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LC–MS/MS method for determination of geldanamycin derivative GM-AMPL in rat plasma to support preclinical development

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ABSTRACT

A LC–MS/MS method for determining the concentration of the small molecule Hsp90 inhibitor, GM-AMPL, has been developed and validated in rat plasma to support preclinical development. 17-[2-(morpholine-4-yl)ethyl]amino-17-demethoxygeldanamycin (GM-AMPL) and the internal standard 17-allylamino-17-demethoxygeldanamycin (17-AAG) were sufficiently separated on a Venusil MP C18 column that was eluted with 80% methanol in water at 40 °C. Quantification studies were performed with a multiple reaction monitoring (MRM) transition of *m/z* 657.3→614.3 and 584.3→541.3 for GM-AMPL and IS, respectively, in the negative ion mode. The present method exhibited good linearity ($R > 0.999$) over the concentration range of 2–600 ng/mL for GM-AMPL in rat plasma with a lower limit of quantification (LLOQ) of 2 ng/mL. The intra-batch and inter-batch assay coefficients of variation (CV) were in range of 1.56–6.84% and 1.62–6.98%, respectively. The plasma samples were extracted with methanol to precipitate protein with extraction recovery in range of 84.09–95.25%. The matrix effect was determined as internal substance (IS) normalized matrix factor of 1.09, 1.18 and 1.05 for samples with three concentration levels of 4, 40 and 400 ng/mL, respectively. This validated method was further applied to successfully determine the pharmacokinetic parameters and oral availability of GM-AMPL in Sprague-Dawley rats following intravenous injection and oral administration.

1. Introduction

Geldanamycin is a benzoquinone ansamycin antibiotic (Fig. 1) [1]. It has potent antiproliferative activity, which correlates to its ability to specifically bind to the heat shock protein hsp90. However, its clinical use has been limited because of its poor oral availability and its obvious hepatotoxicity [2,3]. Two geldanamycin derivatives, 17-allylamino-17-demethoxygeldanamycin (17-AAG) and 17-demethoxy-17-[[2-(dimethylamino) ethyl]amino]-geldanamycin (17-DMAG), were studied clinically for their potential development as therapeutics for cancer (Fig. 1) [4,5]. With the expectation of finding a novel potential candidate for cancer therapy, our group made great efforts toward the design, synthesis and evaluation of geldanamycin derivatives [6,7]. Interestingly, our previous studies indicated that geldanamycin and its derivatives still possessed broad antiviral activities based on a host cell antiviral mechanism. This is an important finding to keep in mind because current antiviral therapy was threatened by severe drug resistance. Furthermore, several 17-alkylamino substituted geldanamycin derivatives exhibited markedly lower toxicity in mice compared to geldanamycin [7]. GM-AMPL, short for 17-(2′-[morpholine-4-yl]ethylamino)-17-demethoxygeldanamycin (Fig. 1A), showed similar antitumor activity and even stronger antiviral activity than 17-AAG *in vitro*. The MTD (maximum tolerable dosage) of GM-AMPL by intravenous administration to Kunming mice is 101 mg/kg (data not published) based on our research, while the MTD of free 17-DMAG was reported as 10 mg/kg in rats [8]. Based on these data, we developed GM-AMPL as a novel potential preclinical candidate.

Although GM-AMPL was previously mentioned in the SAR (structure-activity relationship) research of geldanamycin derivatives developed by Tian et al. [9], the pharmacokinetic profile of GM-AMPL had not been investigated until now. Delineating the pharmacokinetic properties of GM-AMPL is a necessary step toward the development of GM-AMPL as a preclinical drug candidate. Therefore, we developed and validated a sensitive and selective liquid chromatograph–tandem mass spectrometry (LC–MS/MS) method for the quantification of concentrations of GM-AMPL in rat plasma. Additionally, this bioanalytical method was successfully applied to study the pharmacokinetic parameters

