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Development and validation of a highly rapid and sensitive LC–MS/MS method for determination of SZ-685C, an investigational marine anticancer agent, in rat plasma – Application to a pharmacokinetic study in rats

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ABSTRACT

A sensitive and rapid method was developed and validated for the quantitative analysis of the novel anticancer agent SZ-685C in rat plasma using high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) in negative ion mode in order to support the following pre-clinical and clinical studies. SZ-685C and the internal standard (IS, emodin) were extracted from rat plasma by a simple liquid–liquid extraction technique using ethyl acetate as extraction solvent. Chromatographic separation was performed on an Elite Hypersil BDS C18 column (100 mm × 2.1 mm i.d., 3 μm). Elution was carried out using methanol/acetonitrile/2 mM ammonium formate (pH 4) (80:15:5 (v/v/v)) at a flow-rate of 0.3 mL/min with a run time of 2.5 min. This assay was linear over a concentration range of 50–10,000 ng/mL with a lower limit of quantification of 50 ng/mL. The intra- and inter-batch precision was less than 15% for all quality control samples at concentrations of 100, 1000 and 7500 ng/mL. These results indicate that the method was efficient with a short run time and acceptable accuracy, precision and sensitivity. This method was successfully applied to explore pharmacokinetics of SZ-685C in rats after oral and intravenous administration of this agent. The absolute bioavailability is about 54.8–66.8% and the $t_{1/2}$ is 5.7–9.2 h, these results provide basic information for further comprehensive pre-clinical research.

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1. Introduction

SZ-685C, a marine anthraquinone, which is isolated from the secondary metabolites of the mangrove endophytic fungus No. 1403 collected from the South China Sea [1], is a potent inducer of apoptosis with anticancer activity by suppression of the Akt/FOXO pathway with IC_{50} values ranging from 3.0 to 9.6 mM [2]. The pharmacological and toxicological profiles of SZ-685C, as well as its in vivo effectiveness in inhibiting xenografted tumour growth, provide us reasonable optimism that the compound could become an attractive anti-Akt and anti-cancer drug candidate [2].

The importance of pharmacokinetics and drug metabolism (DMPK) has been gradually recognized by pharmaceutical research institutes and regulatory agencies in the past decade [3]. The detailed information and rationale for the metabolic pathway and pharmacokinetics must be clearly illustrated for drug candidates before they may be tested on human. More importantly, accurate and comprehensive understanding of pharmacokinetic and metabolic profiles of new chemical entities should always be provided to support drug discovery and development in a time-sensitive manner. DMPK research now plays a very important role in drug design and is conducted as early in the stages of preliminary biological/pharmacological screening [3]. In order to support the pre-clinical and clinical pharmacokinetic study of SZ-685C, a sensitive and rapid bioanalytical method is warranted.

In the present study, the first method for the quantitative analysis of SZ-685C in rat plasma using HPLC/MS/MS is developed and validated, and the applicability of the method is demonstrated by its implementation in pre-clinical pharmacokinetic studies in rats.

2. Experimental

2.1. Special precaution

All laboratory procedures involving the manipulation of SZ-685C were executed in a dimly lit environment in order to avoid photoisomerization.

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2.2. Chemicals and reagents

SZ-685C (98% purity as determined by HPLC) was obtained from Guangdong Province Key Laboratory of Functional Molecules in Oceanic Microorganism (Sun Yat-sen University) (Fig. 1a). The internal standard (Emodin, 98% purity) was purchased from Kuiqing Company (Tianjin, China) (Fig. 1b). Acetonitrile and methanol (gradient grade) were purchased from Dikma Technology Inc. (Markham, Ontario, Canada). Ultrapure water was prepared using a Milli-Q purification system (Barnstead International Inc., Dubuque, IO). Other reagents were of analytical-grade and purchased from Yonghua Ltd. (Shanghai, China). Agent-free rat plasma was obtained from pre-clinical trials.

2.3. Instrumentation and chromatographic conditions

A Finnigan Surveyor MS pump (San Jose, CA, USA) and a Finnigan Surveyor auto sampler were used for solvent and sample delivery. Chromatographic separation was achieved using a C18 column (Hypersil BDS C-18, i.d. 2.1 mm × 100 mm, 3 μm; Elite HPLC, China) at 25 °C. The mobile phase consisted of methanol/acetonitrile/2 mM ammonium formate in water (pH 4) (80:15:5 (v/v/v)), pumped at a flow-rate of 0.3 mL/min. Total run time was 2.5 min for each injection. A Finnigan TSQ Quantum triple-quadrupole mass spectrometer equipped with an ESI source was used for mass analysis and detection. Mass spectrometric analysis was performed in the negative ion mode (ESI^-) and set up in the multiple reaction monitoring (MRM) mode. Nitrogen was used as the sheath gas (30 psi) and the auxiliary gas (10 psi). The capillary temperature was 350 °C, and the spray voltage was 3500 V. Collision-induced dissociation (CID) studies were performed and argon was used as the collision gas with a collision cell gas pressure of 1.0 mTorr (1 Torr = 133.3 Pa).

