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Quantitation of bentysrepinine (Y101) in rat plasma by liquid chromatography tandem mass spectrometry: Application to pharmacokinetic study

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ABSTRACT

A simple, accurate and sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) method was developed and validated for quantitation of bentysrepinine (Y101) in rat plasma. After the addition of diphenhydramine (internal standard, IS), plasma samples were pretreated by protein precipitation. Chromatographic separation was carried out on an Atlantis® analytical column (4.6 mm × 100 mm, 5 μm, Waters) with methanol: 20 mM ammonium formate consisting of 1.0% formic acid (65:35, v/v) as the mobile phase at an isocratic flow rate of 0.4 mL/min for 7.5 min. The multiple reaction monitoring (MRM) transitions were performed at m/z 490.2 → 339.5 for Y101and m/z 256.0 → 167.0 for IS on a SCIEX API 4000 mass spectrometer in the positive ion mode with electrospray ionization (ESI) source. Good linearity was achieved over the concentration range of 1–2500 ng/mL. The intra- and inter-day precisions were less than 8.3%, and the accuracy ranged from −4.0% to 2.8%. Y101 was stable during the analysis and the storage period. The pharmacokinetic profiles of Y101 at three dose levels were successfully studied for the first time in rats by this method. After single intra-gastric administration of Y101 at the doses of 25, 50 and 100 mg/kg, C_{max} and AUC_{0-t} were proportional to the doses given.

1. Introduction

Hepatitis B virus (HBV)-infected hepatitis is the most common infectious disease and over 400 million people worldwide suffer from the disease [1].

Unfortunately, there is no such an ideal therapeutic drug that is able to act effectively against HBV at present, due to the disadvantages the current medicines have in the treatment of viral suppression, the frequent development of resistance and significant adverse effects [2]. Lamivudine (LMV) and adefovir (ADV) are well-tolerated and may lead to viral suppression in the majority of treated patients [3,4]. LMV is limited by the frequent development of resistance, occurring at the rates of up to 15–30% per year [5]. For ADV, the development of resistance is slower, however, 28% of treated patients develop genotypic resistance [6]. Interferon (IFN) alpha is effective in only one third of patients and the treatment is greatly hampered by significant adverse effects [2].

The natural products, due to their enormous structural diversity, provide a large opportunity for screening anti-HBV agents which attracts drug developers to focus their eyes on its chemical constituents study. *Matijin* (*Dichondra repens* Forst.), a Chinese herb, possesses significant antipyretic effects, cholagogic actions, and immune regulation function. Therefore, it has been widely used in the treatment of chronic liver diseases [7]. Research has been made to identify chemical constituents and pharmacologically active substances, Repenine (Fig. 1A) (a dipeptide compound), isolated from *Dichondra repens* Forst, has been proved to be beneficial to patients with HBV-infected hepatitis [8]. A series of repenine derivatives were synthesized and their bioactivity studies were then performed. Experimental results demonstrated that Y101 (N-[N-benzoyl-O-(2-dimethylaminoethyl)-L-tyrosyl]-L-phenylalaninol hydrochloride) might have potential to act as a novel agent against HBV-infected hepatitis [9].

New compound bentysrepinine (Y101, Fig. 1B) provides obviously inhibitive effects on DHBV-DNA and protection for liver in vivo [10], and has significant inhibitive effect of HBsAg on cultured cell line 2.2.15 [11]. It also has good protective effect on mice with acute liver injury [12], and investigational new drug (IND) status has been granted in china.

In order to define the pharmacokinetic profile of Y101, a LC–MS/MS method for the determination of Y101 in rat plasma needs to be established. In this study, the first analytical method for determination of Y101 concentrations in rat plasma over range of 1–2500 ng/mL has been described. The method we reported is accurate and sensitive, on the basis of a simple protein precipitation approach. This method has been successfully applied

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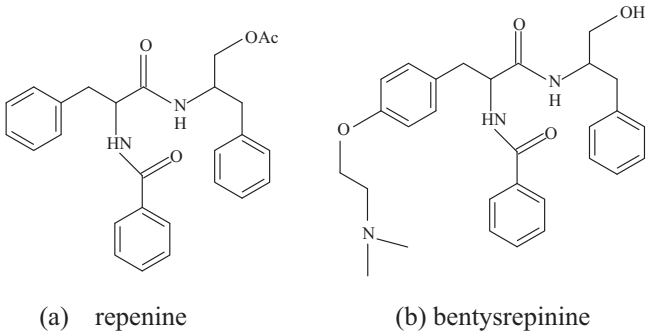

(a) repenine (b) bentysrepinine

Fig. 1. The chemical structures of repenine and bentysrepinine.

to characterize the pharmacokinetics of Y101 in rat following oral administrations of Y101 at three dose levels (25, 50 and 100 mg/kg).