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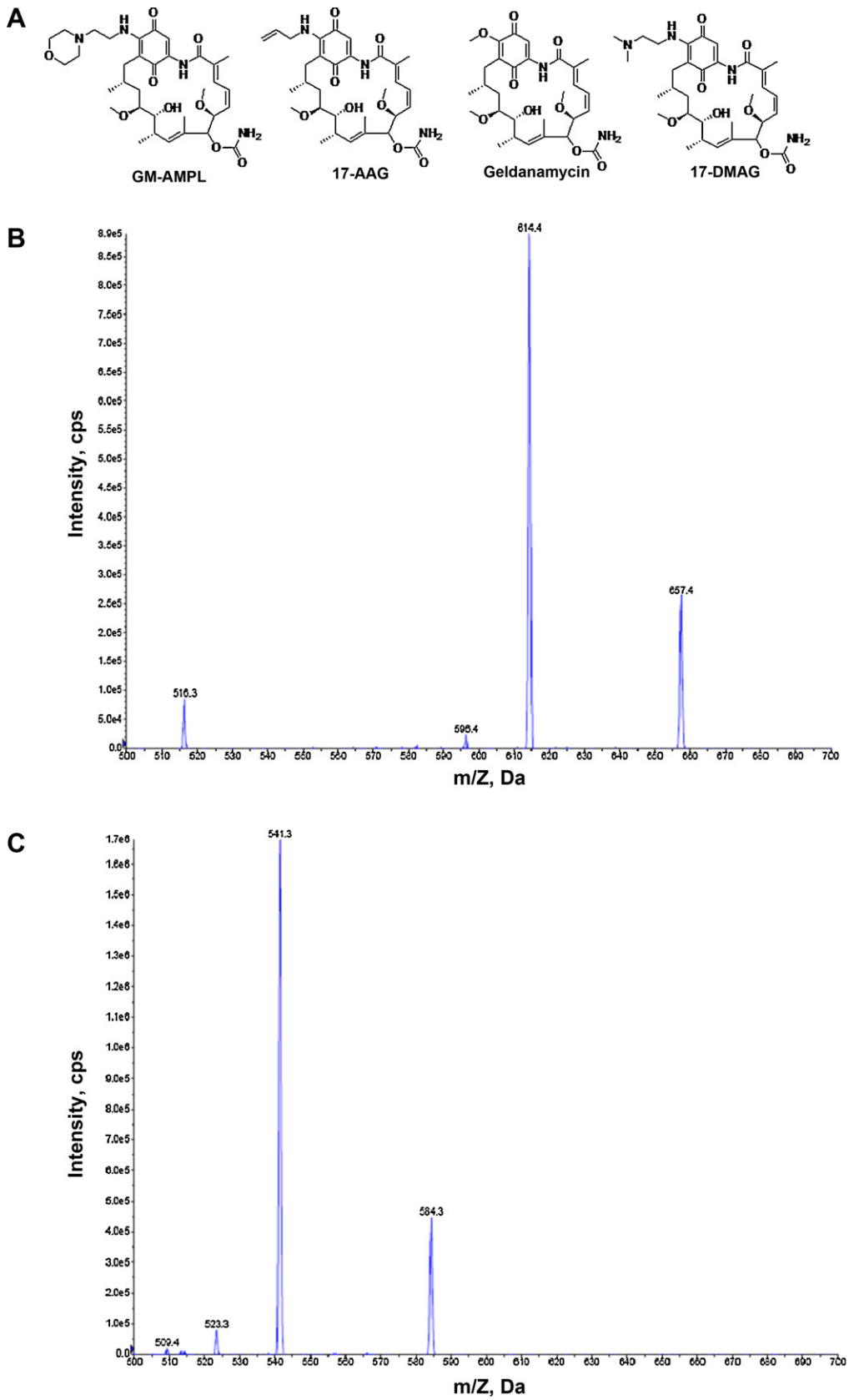

Fig. 1. The chemical structures of GM-AMPL, the internal standard 17-AAG, geldanamycin and 17-DMAG (A) and the MS/MS spectra of GM-AMPL (B) and IS (C).

and oral absorption of GM-AMPL in Sprague-Dawley rats following a single dose intravenous injection and oral administration.

2. Experimental

2.1. Chemicals, reagents and animals

GM-AMPL and the internal standard (IS) were synthesized by ourselves using geldanamycin as the starting material with previously reported method [6]. The purity of the products was >98.5%, as determined by area normalization method of HPLC (high pressure liquid chromatography) analysis. The methanol was HPLC grade and was obtained from Fischer Scientific. The other reagents were analysis grade and purchased from Beijing chemical reagents factory (Beijing, China). The Sprague-Dawley rats were purchased from Beijing Vital River Laboratories (Beijing, China).