The optimized source CID was 15 V for SZ-685C, and 10 V for IS (emodin). The optimized collision energy was 20 eV for both SZ-685C and IS. Based on the full-scan MS and MS/MS spectra of each analyte, the most abundant fragment ions were selected and the mass spectrometer was set to monitor the transitions of the precursors to the product ions as follows: m/z 335.0 → 247.0 for SZ-685C and m/z 268.8 → 224.9 for IS. The scanning time for each analyte was set to 0.3 s. Data acquisition was performed with Finnigan Xcalibur 1.4 software, while peak integration and calibration were processed with Finnigan Lcquan software.

2.4. Preparation of calibration standards and quality control samples

A stock solution of SZ-685C was prepared in methanol at a concentration of 500 μg/mL. This solution was further diluted with methanol to obtain working solutions with concentrations ranging from 0.5 to 100 μg/mL. All the solutions were stored at 4 °C and were brought to room temperature before use. Concentrations of the calibration standards ranged from 50 to 10,000 ng/mL. These working solutions were spiked into rat plasma resulting in quality control samples at three concentration levels (100, 1000 and 7500 ng/mL). Furthermore, quality control samples at the lower limit of quantification (LLOQ; 50 ng/mL) were prepared. A 500 ng/mL solution of the IS (emodin) was similarly prepared in methanol.

2.5. Sample processing

An aliquot of 10 μL of internal standard working solution was added to 100 μL of rat plasma in a 1.5 mL test tube, and the mixture was then vortex-mixed for 10 s. 1.0 mL ethyl acetate was added and the samples were vortexed for 1 min. After standing at room temperature for 3 min, the mixture was centrifuged at 2000 *g* for 5 min. The organic phase was then transferred to a clean brown tube and evaporated to dryness under nitrogen gas at 45 °C. The residues were dissolved in 150 μL methanol/water (50:50 (v/v)) and an aliquot (10 μL) of the reconstituent was injected onto the LC/MS/MS for analysis.

2.6. Method validation

The method validation assays were performed according to US Food and Drug Administration (FDA) bioanalytical method validation guide [4].

2.6.1. Selectivity, sensitivity and linearity

The selectivity was investigated by preparing and analyzing six individual rat blank plasma samples at the LLOQ. The LLOQ was defined as the lowest concentration on the calibration curve of the analytes measured with acceptable precision and accuracy (i.e., relative standard deviation (RSD) and relative error <20%) and with at least five times the response compared to blank response (noise).

The calibration curve was obtained by plotting the ratio of peak area of analyte to that of IS against the nominal concentration of calibration standards, including 50, 100, 500, 1000, 5000, 7500 and 10,000 ng/mL. The standard curve was fitted to linear regression ($y = ax + b$) using $1/x$ as weighting factor.

2.6.2. Accuracy and precision

The intra-run accuracy and precision were evaluated by QC samples at three concentration levels (100, 1000, and 7500 ng/mL). The inter-run accuracy and precision were determined by analyzing the QC samples at three concentrations on three different days. The precision was determined as the RSD or coefficient of variation (CV (%)) and the accuracy was expressed as a percentage of the measured concentration over the nominal (theoretical) concentration.

2.6.3. Stability

The stability of analyte in rat plasma and extraction solvent was estimated by QC samples at three concentrations mentioned previously, using six replicates for each concentration. The stability experiments of analyte included: (a) stability at room temperature for 4 h; (b) stability after three freeze–thaw cycles; (c) stability in the extraction solvent at 4 °C for 12 h; and (d) the long-term stability after storage at −20 °C for 3 weeks.

2.6.4. Recovery

The recovery of analyte and IS was determined by comparing the responses of the analytes from QC samples ($n = 6$) to the responses of analytes spiked in post-extracted blank rat plasma at equivalent concentrations. The recovery of analyte was determined at 100, 1000, and 7500 ng/mL concentrations.

2.6.5. Matrix effect

The absolute and relative matrix effect (ME) on the spectral response of SZ-685C was assessed as described by Matuszewski et al. [5] with slight modifications as mentioned previously [6]. Since ME is a concern with the fast isocratic system, the co-elution effect and potential ion suppression were evaluated. To assess the co-elution effect, pooled blank plasma was spiked with analyte or IS, and the corresponding peak area was compared to that from the spiked sample of combined IS and analyte. QC samples of the three concentrations were analyzed. To assess the 'absolute' ME, i.e., the potential ion suppression due to the matrix components, six different batches of blank plasma were extracted by ethyl acetate and then spiked with SZ-685C at 100, 1000, and 7500 ng/mL (B). The corresponding peak areas were then compared to those of the aqueous standards at equivalent concentrations (A). The peak area ratio of B/A (as a percentage) was used as a quantitative measure

of the matrix effect. A ME value of 100% indicates that the response in the mobile phase and in the plasma extracts was the same and no absolute matrix effect was observed. A value of >100% indicates ionization enhancement, and a value of <100% indicates ionization suppression.