2. Methods

2.1. Chemicals and reagents

Bentysrepinine (Y101, Purity: 99.8%) was kindly provided by the Key Laboratory of Chemistry for Natural Products of Guizhou Province (Guizhou, China). Diphenhydramine (IS, Purity: 99.5%), was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC grade) and formic acid (purity ≥ 98%) were purchased from Tianjin Concord Tech. Reagent Co. Ltd. (Tianjin, China). Analytical grade ammonium formate was purchased from Tianjin Guang Fu Fine Chemical Research Institute (Tianjin, China). Deionized water was produced with a SYZ550 quartz pure water distiller (Tianjin Xinzhou Tech Co. Ltd. Tianjin, China). Freshly obtained drug-free heparinized rat plasma was collected from male Sprague-Dawley rats in our laboratory and stored at −20 °C before use.

2.2. Instrumentation and analytical conditions

Liquid chromatography tandem mass spectrometry (LC–MS/MS) analyses were performed using a Shimadzu high performance liquid chromatograph coupled with a SCIEX API 4000 Q-Trap mass spectrometer equipped with a turbo-ionspray probe; and Analyst Ver. 1.5 software was used for data collection and processing (Applied Biosystems, Toronto, Canada). The analyte Y101 and IS were separated at 40 °C by injection of 5 μL sample onto an Atlantis® T3 analytical column (4.6 mm × 100 mm, 5 μm, Waters) with methanol: 20 mM ammonium formate consisting of 1.0% formic acid (65:35, v/v) as the mobile phase at an isocratic flow rate of 0.4 mL/min for 7.5 min. The samples were kept at 4 °C in the autosampler.

The mass spectrometer (mass range from 50 Da to 3000 Da) was equipped with electrospray ionization (ESI) in positive mode. The protonated ions $[M+H]^+$ for Y101 and IS were selected in the first quadrupole, and the collision energy were 28 eV and 25 eV for Y101 and IS respectively which were adjusted to achieve maximum sensitivity for each ion transition. The multiple reaction monitoring (MRM) modes were used to detect a specific transition of the precursor ion to the product ion at m/z 490.2 → 339.5 for Y101and m/z 256.0 → 167.0 for IS. Optimized instrument settings specific for Y101 and IS were as follows: Curtain Gas was 10 psi, Ion Source Gas 1 was 70 psi, Ion Source Gas 2 was 70 psi, IonSpray Voltage was 4000 V, Turbo Heater Temperature was 600 °C. Quantification and confirmation transitions and their optimized values for Entrance Potential (EP), Declustering Potential (DP), Collision Cell Exit Potential (CXP) were 10 V, 100 V and 8 V, respectively.

2.3. Preparation of standards and calibration curves

Two stock solutions for standard solution and quality control (QC) samples were prepared for Y101 from independent preparations. The stock solutions were prepared by dissolving 10 mg of Y101 into 10 mL of methanol to a final concentration of 1.0 mg/mL. A series of Y101 working standard solutions were prepared by dilutions of the stock solution with methanol to obtain the following Y101 concentrations: 1, 2, 10, 50, 200, 1000 and 2500 ng/mL. Working solutions for QC samples with concentrations of 2, 200 and 2000 ng/mL were prepared in the same manner. An IS working solution was also prepared by diluting the IS stock solution (1.0 mg/mL) to a final concentration of 200 ng/mL with methanol. All the solutions were kept at 4 °C and were brought to room temperature before use.

Aliquot (100 μL) working solutions were spiked in 100 μL blank rat plasma to obtain calibration standard samples over the concentration range from 1 to 2500 ng/mL. QC samples were prepared in the same manner at three levels (2, 200 and 2000 ng/mL). All of the spiked plasma samples were then treated according to sample preparation procedure. Both the calibration standard samples and the QC samples were applied in the method validation and the pharmacokinetic study.

