Brain tumor	
	Mean	CV	Mean	CV	Mean	CV
1.4[a] or 4.1[b]	96.1	11.6	89.2	8.3	40.3	13.9
111.1	93.1	8.5	84.9	10.4	42.4	12.6
1000.0	94.4	9.7	83.1	9.6	43.5	8.7

[a] Sunitinib in plasma.
[b] Sunitinib in normal brain and brain tumor.

3. Results and discussion

3.1. Mass spectrometry and chromatography

ESI operated in positive ion mode was used for the LC–MS/MS analysis to provide optimum sensitivity and selectivity. The mass spectrum of sunitinib showed protonated molecular ions ($[M+H]^+$) at *m*/*z* 399. One of the major fragments observed was at *m*/*z* 326, which was selected for subsequent monitoring in the third quadrupole. The mass spectrum of the IS, camptothecin, showed a $[M+H]^+$ at *m*/*z* 349, and the high collision energy gave one major product ion at *m*/*z* 305 (Table 1).

Under the optimal LC conditions, sunitinib eluted at 1.73–1.93 min, and the IS at 1.90–2.10 min, with a total chromatographic run time within 3 min. Carryover was not obvious in either blank matrices or zero-level standard (blank with the IS). Representative chromatograms of extracted blanks and sunitinib PK study samples in mouse plasma, normal brain and brain tumor are presented in Fig. 1.

3.2. Sample preparation and extraction efficiency

In mice bearing indwelling cannulas for serial blood sampling to characterize the PK of a given drug, the blood sample volume is limited to ∼20 μL at each time point. In this regard, protein precipitation was selected in this study as the sample preparation method for determination of sunitinib in mouse plasma. The extraction efficiency of sunitinib at various concentrations from mouse plasma using protein precipitation was 93–96% (Table 2), which was higher than the reported sunitinib extraction efficiency from human plasma using liquid–liquid extraction (i.e. 39–46%) [8]. The mean extraction efficiency for the IS was 93.0% (CV%, 6.2; $N = 20$).

The relatively more complex nature of the tumor tissue samples prevented the successful application of a protein precipitation method, and thus, a liquid–liquid extraction procedure was applied as sample pretreatment in order to improve the selectivity and sensitivity of the LC–MS/MS assay. Sunitinib is a weak base with the reported solubility of 3022 μg/mL at pH 2 and 511 μg/mL at