2.6.6. Dilution effect

Dilution effect was investigated to ensure that samples could be diluted with blank matrix without affecting the final concentration. SZ-685C spiked rat plasma samples prepared at two concentrations (20 and 50 μg/mL) of SZ-685C were diluted with pooled rat plasma at dilution factors of 10 and 25 in six replicates and analyzed. The precision and accuracy of six replicates should be less than or equal to 15%, or 100 ± 15%, respectively, similar to other QC samples.

2.7. Pharmacokinetics study

Adult Sprague-Dawley rats (250–300 g) were obtained from the Animal Center of Sun Yat-sen University (Guangzhou, China) and maintained on a 12 h light–dark cycle with free assess to food and water for one week. On the day before the pharmacokinetic study, a polyethylene tube (i.d. 0.58 mm, o.d. 0.965 mm, Becton Dickinson, Sparks, MD, USA) was implanted into the right jugular vein through surgery. This catheter was used for blood sampling. Twenty-four rats were divided into four groups with three male and female rats in each. Group 1 received a single bolus intravenous injection of SZ-685C at the dose of 12.5 mg/kg through rat tail vein; serial blood samples (150 μL) were collected before dosing and at 2 min, 5 min, 10 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h and 24 h after intravenous administration. Groups 2, 3 and 4 received a single oral administration of SZ-685C at a dose of 25, 50 and 100 mg/kg through gavage, respectively. Serial blood samples were collected before dosing and at 5 min, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h and 36 h after oral dosing. After each intravenous dosing or blood sampling, 0.2 mL heparin-saline (10 IU/mL) was used to flush the catheter. The blood samples were centrifuged at 1500 g for 10 min and the plasma was collected and frozen at −20 °C. All plasma samples were analyzed within 3 weeks after sample collection. The study was approved by the Animal Ethics Committee of Sun Yat-sen University.

Rat plasma samples (100 μL) were thawed at room temperature and spiked with IS and processed as described above, then analyzed along with calibration standard samples and QC samples in duplicate at three concentrations. The criteria for acceptance of analytical runs are that two out of six QC samples were permitted beyond 15% of the nominal concentration but should not in the same concentration level. Non-compartmental pharmacokinetic parameter calculations were performed using the NONMEM Program version 1.1 (GloboMax Inc., Ellicott City, MD). The area under the plasma concentration–time curve (AUC) from time zero to the last quantifiable time point (AUC_{0-t}) and from time zero to infinity ($AUC_{0-\infty}$) were estimated using the log-linear trapezoidal rule. The elimination half-life ($t_{1/2\beta}$) was calculated as $0.693/\beta$ where β is the elimination rate constant calculated from the terminal linear portion of the log plasma concentration–time curve. The time to peak plasma concentration (T_{max}) and peak plasma concentration (C_{max}) was determined by visional inspection. The mean residence time (MRT) was calculated by the total area under the first-moment curve divided by AUC_{0-t}.

3. Results and discussion

3.1. HPLC–MS/MS system

In pharmacokinetic study, plasma samples are most frequently analyzed by liquid chromatography (LC) with UV detection or with mass spectrometry (MS). Compared with LC–UV, LC–MS/MS has better sensitivity, selectivity and higher throughput. Especially in drug discovery and development, tandem mass spectrometry has proven to be superior to LC–UV to characterize the parent drugs and their metabolites [7]. LC–MS/MS has become the method of choice for quantitation to support drug discovery, and has completely replaced high performance liquid chromatography (HPLC) with UV detection in this process.

3.1.1. MS/MS optimization

SZ-685C can be ionized under both positive and negative ionization conditions. The negative mode yielded a signal 20-fold higher for the deprotonated quasimolecule ion $[M-H]^-$ (m/z 335) than that for the protonated quasimolecule ion $[M+H]^+$ (m/z 337) in positive mode, $[M-H]^-$ was the predominant ion in the Q1 spectrum and was used as the precursor ion to obtain ion spectra. The full scan mass spectrum and product ion mass spectra of SZ-685C and IS are shown in Figs. 2 and 3. For SZ-685C, four species of major daughter ions (m/z 247.0, m/z 248.0, m/z 262.0 and m/z 233.0) were recorded, the fragment ion of m/z 247 was selected to monitor the transition because it was more abundant and reliable to measure in terms of signal to noise ratio. For the IS, emodin, a few species of daughter ions (m/z 224.9, m/z 240.9, m/z 210.0, m/z 196.6 and m/z 181.0) were recorded, but only m/z 224.9 was the most abundant and stable, therefore it was selected for monitoring its transition. The collision energy in the product MS/MS mode was also investigated from 2 to 30 eV to optimize the sensitivity, and the optimal values were 20 eV for both SZ-685C and IS.