2.2. LC–MS/MS system

Chromatographic separations were performed using a SHIMADZU UFLC20A (SHIMADZU, Tokyo, Japan) instrument equipped with binary pumps, SIL-20A(HT) autosampler, CTO-20A column oven and DGU-20A3 online degasser. A Venusil MP C18 column (3.5 μm, 2.1 mm × 50 mm; Agela Technologies, Wilmington, DE, USA) was used, and 80% methanol in water was used as an eluent at 40 °C. The flow rate was set to 0.3 mL/min. The API 4000 Qtrap MS/MS spectrometer (AB SCIEX, CA, USA) was equipped with an electrospray ion (ESI) source and run in negative ion mode. Compounds were quantified using multiple reaction monitoring (MRM). Data acquisition and quantification was performed with Analyst 1.5.1 program.

2.3. Preparation of calibration curve and quality control samples

GM-AMPL was dissolved in methanol to prepare the standard work solutions (10, 30, 100, 300, 1000 and 3000 ng/mL) and quality control solutions (20, 200 and 2000 ng/mL). The IS solution of 17-AAG in methanol was prepared at a concentration of 1500 ng/mL. These solutions were kept away from light at 4 °C. These solutions were equilibrated to room temperature immediately prior to use. The calibration standards samples (2, 6, 20, 60, 200 and 600 ng/mL) and quality control (QC) samples (4, 40 and 400 ng/mL) were prepared by spiking 20 μL of the appropriate work solution, 40 μL of methanol and 20 μL of IS solution into 100 μL of blank rat plasma. The final concentration of IS in plasma samples was 300 ng/mL.

2.4. Sample preparation

Twenty microliters of IS solution and 60 μL of methanol were added to 100 μL of rat plasma sample and vortexed for approximately 0.5 min. Two hundred microliters of methanol was then added to the vortexed sample to precipitate the protein. The samples were then mixed (linear velocity: 4 m/s, 40 s/circle) for 40 s, and finally centrifuged for 5 min at 14,000 rpm. Ten microliters of the supernatant was injected into LC system for analysis.

2.5. Validation assay

Following the criteria suggested by the US Food and Drug Administration (FDA) bioanalytical method validation guidance [10], we validated this method for selectivity, linearity, LLOQ (lower limit of quantification), accuracy and precision, matrix effect, extraction recovery and stability.

2.5.1. Selectivity

Six different lots of blank plasma samples were compared with plasma samples spiked with GM-AMPL and IS to verify the selectivity of this method. This method was determined to be selective when there were no interference signals from endogenous substances at the retention times of GM-AMPL and IS. Otherwise, selectivity was acceptable only if the peak area ratio of the analyte at LLOQ and interference substance was >5.

2.5.2. Calibration curve

Six calibration curves of corresponding six concentrations for GM-AMPL (2, 6, 20, 60, 200 and 600 ng/mL) were assayed in establishment of this bioanalytical method. The peak areas ratio of GM-AMPL to IS as the assay response (y) against nominal concentration of GM-AMPL (x) was plotted to setup the calibration curve. The data was fitted into linear regression analysis with a weighting factor of $1/x$ to calculate the calibration equation and correlation coefficients (r). Good linearity was determined as $r > 0.995$.

The lowest concentration level of calibration curve was defined as the LLOQ of the assay. Five replicates of plasma samples were used and resulted in acceptable accurate and precision measurement of LLOQ.

2.5.3. Precision and accuracy

The precision and accuracy were assessed at different day by analyzing QC samples of three individual batches at concentrations of 4, 40 and 400 ng/mL, respectively. Five replicates of each QC concentration of GM-AMPL were used for both intra-batch and inter-batch assays. Accuracy was expressed by the percentage of the mean determined concentration to nominal concentration, and precision was expressed as CV. Five replicates of freshly prepared LLOQ samples were also determined and resulted in accuracy and precision measurements at the concentration of LLOQ based on nominal concentration.

Five replicates of dilution integrity sample of GM-AMPL with a concentration of 2000 ng/mL were prepared to evaluate the dilution effect. The diluted samples, obtained by diluting the 2000 ng/mL samples 10-fold with blank plasma, were analyzed for a dilution assay. The concentration of dilution integrity samples was back-calculated from diluted samples. Dilution effect was determined with accuracy and precision measurement of back-calculated concentrations based on nominal concentration.

The precision determined for QC samples should not exceed 15% of the CV value except for LLOQ, where it should not exceed 20% of CV. The calculated mean concentration value should be within 15% of the nominal value for QC sample except for LLOQ where it should not deviate by more than 20%.