2.4. Sample preparation

Plasma sample was prepared with protein precipitation. An aliquot (100 μL) methanol or 100 μL working standard solutions and 200 μL IS working solution were added to 100 μL plasma samples in polypropylene tubes. These tubes were then vortex mixed for 30 s, followed by centrifugation at 12,000 rpm at 4 °C for 5 min, and then a volume of 5 μL of the supernatant was injected into the LC–MS/MS system for analysis.

2.5. Method validation

Method validation and documentation were performed according to guidelines set by the United States Food and Drug Administration (FDA) for bioanalytical method validation [13]. This method was validated in terms of linearity, specificity, lower limit of quantification (LLOQ), recovery, matrix effect, intra- and inter-day accuracy and precision, and stability of the analyte during the sample storage and processing procedures.

2.5.1. Specificity

The specificity was defined as non-interference at retention times of Y101 and IS from the endogenous plasma components and no cross-interference between Y101 and IS using the proposed extraction procedure and LC–MS/MS conditions. Six different lots of blank (Y101-free rat plasma) were analyzed without IS to assess the specificity of the method.

2.5.2. Linearity and lower limit of quantification

For the evaluation of the linearity of the calibration curve over a linear range from 1 to 2500 ng/mL, the analyses of Y101 in plasma samples were performed in six replicates on three consecutive days using fresh preparations. Each calibration curve consisted of a blank sample, a zero sample and seven calibrator concentrations. Another blank sample was analyzed immediately following the highest concentration standard in each run to monitor the carry-over of Y101 or the internal standard. Each calibration curve was constructed by plotting the analyte to IS peak area ratio (y) *vs* analyte concentrations (x). The calibration curves were fitted using a least

square linear regression model $y = ax + b$, weighted by $1/x^2$ using the Analyst® software. The deviations of these back-calculated concentrations from calibration standard samples should be within ±15% of the theoretical value. LLOQ for the assay was the lowest concentration of the calibration curve at which Y101 spiked in six different lots of plasma and then treated using the same sample processing procedure. The LLOQ could be measured with precision and accuracy within 20%.

2.5.3. Accuracy and precision

The intra- and inter-assay precisions and accuracies were assessed from results of six replicates of the low, medium and high QC samples (2, 200, 2000 ng/mL) on three consecutive days. Intra-assay precision and accuracy were calculated using replicate ($n = 6$) determinations for each concentration of the spiked plasma sample during a single analytical run. Inter-assay precision and accuracy were calculated using replicate ($n = 18$) determinations of each concentration made on three separate days. The precisions were expressed using the relative standard deviation (RSD), and the accuracies were expressed as the percent difference between the measured concentration and the nominal concentration described as the relative error (RE). The acceptable precision and accuracy should be within 15%.

Sample dilution was validated with samples of concentration 10,000 ng/mL, which were diluted ten-fold with blank rat plasma and processed as other QC samples.

2.5.4. Recovery and matrix effect

The extraction efficiency of Y101 was determined by analyzing six replicates of Y101 plasma samples at three QC concentration levels of 2, 200, 2000 ng/mL, respectively. Recovery was calculated by comparing the peak areas of Y101 added into blank plasma and extracted using the protein precipitation procedure with those obtained from Y101 spiked directly into post-protein precipitation solvent.

The matrix effect (ME) was measured by comparing the peak response of the post-extracted spiked sample with those of the pure standards containing equivalent amounts of the Y101 prepared in mobile phase. The experiments were performed at the three QC levels in six replicates.

2.5.5. Stability study

The stability of stock solutions of Y101 and the IS was evaluated at room temperature for 24 h and in refrigerator (4 °C) for 1 month. The stability of Y101 in rat plasma was assessed by analyzing replicates ($n = 6$) of QC samples at concentrations of 2, 200 and 2000 ng/mL during the sample storage and processing procedures. For all stability studies, freshly prepared stability testing QC samples were evaluated by using freshly prepared standard curve for the measurement. The short-term stability was assessed after exposure of the plasma samples to ambient temperature for 24 h. The long-term stability was assessed after storage of the plasma samples at −20 °C for 30 days. The freeze/thaw stability was determined after three freeze/thaw cycles (room temperature to −20 °C). The sample stability in the autosampler tray was evaluated at 4 °C for 24 h. This sample stability evaluation mimics the residence time of the samples in the autosampler for each analytical run. The concentrations obtained from all stability studies were compared with the spiked concentration, and the percentage concentration deviation was calculated. The analytes were considered stable in rat plasma when the concentration difference was less than 15%.