3.1.2. Selection of IS

It is necessary to use an IS to obtain high accuracy and precision when a mass spectrometer is used as detector. Emodin (Fig. 1) was adopted as the IS, because of its similarity of retention time and ionization characteristics with those of the analyte, and because of the minimal endogenous interferences at product ion of $[M-H]^-$ with m/z 224.9.

3.1.3. Sample preparation

Sample preparation is a critical step for accurate and reliable LC–MS/MS assays. The most widely employed biological sample

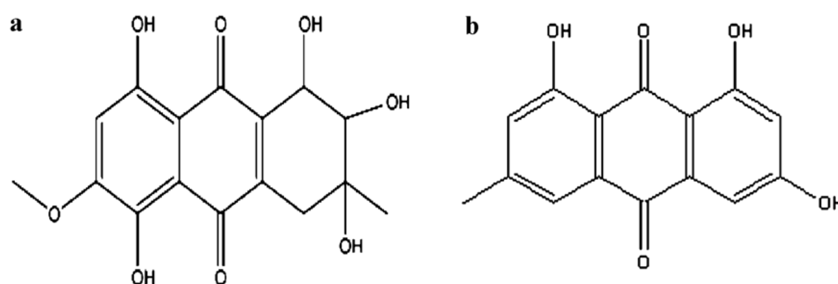

Fig. 1. Chemical structure of SZ-685C and IS (emodin).

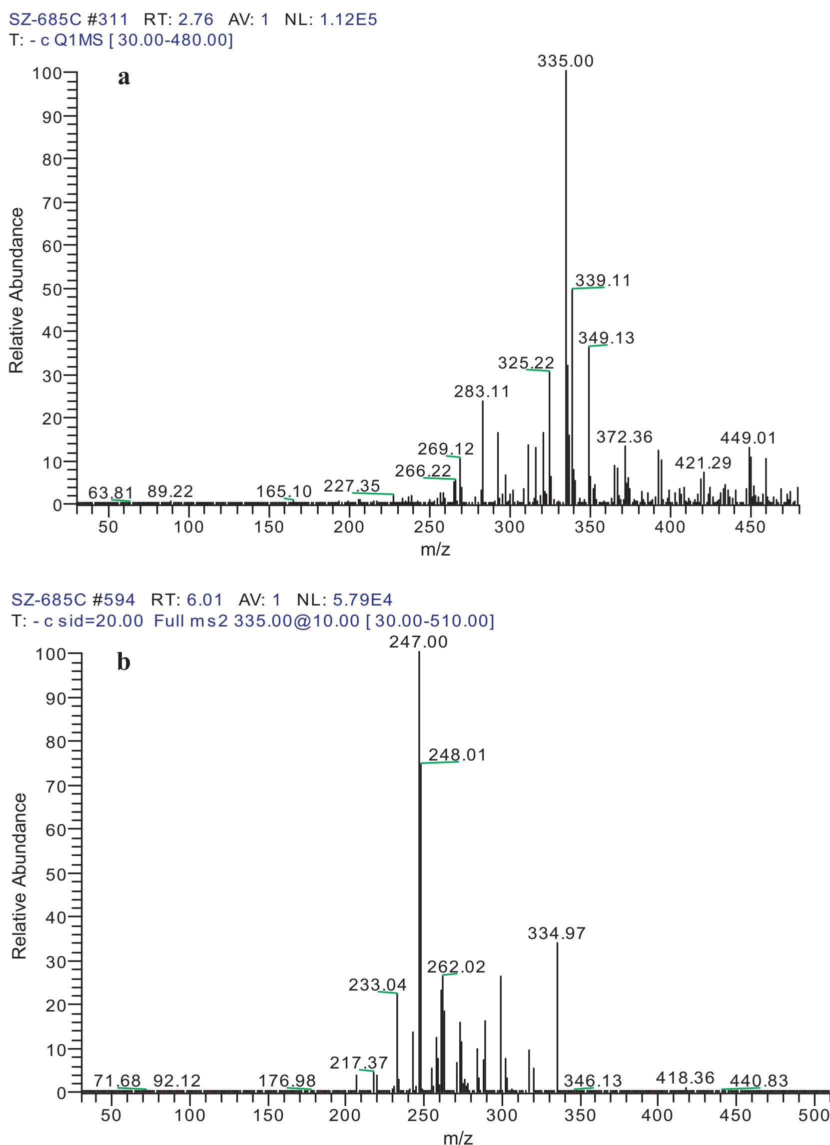

Fig. 2. (a) Negative-ion ESI mass spectrum for SZ-685C; (b) MS/MS product-ion spectrum of SZ-685C with $[M-H]^-$ at m/z 335 as the precursor ion.

preparation methodologies currently are liquid–liquid extraction (LLE), and protein precipitation (PPT). Compared with PPT, LLE can be more helpful in producing a relatively clean sample, enhancing the sensitivity, and avoiding the introduction of highly polar materials onto the column and MS system. We investigated several organic extraction solvents including ethyl ether, *n*-hexane, ethyl acetate, and dichloromethane in different pHs environment. It was found that ethyl acetate with no pH adjustment could yield the highest recovery (>75%) (Table 1).