2.5.4. Extraction recovery and matrix effect

In this study, five different lots of plasma samples were used for measurement of extraction recovery and matrix effect.

The extraction recovery of GM-AMPL at 4, 40 or 400 ng/mL and IS at 300 ng/mL was determined by comparing the peak area of GM-AMPL/IS of extracted plasma QC samples to those of extracted blank plasma samples spiked with equal amounts of GM-AMPL and IS. The percent recovery at each concentration level was calculated using the following equation:

$$\text{Recovery}\,(\%) = \left[\frac{\text{peak area of extracted sample}}{\text{peak area of extracted blank sample spiked with analyte and IS}}\right] \times 100.$$

The matrix effect results from the co-elution of components present in biological samples with the target analyte. These extraneous components may not give a signal in MRM of the analyte

but they can affect the MRM response dramatically. The matrix effect was determined by measuring the IS normalized matrix factor (MF) using five different lots of plasma samples. The peak area ratio (A) of GM-AMPL (4, 40 or 400 ng/mL) to IS (300 ng/mL) in the mobile phase was compared with the peak area ratio (B) measured in extracted samples of blank plasma spiked with the same amounts of GM-AMPL and IS. The IS normalized MF at each concentration was calculated from the following equation: MF = B/A.

2.5.5. Stability

The stability study of GM-AMPL was performed under different storage conditions at three concentration levels (4, 40 and 400 ng/mL) in three replicates. Short-term and long-term stability of GM-AMPL in plasma were assessed after plasma samples were stored at the ambient temperature for 4 h and kept at −20 °C for 15 days. The post-preparative stability of samples was tested by analyzing extracted samples kept in auto-sampler vials for 8 h at room temperature.

2.6. Pharmacokinetic study

For pharmacokinetic studies, the SD rats were divided into two groups. Single dosage of GM-AMPL by oral gavage (po) was administered to one group (n = 6, 3 males, 3 females), while the other group (n = 6, 3 males, 3 females) was administered GM-AMPL *via* tail vein injection (iv). The rats were housed under standard conditions, had free access to water and consumed a standard laboratory diet throughout the experiments. Both groups of rats were dosed with GM-AMPL dissolved in a solvent mix of DMSO (dimethyl sulfoxide), Tween-20 and saline (5/5/90). The dosage was 5 mg/kg for intravenous administration and 25 mg/kg for oral administration. Rat blood samples were collected from the retro-orbital sinus and collected into test tubes containing the anticoagulant heparin at the time points 0.33, 0.67, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 10.0 h after oral administration and 0.03, 0.17, 0.50, 1.0, 1.5, 2.0, 4.0, 6.0 and 10.0 h after intravenous administration. Plasma was separated by centrifugation with 4500 × g for 5 min at low temperature (4 °C) and stored at −20 °C until analysis. Plasma samples with concentrations greater than the upper limit of quantification (ULOQ) were diluted with blank plasma to adjust the concentrations down to the range of the standard curve before sample preparation and reanalyzed; then, the original concentrations were back-calculated. The pharmacokinetic curves were described as Log concentration *vs.* time plots. The non-compartmental model analysis was used for calculating pharmacokinetic parameters with WinNolin (V5.2.1) program. The oral bioavailability (F) was valued using the following equation:

$$F(\%) = \left[\frac{D_{iv} \times AUC_{oral}}{D_{oral} \times AUC_{iv}}\right] \times 100,$$

where D, dosage and AUC, area under concentration–time curve.

3. Results and discussion

3.1. Optimization of bioanalytical method

Regarding IS selectivity, geldanamycin should be the first choice for consideration. However, geldanamycin is much more light sensitive and unstable than GM-AMPL, especially when it is dissolved in solution. Geldanamycin displayed a photodegradation half life of about 60 min under light intensity of 4500 lx [11], while GM-AMPL only degraded 10% or even less under light intensity of 4500 lx for 120 min. Therefore, 17-AAG was considered as a more suitable choice as IS than geldanamycin due to its similar chemical structure with GM-AMPL. In addition, its solubility and stability of 17-AAG also appear to be much more similar with GM-AMPL. Furthermore, we chose 17-AAG as the IS in this study because it is readily available in our laboratory.