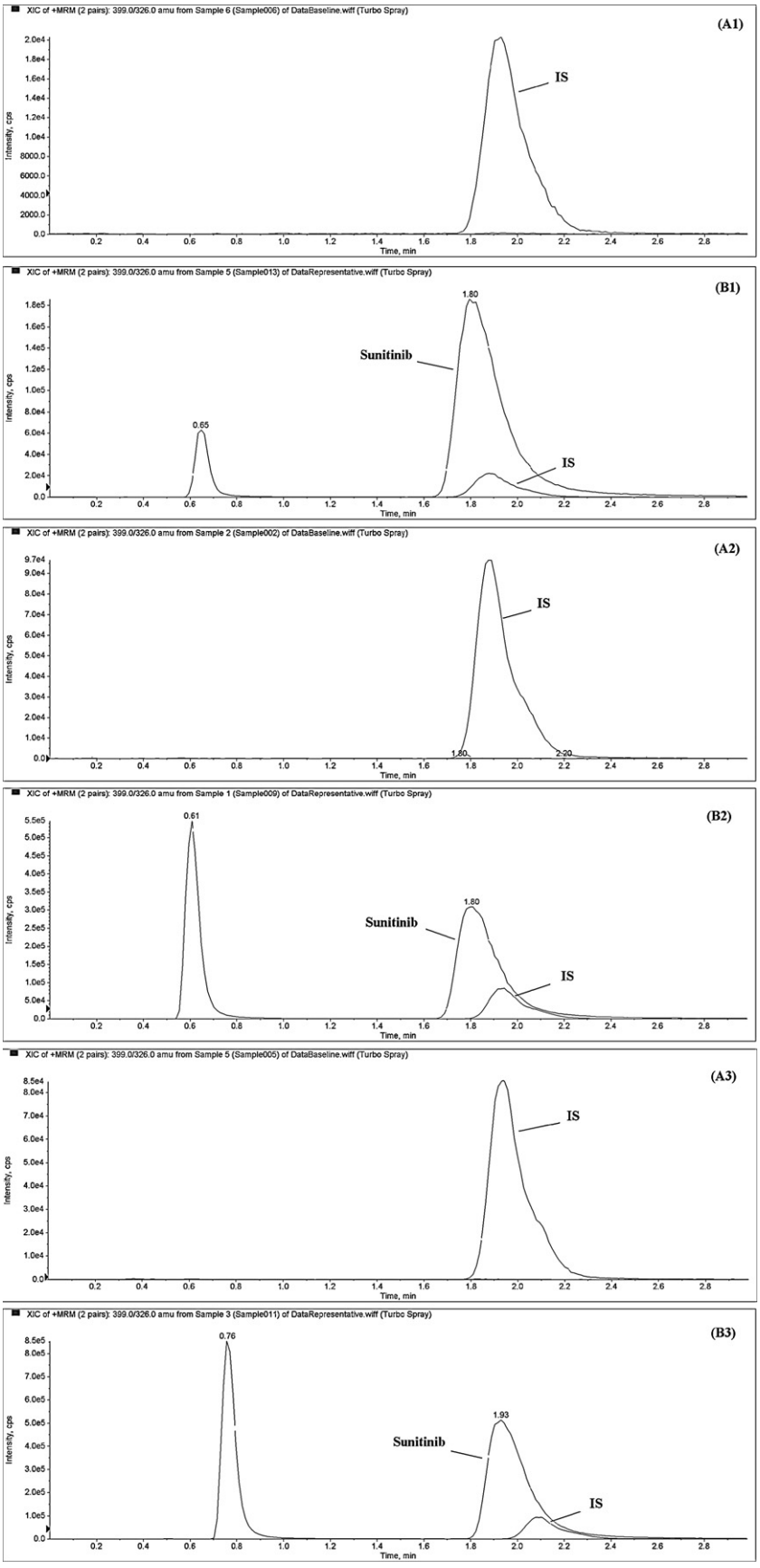

Fig. 1. Representative chromatograms of zero-level sunitinib concentrations (A) and sunitinib PK study samples (B) in mouse plasma (1), normal brain (2) and brain tumor (3).

Table 3
Limit of quantification and calibration curve results ($N = 5$) for sunitinib in mouse plasma, normal brain and brain tumor.

Biological matrix	LOQ	Slope (mean ± SD)	*Y*-intercept (mean ± SD)	R^2 (min–max)
Plasma	1.372 ng/mL	0.1704 ± 0.0080	0.0533 ± 0.0378	0.9954–0.9994
Normal brain	4.115 ng/g	0.0265 ± 0.0063	0.0298 ± 0.0214	0.9903–0.9953
Brain tumor	4.115 ng/g	0.0740 ± 0.0053	0.1133 ± 0.0741	0.9925–0.9987

Table 4
Intra-day ($N = 6$) and inter-day ($N = 5$) precision and accuracy for sunitinib in mouse plasma, normal brain and brain tumor.

Matrix	Nominal concentration	Precision (%)		Accuracy (%)			
		Intra-day	Inter-day	Intra-day		Inter-day	
				Mean	Range	Mean	Range
Plasma	1.4 ng/mL	6.0	4.9	4.9	−4.4 to 12.4	−1.6	−7.6 to 4.2
	111.1 ng/mL	1.6	2.5	3.2	0.9 to 5.4	7.9	5.7 to 12.7
	1000.0 ng/mL	2.7	1.7	−13.1	−14.9 to −8.4	−12.0	−13.1 to −9.4
Normal brain	4.1 ng/g	3.3	8.4	11.2	4.1 to 13.8	1.3	−10.6 to 10.3
	111.1 ng/g	11.7	4.9	5.8	−12.2 to 14.4	8.3	0.6 to 13.3
	1000.0 ng/g	10.6	2.6	3.3	−13.9 to 15.0	−10.0	−13.5 to −7.7
Brain tumor	4.1 ng/g	7.2	7.8	6.7	−6.1 to 13.3	−1.4	−13.2 to 4.8
	111.1 ng/g	4.7	6.1	3.8	−2.7 to 9.0	1.7	−9.1 to 6.7
	1000.0 ng/g	7.7	8.0	−1.2	−6.3 to 14.0	−6.2	−13.3 to 2.3

pH 6 [13]. In this study, alkalization of the sample with the addition of 0.05N of NH_4OH followed by the extraction with MTBE was found to improve drastically the recovery of sunitinib from tumor homogenates, whereas the recovery of the IS was somehow decreased (data not shown). Therefore, the two-step liquid–liquid extraction scheme with the addition of 0.05N NH_4OH in the second step was employed to achieve the maximum extraction efficiency for both sunitinib and the IS. The resultant mean extraction efficiencies were 83–89% for sunitinib (Table 2) and 51.3% for the IS (CV%, 4.7; $N = 20$).