3.2. Method validation

3.2.1. Selectivity and LLOQ

The selectivity of the method was demonstrated by comparing MRM chromatograms of blank samples with those of the corresponding samples spiked with SZ-685C and IS. Fig. 4 shows typical chromatograms for a blank rat plasma sample, a spiked plasma sample, and a rat plasma sample at 1 h after an intravenous administration of 5 mg/kg SZ-685C. There were no significant endogenous peaks directly interfering with the detection of analytes. The retention times of SZ-685C and IS were about 1.0 min and 1.6 min, respectively.

The present LC/MS/MS method provided an LLOQ of 50 ng/mL with an accuracy of 9.5% in terms of relative error (RE) and a precision of 9.0% in terms of RSD ($n = 6$). These data indicate that the

Table 1
Extraction efficiency in different sample preparation methods.

pH	Solvent	Recovery (%)
5	Ethyl ether	35.2
	n-Hexane	40.5
	Ethyl acetate	61.5
	Dichloromethane	53.3
7.4	Ethyl ether	45.1
	n-Hexane	43.2
	Ethyl acetate	78.8
	Dichloromethane	63.1
8.5	Ethyl ether	44
	n-Hexane	48.1
	Ethyl acetate	70.6
	Dichloromethane	59.1

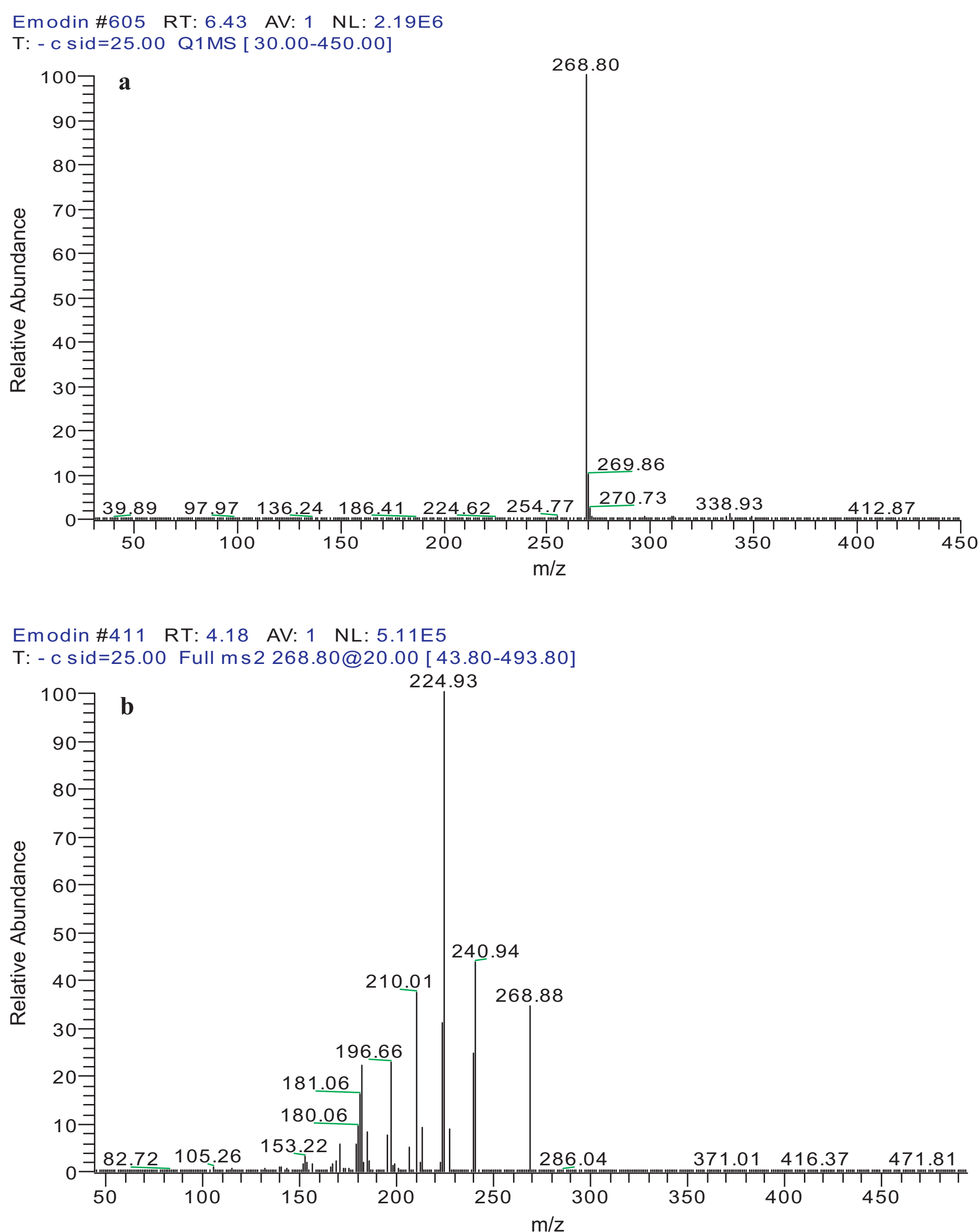

Fig. 3. (a) Negative-ion ESI mass spectrum for emodin (IS); (b) MS/MS product-ion spectrum of emodin with $[M-H]^-$ at m/z 268.9 as the precursor ion.

assay is sensitive and reproducible and will meet the requirement in pre-clinical pharmacokinetic study.

3.2.2. Linearity and sample preparation

In pre-clinical study, SZ-685C will be administered to subjects through various route (e.g., bolus injection, oral administration, etc.) in multiple dosage, and consequently the plasma concentration will vary to a large extent. Therefore, a correspondingly wide linear range was needed. Initially the linear range was set from 50 to 40,000 ng/mL. It was found that the response of SZ-685C showed a nonlinear dependence on the nominal plasma concentration over such a broad range, so the calibration curve was re-established with the concentration range of 50–10,000 ng/mL with correlation coefficients greater than 0.997. Those samples with higher concentrations (10.0–50.0 μg/mL) were diluted several times by using rat plasma and the dilution integrity was confirmed with the precision and accuracy for six replicates of diluted samples within the acceptable range (data not shown). The results suggested that reanalysis of samples whose concentrations were above the upper limit of quantification (10.0 μg/mL) by appropriate dilution could be fulfilled.

3.2.3. Accuracy and precision of the assay

The intra-run and inter-run precision and accuracy in rat plasma were evaluated using the low, medium, and high quality control spiked plasma samples. The intra-run ($n=5$) and inter-run ($n=5$) results obtained when the quality control samples were analyzed for SZ-685C are summarized in Table 2. All accuracy and precision values for the intra- and inter-run studies were within acceptable limits (±15% relative error, RSD ≤ 15%).

3.2.4. Recovery and matrix effect

The recovery was determined in spiked plasma samples ($n=6$) at three concentrations of 100, 1000, and 7500 ng/mL by dividing