Because GM-AMPL and 17-AAG are highly lipophilic compounds, suitable retention times and adequate separation of GM-AMPL and IS were achieved simply by using a mobile phase consisting of a high proportion of organic solvent (methanol/water: 80/20) at a flow rate of 0.3 mL/min on a UFLC (Ultra Fast Liquid Chromatograph) instrument. The retention time were 1.24 and 1.41 min for GM-AMPL and IS, respectively. A single run was performed in only 3 min, thereby significantly reducing the consume amount of mobile phase and shortening the necessary time for the whole assay comparing with flow rate of 1 mL/min and delayed retention time of analyte presented by HPLC instrument.

Negative ion mode was used to give $[M-H]^-$ at m/z 657.3 and m/z 584.3 for GM-AMPL and IS, respectively, as precursor ions for fragmentation in MRM, which produced better ion responses for quantification analysis in MS/MS spectra than positive ion mode. The collision energies were optimized for both GM-AMPL and the IS to obtain the most intense fragment ions. MS/MS product-ion spectra of GM-AMPL and IS were shown in Fig. 1. Their characteristic ion dissociation transitions, m/z 657.3→614.3 and m/z 584.3→541.3, were selected for GM-AMPL and the IS, respectively, for their quantitative measurement (Fig. 1). The m/z 584.3→541.3 for precursor and product ion of 17-AAG was in line with reported values in the literature [12]. Dwell time was 20 ms, duty cycle was 0.1 s on MRM transitions, and no smoothing was applied.

Some reported methods used a liquid–liquid extraction with ethyl acetate followed by evaporation for other geldanamycin derivatives such as 17-AAG and 17-DMAG [13–15]. In our study, samples were processed by one-step extraction with methanol to precipitate protein. It is a simple, rapid and efficient way for removal of most plasma protein. The samples were effectively deproteinized, and analyte/IS was sufficiently extracted with methanol rather than with acetonitrile due to the higher solubility of analyte/IS in methanol.

3.2. Selectivity

Fig. 2 shows the representative chromatograms obtained from blank rat plasma, rat plasma samples spiked with GM-AMPL and IS. GM-AMPL and IS were sufficiently separated from each other, and there were no significant interferences from endogenous substances in both female and male rat plasmas. An unknown plasma sample collected at 1.5 h after oral administration of GM-AMPL was also shown in Fig. 2, which indicated a calculated plasma concentration of 3290 ng/mL.

3.3. Linearity and LLOQ

Good linearity of this method was obtained in the concentration range from 2 to 600 ng/mL. The mean regression equation from six calibration curves was: $y = 0.00498x - 0.00226$ with $r > 0.9991$. The deviation of all back-calculated values for calibration curve was within 15% from the actual concentration. LLOQ for GM-AMPL was established at 2 ng/mL. The LLOQ was independently measured with acceptable intra-batch precision and accuracy of 7.59% and 104.40%, respectively (Table 1). And the ratio of S/N (signal to noise) was >10 (Fig. 2A and B).

3.4. Accuracy and precision

The intra-batch and inter-batch precision and accuracy of three QC levels were shown in Table 1. The intra- and inter-batch precision were found to be 6.98% or less, while the intra- and inter-batch accuracy ranged from 96.87% to 116.60% for mean values of each