2.5.6. Pharmacokinetic study

Sprague-Dawley rats (220 ± 20 g) were purchased from Institute of Radiation Medicine, Chinese Academy of Medical Sciences (Tianjin China). All animals were housed in an environmentally controlled room (temperature: 25 ± 2 °C, humidity: 50 ± 20%) with a natural light-dark cycle for 1 week before the experiment. All animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals of Tianjin Institute of Pharmaceutical Research, which was approved by Tianjin Municipal Science and Technology Commission (Tianjin, China). All animals were fasted overnight with water allowed *ad libitum*. All rats were randomized into three groups (eight in each group) of 25, 50 and 100 mg/kg dose groups. The Y101 solution for oral administration was prepared by suspending Y101 in carboxymethyl cellulose sodium (CMC-Na) aqueous solution. After a single dose was administered by oral gavage, blood samples (0.3 mL) were collected in heparinized tubes via the orbital vein at 0.033, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, 12, 24 h. After all blood samples were centrifuged at 12,000 rpm for 10 min, the plasma samples were collected, and then immediately stored in a −20 °C freezer until analysis. Major pharmacokinetic parameters, including $t_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$ and *CL* were analyzed by non-compartmental methods using the DAS (Drug Statistics) 2.0 software (edited by Chinese Mathematical Pharmacology Society). The peak plasma drug concentration (C_{max}) and time to C_{max} (T_{max}) were obtained directly from the detected concentration *vs* time data.

3. Results and discussion

3.1. Mass spectrometric detection

In order to optimize electrospray positive ionization (ESI) mode conditions, Y101 and IS were dissolved in methanol, and then infused into the mass spectrometer to perform full scans in positive ion mode. The ESI positive ion mode was chosen for ion product since there is one basic-N atom in the molecule of Y101, and the response of Y101 in the positive ion mode was much higher than that in the negative mode. When Y101 and IS were injected directly into the mass spectrometer along with the mobile phase, the analytes yielded predominantly $[M+H]^+$ ions at m/z 490.2 for Y101 and at m/z 256.0 for IS. Each of the precursor ions was subjected to collision induced dissociation to determine the resulting product ions from the product ion mass spectra in Fig. 2, the most abundant and stable fragment ions were generated at m/z 339.5 for Y101 and m/z 167.0 for IS. So the mass transitions chosen for quantitation were m/z 490.0 → 339.5 for Y101 and m/z 256.0 → 167.0 for IS, respectively.

3.2. Chromatographic separation

The chromatographic conditions, especially the chromatographic column and the composition of the mobile phase, were optimized through several trials to achieve rapid and selectively separations with satisfactory peak shape, and appropriate ionization for Y101 and IS as well as to eliminate the matrix effect, which was found to significantly increase the response of Y101. Methanol was chosen according to the optimal chromatographic separation generated from LC and the highest signal-to-noise (S/N) ratio produced from ESI source. An acidic modifier (1.0% formic acid) in the mobile phase enhanced sensitivity approximately two-fold, by comparing with solvent with no additive, while maintaining the separation pattern. However, the low pH may result in column instability, and it was overcome by the using of an Atlantis T3 column which can provide not only good peak shape but also exceptional chemical stability across a wide pH range. The addition of 20 mM ammonium formate was to overcome the asymmetric peak shape by simple protein precipitation. Hence, a mobile phase consisting of methanol and 20 mM ammonium formate containing 1.0% formic acid as described in experimental section was