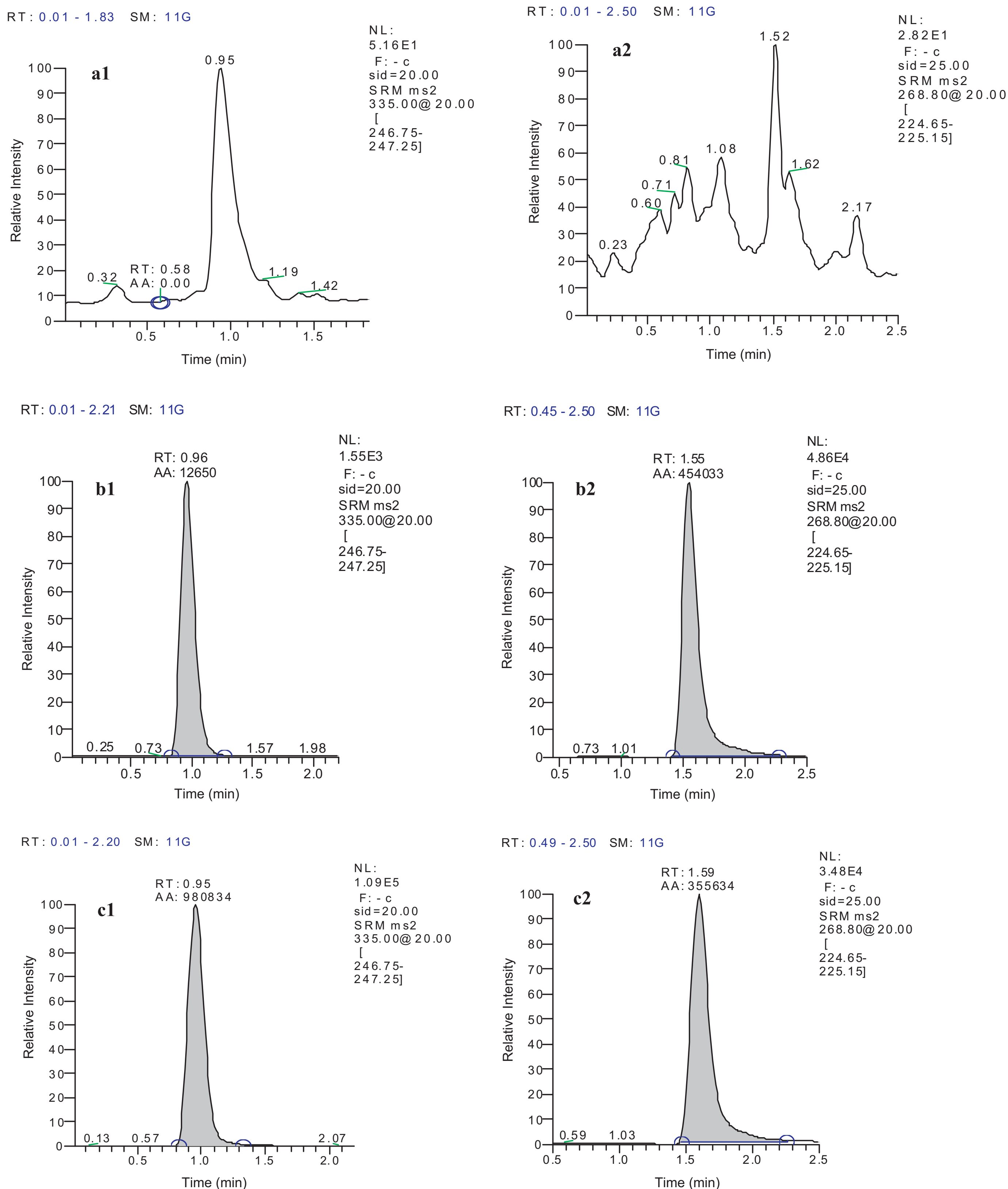

Fig. 4. Representative multiple reaction monitoring (MRM) chromatograms of SZ-685C and IS in rat plasma. (A) A blank plasma sample; (B) a plasma sample with added SZ-685C at an LLOQ level (50 ng/mL); and (C) a plasma sample from a rat 1 h after an intravenous administration of 12.5 mg/kg SZ-685C.

the peak area of SZ-685C sample spiked before extraction by the peak area of an equal concentration of SZ-685C sample spiked in the same matrix after extraction. The overall recovery was about 75.6–81.0% (Table 3).

The absolute matrix effect factor was about 94.3 ± 2.7% and found to be independent of SZ-685C plasma concentration and rat plasma lot (Table 3). The results indicate that the ion suppression is so minor as to be negligible in the method. The co-elution effect

Table 2
Precision and accuracy of the method for the analysis of SZ-685C (mean ± SD, $n = 5$).

Nominal conc. of SZ-685C (ng/mL)	Precision		Accuracy
	Mean ± SD	RSD%	Mean relative error%
Intraday			
100	114.5 ± 9.8	8.6	14.5
1000	1116.3 ± 36.0	3.2	11.6
7500	7191.9 ± 299.3	4.2	−4.1
Interday			
100	97.7 ± 13.9	14.3	−2.3
1000	1069.4 ± 60.2	5.6	6.9
7500	7384.7 ± 275.0	3.7	−1.5

Table 3
Recovery (extraction efficiency) and matrix effect of the method (mean ± SD, $n = 6$).