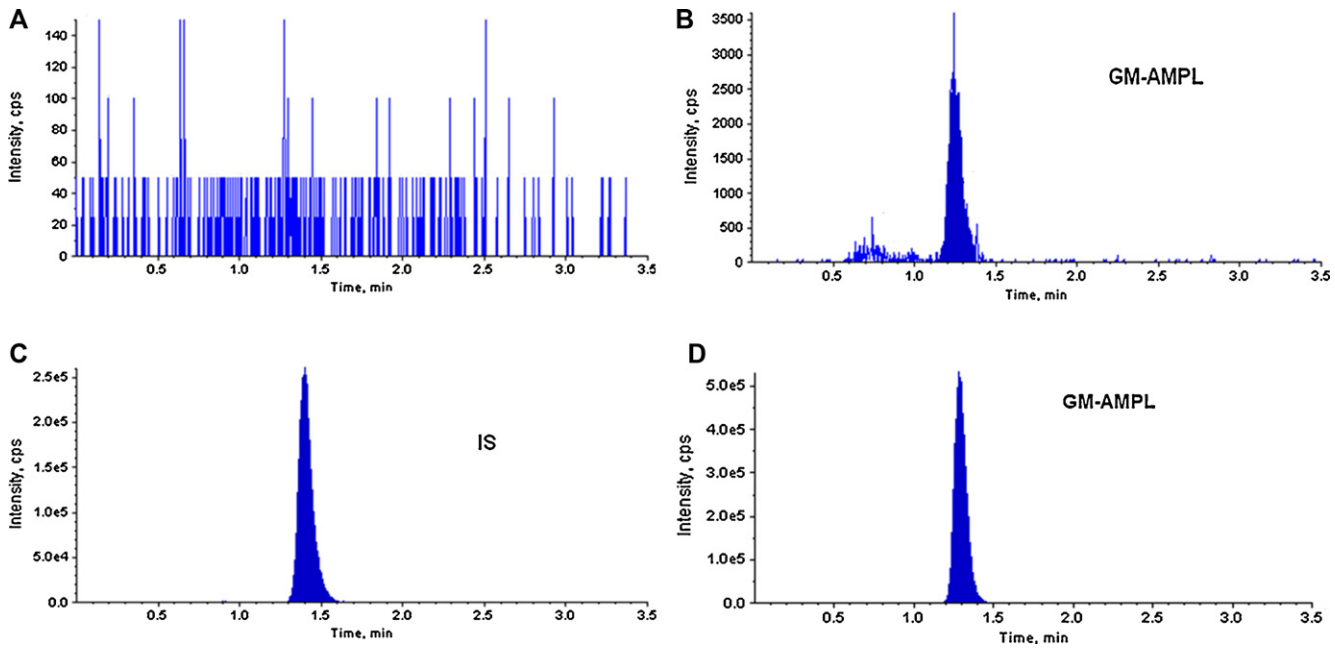

Fig. 2. The representative chromatograms of GM-AMPL in rat plasma: blank rat plasma (A); rat plasma spiked with GM-AMPL at a concentration of 2 ng/mL (B); rat plasma spiked with IS at a concentration of 300 ng/mL (C) and GM-AMPL (3290 ng/mL) in female rat plasma at 1.5 h after oral administration (D).

QC levels. It was noted that average accuracy at low QC concentration of 4 ng/mL ranged from 114.10% to 116.60% which was near or beyond the suggested criteria range of 85–115%. Higher deviation of accuracy at low QC samples was ascribed to sample processing. The intra-batch accuracy of dilution integrity samples at 2000 ng/mL was 105.3% of the nominal value and the intra-batch precision was within 7.23% (Table 1). Moreover, the back-calculated concentrations of 43 QC samples were within ±15% of nominal concentrations, which was more than 67% of all QC samples (total $n = 50$). Thus, we considered that accuracy and precision of QC samples were within the acceptable range for bioanalytical purposes.

3.5. Extraction recovery

Extraction recovery was evaluated at low, medium and high QC concentrations (4, 40 and 400 ng/mL) for GM-AMPL and at single concentration (300 ng/mL) for IS. The mean extraction recovery of analytes was calculated with 94.58 ± 5.17%, 84.09 ± 8.59% and 95.25 ± 4.23% for low, middle and high QC samples, respectively ($n = 5$). The mean extraction recovery of IS on all QC levels ($n = 15$) was 92.11 ± 7.08%. A one-step extraction with methanol to precipitate protein was sufficient to extract analytes from plasma.

3.6. Matrix effect

The matrix effect was calculated with internal standard normalized matrix effect factor in this study. The MF was determined to values of 1.09, 1.18 and 1.05 for low, middle and high QC samples, respectively ($n = 5$). The mean MF value was 1.11. These results demonstrated that the matrix effect imposed no significant adverse impact on the quality of data although there was a weak signal enhancement attributed to rat plasma, as determined by the MF value.

3.7. Stability

The stability of GM-AMPL after storage under different conditions was shown in Table 2. The calculated concentrations of GM-AMPL ranged from 91.33% to 100.26% of nominal concentration for long-term stability and post-preparative stability. The

Table 1
Intra-batch and inter-batch assay for accuracy and precision of GM-AMPL in rat plasma.