Protein precipitation and liquid–liquid extraction were initially considered for the isolation of sunitinib and the IS from normal brain homogenates. However, satisfactory results were not obtained for the peak shape and recovery. This may be attributable to the non-specific binding of sunitinib to the brain tissue, which is known to be rich in fats, lipids and proteins, as sunitinib is a highly lipophilic compound with a partition coefficient ($\log P$) value of 5.2 [14]. The SPE method was thus used in an attempt to improve the extraction efficiency. The C8 sorbent was found to give satisfactory results for sample cleanup and recovery for both sunitinib and the IS although the extraction efficiency of sunitinib was relatively low (i.e., 40–44%) (Table 2). This may be due to the complex nature of the brain tissue. The mean extraction efficiency for the IS from brain homogenates was 29.6% (CV%, 10.5; $N = 20$). Overall, the extraction efficiency of sunitinib was concentration-independent irrespective of the matrices used.

3.3. Method validation

3.3.1. Linearity and LOQ

In this study, the calculated peak area ratios of sunitinib to camptothecin versus the nominal concentration of the analyte displayed a good linear relationship with coefficients of determination ≥0.99 over the concentration ranges of 1.37–1000 ng/mL for plasma and 4.12–1000 ng/g for the normal brain and brain tumor using a weighting factor of $1/x^2$ (Table 3). The LOQs were established at the lowest points of the standard curves, i.e., 1.37 ng/mL for plasma, 4.12 ng/g for normal brain and brain tumor (Table 3).

3.3.2. Accuracy and precision

The results of intra-day and inter-day accuracy and precision are presented in Table 4. The intra- and inter-day precisions for all three matrices of interest were less than 11.7%. Over the range of concentrations from 1.37 to 1000 ng/mL for plasma, 4.12 to 1000 ng/mL for normal brain and brain tumor, the average intra-day accuracies ranged from −13.1% to 11.2%, and inter-day accuracy ranged from −12.0% to 8.3% (Table 4). The results indicate that the precision and accuracy of this method are adequate for bio-analytical purposes.

3.3.3. Freeze/thaw cycle stability

The aim of the stability study was to obtain information on the stability of the analyte in the matrix of interest during the multiple freeze/thaw cycles. The percentage decrease in analyte peak area between the freshly prepared sunitinib-spiked samples and those after three freeze–thaw cycles was <15% in all tested samples (Table 5), suggesting that the reanalysis of sunitinib in plasma, normal brain and brain tumor samples, which may entail freeze–thaw cycles, can be undertaken if the number of cycles is three or less.

3.4. Application to pharmacokinetic study

The validated method was applied to the analysis of plasma, normal brain and brain tumor samples obtained from the intracerebral tumor-bearing nude mice receiving 20 mg/kg of sunitinib as a 20-min IV infusion. Prior to the animal sample analysis, the plasma and tumor homogenate samples were diluted 10 times with the corresponding blank matrices because some of the plasma and tumor sunitinib concentrations exceeded the standard curve

Table 5
Freeze/thaw cycle stability of sunitinib in mouse plasma, normal brain and brain tumor at three quality control concentrations.

Matrix	Nominal concentration	% Analyte decrease (mean, $N = 3$)		
		Cycle 1	Cycle 2	Cycle 3
Plasma	1.4 ng/mL	0.7	1.3	3.3
	111.1 ng/mL	0.7	7.6	12.6
	1000.0 ng/mL	7.5	12.0	13.0
Normal brain	4.1 ng/g	8.2	11.0	13.6
	111.1 ng/g	4.3	6.2	11.5
	1000.0 ng/g	2.8	8.9	11.0
Brain tumor	4.1 ng/g	1.6	7.8	13.7
	111.1 ng/g	4.3	8.1	14.2
	1000.0 ng/g	0.2	1.4	10.8