Spiked plasma concentration (ng/mL)	Recovery (%)	Matrix effect (%)
100	81.0 ± 4.1	97.6 ± 6.1
1000	77.7 ± 3.5	90.4 ± 4.4
7500	75.6 ± 3.4	94.2 ± 2.5

Table 4
Stability of SZ-685C in rat plasma and extraction solvent under various storage conditions ($n = 5$).

Storage condition	Nominal conc. of SZ-685C (ng/mL)	Calculated conc. (ng/mL)	
		Mean ± SD	Mean relative error (%)
−20 °C/21 d	100	95.7 ± 13.81	−4.35
	1000	928.2 ± 61.69	−7.19
	7500	6903.6 ± 196.1	−8.13
−20 °C/3 freeze–thaw cycles	100	91.6 ± 13.3	−8.84
	1000	936.1 ± 61.69	−7.13
	7500	6460 ± 196.5	−13.87
Room temperature/4 h	100	101.6 ± 14.7	1.6
	1000	993.3 ± 84.9	−0.67
	7500	6699.0 ± 297.5	−10.68
4 °C/12 h (extraction solvent)	100	110.6 ± 14.2	10.6
	1000	1071.7 ± 64.6	7.2
	7500	8045.9 ± 258.3	7.3

of SZ-685C and IS was assayed and there is no cross-talk between SZ-685C and IS (data not shown).

3.2.5. Stability

The stock solution of SZ-685C in methanol (500 μg/mL) was stable for at least 1 month at −20 °C. Stability results in plasma are summarized in Table 4. SZ-685C was stable in the extracted plasma samples at 4 °C for at least 12 h. The SZ-685C quality control samples were not affected after three freeze–thaw cycles. SZ-685C was stable in plasma at room temperature for up to 4 h, and was stable for up to 3 weeks when stored at −20 °C.