Assay	Concentration (ng/mL)	QC			LLOQ	Dilution effect
		4	40	400	2	2000
Intra-batch day 1 ($n = 5$)	Mean	4.56 ± 0.18	44.02 ± 0.76	416.24 ± 28.49	2.09 ± 0.16	2106.22 ± 152.37
	Accuracy (%)[a]	114.10 ± 4.48	110.05 ± 1.90	104.06 ± 7.12	104.41 ± 7.92	105.31 ± 7.62
	Precision (CV, %)	3.93	1.73	6.84	7.59	7.23
Intra-batch day 2 ($n = 5$)	Mean	4.66 ± 0.12	43.78 ± 0.68	387.49 ± 13.96		
	Accuracy (%)	116.60 ± 3.02	109.46 ± 1.71	96.87 ± 3.49		
	Precision (CV, %)	2.59	1.56	3.60		
Intra-batch day 3 ($n = 5$)	Mean	4.61 ± 0.10	44.11 ± 0.82	441.71 ± 9.78		
	Accuracy (%)	115.35 ± 2.38	110.28 ± 2.04	110.43 ± 2.44		
	Precision (CV, %)	2.06	1.85	2.21		
Inter-batch ($n = 15$)	Mean	4.61 ± 0.13	43.97 ± 0.71	415.15 ± 28.99		
	Accuracy (%)	115.35 ± 3.33	109.93 ± 1.78	103.79 ± 7.25		
	Precision (CV, %)	2.89	1.62	6.98		

[a] Expressed as percent of tested values to nominal values.

Table 2
Stability of GM-AMPL in rat plasma and in post-preparation storage ($n = 3$).[a]

Storage conditions	Concentration (ng/mL)		
	4	40	400
Stored 4 h at room temperature	116.92 ± 1.84	109.42 ± 1.48	111.29 ± 2.01
Stored 15 days at −20 °C	91.42 ± 5.76	91.33 ± 6.40	92.30 ± 6.52
Post-preparation storage for 8 h at room temperature	103.53 ± 4.81	104.02 ± 2.97	100.26 ± 5.66

[a] Stability was calculated as percentage of calculated concentrations after certain storage treatment to nominal concentrations, and expressed with the mean and standard error of three samples.

short-term stability at middle and high concentration levels for GM-AMPL was also with acceptable average percent recovery of 91.42% and 103.53%, respectively. However, the average percent recovery (116.92%) was beyond ±15% of the nominal low concentration level (4 ng/mL). The degradation of GM-AMPL itself during the extraction process should not result in this higher percent recovery at low concentration level. It might be induced by little high matrix effect or sample preparation process. On consideration of the concentration level of samples with higher percent recovery was closing with LLOQ, we considered GM-AMPL was stable under above test conditions.

3.8. Pharmacokinetics application

The analytical method described in this paper was used to determine the plasma concentration of GM-AMPL administrated to SD rats by oral and intravenous routes. The representative plots of Log concentration *vs.* time were shown in Fig. 3.

It should be noted that there was significant gender-related difference appeared in pharmacokinetic curves of GM-AMPL between female and male rats. Oral or intravenous administration of GM-AMPL with single dosage produced a higher plasma concentration level for female rats than that of male rats (Fig. 3A and B). It is consistent with gender effect on substrate metabolized by CYP3A, and metabolism of geldanamycin analogs was dependent on CYP isozymes [16,17]. The pharmacokinetic profile of GM-AMPL, ignoring the gender disparity, is shown in Table 3 using a non-compartmental analysis. The plasma elimination half life ($t_{1/2}$) of GM-AMPL was 1.93 h following the iv dose. Total plasma clearance (Cl) was 1.49 L/h/kg and mean volume of distribution (Vd) was 4.06 L/kg. Following the po dose, GM-AMPL was absorbed and reached a peak concentration (C_{max}) of 1.73 mg/L at time of maximum concentration (t_{max}) of 1.45 h. The plasma $t_{1/2}$ was 1.53 h. The mean retention time (MRT) of GM-AMPL was 1.49 and 2.30 h for intravenous and oral dosages, respectively.

The oral bioavailability was valued by comparing the area under the concentration–time curve (AUC_{inf}) of oral administration of GM-AMPL with the AUC of intravenous administration. The individual *F* values of female and male rats were 38.22% and 7.93%, respectively. The average *F* calculated with the average $AUCs_{inf}$ of all rats was approximately 26.36%.

Table 3
Pharmacokinetic parameters of GM-AMPL in rats ($n = 6$).