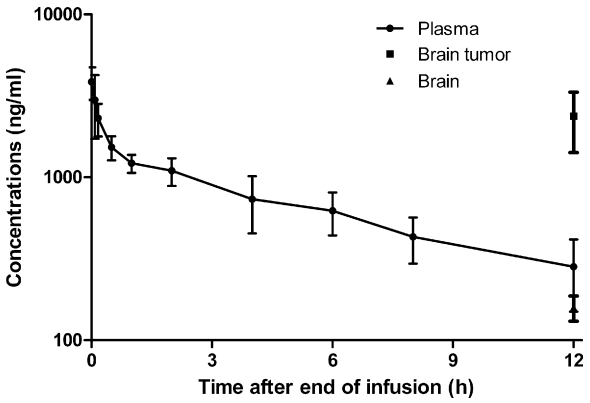

Fig. 2. Representative sunitinib 12-h plasma concentration profile and concentrations in brain tumor and normal brain at 12 h. Sunitinib concentrations in plasma (solid dots), brain tumor (solid square) and normal brain (solid triangle) were determined in intracerebral tumor-bearing mice ($N = 4$) receiving 20 mg/kg of sunitinib as a 20-min IV infusion. *Bars*, SD.

range. The mean plasma concentration–time profile of sunitinib and its concentrations in brain and brain tumor at 12 h are shown in Fig. 2. Following a 20-min IV infusion of 20 mg/kg sunitinib, the plasma concentration versus time profile of sunitinib declined rapidly within the first 1 h and was then gradually eliminated with an elimination half-life (mean ± SD, $N = 4$) of 5.11 ± 0.94 h. Sunitinib in mice exhibited extensive tissue distribution with a steady-state volume of distribution of 12.0 ± 2.4 L/kg. Total clearance was 1.86 ± 0.59 L/h/kg and the $AUC_{0-\infty}$ was 11.5 ± 3.2 μg h/mL. The concentration ratios of brain tumor to plasma, normal brain to plasma, and brain tumor to normal brain at 12 h were 8.4, 0.56 and 14.9, respectively. The observed high brain tumor to plasma concentration ratio are likely predicated upon compromised blood–brain barrier function in the brain tumor that enabled sunitinib to accumulate in brain tumor, possibly due to binding to tissue components.

In a recent study by Haouala et al. [9], sunitinib was separated as two distinct peaks corresponding to Z/E isomers using a gradient elution on a C18 analytical column. Sunitinib is a 5-fluoro-2-oxindole with an exocyclic double bond attaching the molecule to a dimethyl pyrrole carboxamide thereby showing geometric isomerism. Understanding the kinetics of sunitinib Z–E isomerism can help define the experimental conditions so that the ratio of Z- to E-isomer would be kept constant in processed samples. However, the kinetics of sunitinib Z–E isomerism have yet been published so far and characterization of such kinetics in biological matrices is beyond the scope of this study, Nonetheless, based on studies on the Z–E isomerism of SU5416 [15,16], an analogue of sunitinib, it is possible that, like SU5416, the solid substance of sunitinib may exist only as the Z-isomer, which is the thermodynamically stable form, whereas in solution it converts to the E-isomer following light exposure and reverts to the Z-isomer in the dark. Therefore, in order to minimize the isomerization of sunitinib, sample preparation was done under light-protected conditions, and LC sample vials were placed in the autosampler tray protected from light. Moreover, studies have shown that the Z-isomer of SU5416 is completely stable in acidified (pH 2) methanol or acetonitrile, while the E-isomer is not stable in solution and readily converted back to the Z-isomer when the solution is protected from light [15,17]. Therefore, in order to further stabilize the Z-isomer, we used methanol containing 0.1% acetic acid for protein precipitation, and the mobile phase consisting of 75% acetic buffer at pH about 2 for reconstituting residues obtained from the liquid–liquid and solid-phase extractions. The assay performance data for the determination of sunitinib in all three tested biological matrices suggest that the isomerization of sunitinib in the processed samples has no apparent influence on the sensitivity and repeatability of the assay.

4. Conclusions

A robust, sensitive and specific LC–MS/MS assay for sunitinib in mouse plasma, normal brain and brain tumor was developed and validated. In contrast to recently published sunitinib LC–MS/MS assay methods [8–10], the present method showed satisfactory extraction efficiency of sunitinib from a small volume of plasma using a simple and rapid protein precipitation and from tumor tissue homogenates using a two-step liquid–liquid extraction. The SPE method with adequate extraction efficiency developed for recovering sunitinib from brain tissue could be an option for extracting sunitinib from other biomatrices that are rich in fat, lipids and proteins when the liquid–liquid extraction is problematic. The established method can be used to study the systemic PK properties and brain distribution of sunitinib in intracerebral tumor-bearing mice.

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