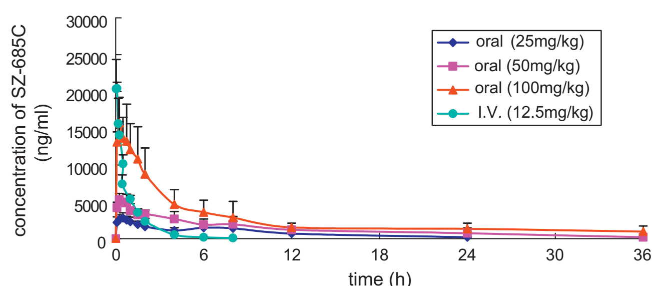

Fig. 5. Mean plasma concentration–time profiles of SZ-685C in rats plasma after oral and intravenous administration of SZ-685C.

3.3. Pharmacokinetic study

3.3.1. Application to pre-clinical pharmacokinetic study

In this study, we explored the pharmacokinetics of SZ-685C in Sprague-Dawley rat after a single intravenous or oral administration. To our knowledge, this is the first attempt at determining its pharmacokinetics. The mean plasma concentration–time curves after an oral administration of 25, 50 and 100 mg/kg and a bolus injection of 12.5 mg/kg SZ-685C are shown in Fig. 5, while the major pharmacokinetic parameters are listed in Table 5. After oral gavage, SZ-685C was rapidly absorbed and the time to maximal plasma concentration (T_{max}) was about 30 min post-dosing. Meanwhile, the maximal plasma concentration was high enough (C_{max} = 3148.4–16,395.9 ng/mL) to reach effective levels for sensitive cancer cell lines (IC_{50} = 2700 ng/mL) [2]. The $AUC_{0–24}$ of the three group (25, 50 and 100 mg/kg) was 18,296.5 ± 6148.3, 36,848.2 ± 4933.0 and 98,516.2 ± 33,097.2 ng h/mL, respectively. $AUC_{0–\infty}$ of the corresponding group was 18,598.6 ± 6290.7, 37,287.5 ± 5082.8 and 98,897.3 ± 33,173.0 ng h/mL, respectively; and elimination half-life was 5.7 ± 2.1, 7.7 ± 2.4 and 9.2 ± 2.2 h, respectively. After bolus intravenous injection of 12.5 mg/kg SZ-685C, the $AUC_{0–24}$, $AUC_{0–\infty}$ and $t_{1/2}$ were 18,416.2 ± 2462.2 ng h/mL, 18,465.5 ± 2460.1 ng h/mL and 4.0 ± 2.3 h, respectively. The absolute oral bioavailability was 54.8–66.8%. Although it requires more in-depth studies to clarify whether the compound is an ideal anticancer drug, the basic pharmacokinetic parameters are positive for its further research. Additional pharmacokinetic studies are undergoing to characterize the DMPK of the drug.

Meanwhile, $AUC_{0–24}/AUC_{0–\infty}$ of the three dosage group was more than 95%, far higher than 80%, which means the time for blood sampling is long enough to obtain efficient and precise pharmacokinetic parameters of SZ-685C, and the LC/MS/MS method is sensitive enough to support further comprehensive pre-clinical and clinical DMPK study.

Table 5
Pharmacokinetic parameters in rats after oral and intravenous administration of SZ-685C ($n = 6$).

Parameter	Oral administration		i.v. administration	
	25 mg/kg	50 mg/kg	100 mg/kg	12.5 mg/kg
T_{max} (h)	0.4 ± 0.2	0.5 ± 0.3	0.3 ± 0.3	
C_{max} (ng/mL)	3148.4 ± 555.3	5952.2 ± 1447.4	16,395.9 ± 3831.0	21,122.6 ± 3434.8
$t_{1/2}$ (h)	5.7 ± 2.1	7.7 ± 2.4	9.2 ± 2.2	4 ± 2.3
CL (or CL/F) (L/h kg)	1.5 ± 0.5	1.4 ± 0.2	1.1 ± 0.3	0.7 ± 0.1
$AUC_{0–t}$ (μg h/L)	18,296.5 ± 6148.3	36,848.2 ± 4933.0	98,516.2 ± 33,097.2	18,416.2 ± 2462.6
$AUC_{0–\infty}$ (μg h/L)	18,598.6 ± 6290.7	37,287.5 ± 5082.8	98,897.3 ± 33,173.0	18,465.5 ± 2460.1
MRT (h)	5.2 ± 2.0	7.2 ± 2.2	11.8 ± 3.4	1.51 ± 0.17
Vd (or *Vd/F*) (L/kg)	11.1 ± 2.3	14.7 ± 2.7	14.5 ± 5.0	4.0 ± 2.3
F (%)	52.0 ± 15.4	54.8 ± 6.9	66.8 ± 22.5	

4. Conclusion

A simple, sensitive and rapid LC–MS/MS method was developed and validated for the quantification of SZ-685C in rat plasma. This method had been used to assess the pharmacokinetic profile of SZ-685C in rats. Further investigation on SZ-685C as a cancer chemopreventive agent is warranted.

Conflict of interest

The authors declared no conflict of interest.

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