Parameters	iv (5 mg/kg)	po (25 mg/kg)
$t_{1/2}$ (h)	1.93	1.53
t_{max} (h)	/	1.45
C_{max} (mg/L)	/	1.73
AUC_{0-t} (mg/L h)	3.57	4.70
AUC_{inf} (mg/L h)	3.63	4.74
Vd (L/kg)	4.06	31.41
Cl (L/h/kg)	1.49	13.51
MRT (h)	1.49	2.30
F (%)	26.36	

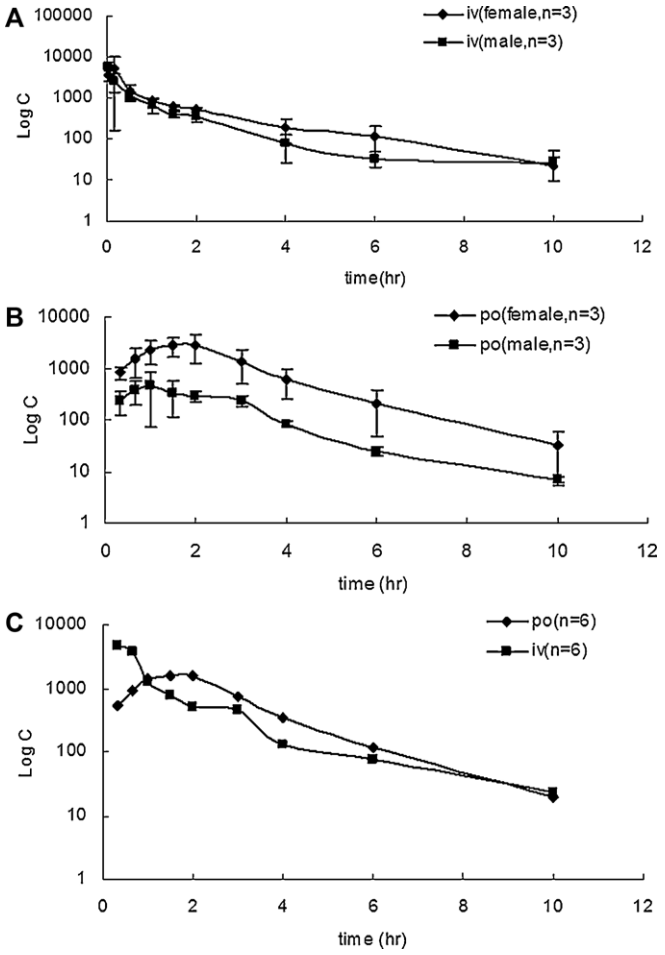

Fig. 3. Pharmacokinetic curves of GM-AMPL with single oral or intravenous treatment were described as Log concentration *vs.* time plot. Pharmacokinetic curves of GM-AMPL for female and male rats individually with single intravenous administration (A, 5 mg/kg, $n = 3$) or single oral administration (B, 25 mg/kg, $n = 3$) and average oral and intravenous pharmacokinetic curves of GM-AMPL ignoring the gender disparity (C, $n = 6$). Data represent the mean and standard error of three animals in Fig. 3A and B.

4. Conclusions

A rapid and accurate LC–MS/MS method was developed for the first time to quantify the amount of the Hsp90 inhibitor, GM-AMPL, in SD rat plasma. Isocratic chromatographic condition was used with a mobile phase composed of 80% methanol and 20% water. It facilitated the analysis process to be more simple and easy compared with salt-containing mobile phase and gradient elution [11,18,19]. The sample preparation, which used methanol to precipitate protein, gave high extraction recoveries (>90%) for all QC samples, and no significant matrix effect was observed in this method. This method produced excellent reproducibility, rapid sample preparation and accurate quantification for this study.

The method was successfully applied to the pharmacokinetic study of GM-AMPL. The higher plasma concentration curve was observed in female rats than in male rats after both oral and intravenous administration of GM-AMPL in this study. This gender-related difference could be resulted from the metabolism clearance of geldanamycin analogs mediated by CYP3A. The oral bioavailability of GM-AMPL at 26% in rats was comparable with 17-AAG, which was reported as 24% but was lower than 17-DMAG, which was reported as 50% [15,20]. Therefore, further efforts are still necessary to improve the oral availability of geldanamycin analogs.

Overall, this study produced valuable information about the pharmacokinetic properties of GM-AMPL, which is important for its further development possibility as a drug candidate. The method described in this study possessed of good selectivity, reproducibility, acceptable accuracy and precision, chemical stability and simple and liable extraction process. Thus it provided a convenient tool for future practical pharmacokinetic applications of geldanamycin analogs.

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