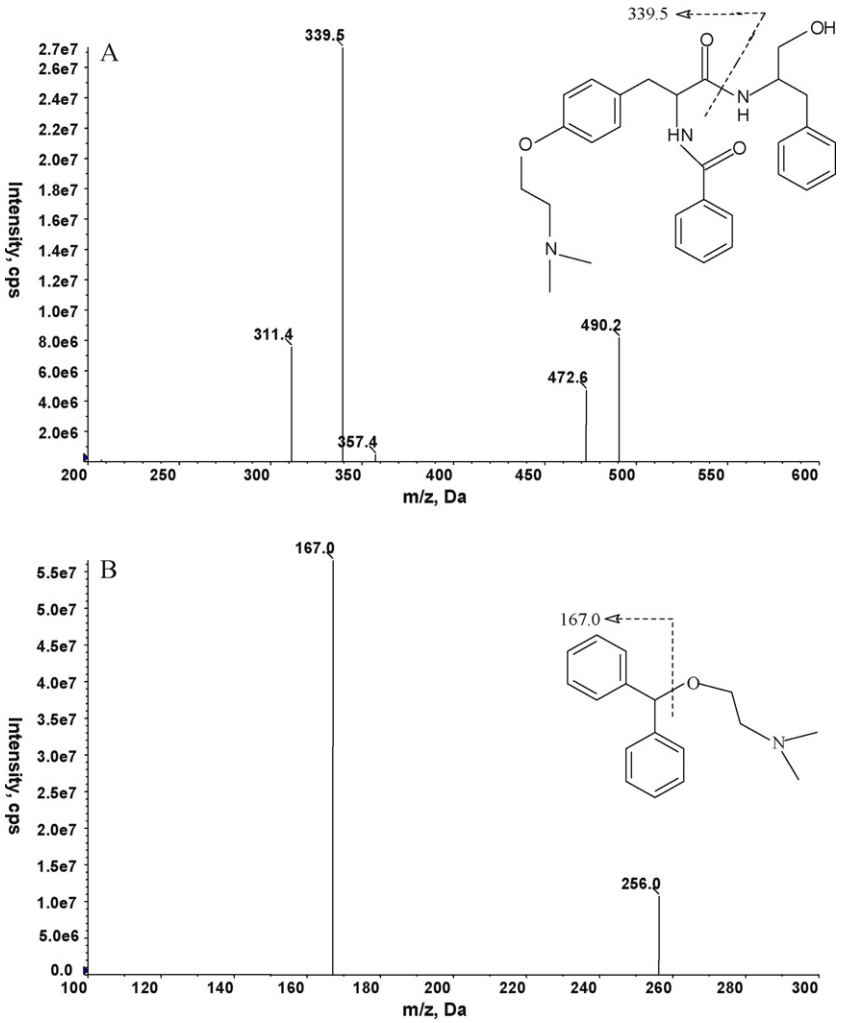

Fig. 2. The chemical structures and product ion mass spectra of the $[M+H]^+$ ions of (A) Y101 and (B) IS.

selected. The flow rate 0.4 mL/min and the injection volume 5 μL were selected for the optimal chromatographic separation of Y101. To avoid or reduce the ion enhancement, the proportion of organic phase in the mobile phase was lower, but the run time was significantly prolonged [14]. Diphenhydramine was selected as the internal standard for its similar chromatographic retention and mass spectrometric behavior to Y101; and the subsequent results demonstrated that the extraction recovery and matrix effect of diphenhydramine were similar to the Y101 and was stable in the sample process. Therefore, under optimized LC and MS conditions, Y101 and IS were separated with retention times of 5.04 and 5.45 min, respectively, as shown in Fig. 3.

3.3. Sample preparation

Protein precipitation was chosen to pretreat samples, since it is easy to manipulate and not time-consuming. After investigation of matrix effects and recoveries of Y101 and IS, methanol was then chosen as a precipitant in the current study, as it could not only ensure a good cleanup of the plasma samples, adequate recoveries and high sensitivity but also effectively eliminate matrix effect. Since a large amount of biological samples need to be determined in the pharmacokinetic study, protein precipitation established in the study can satisfy all the needs as required by guidelines of FDA.

3.4. Method validation

3.4.1. Specificity

The specificity of the method was evaluated by analyzing individual blank plasma samples from six different sources. All samples were found to have no interferences from endogenous substances at the retention time of either Y101 or the IS. Representative chromatograms of a rat blank plasma, a spiked rat plasma sample with Y101 and IS, and a plasma sample from a rat are shown in Fig. 4.

3.4.2. Linearity and lower limit of quantification

The method exhibited good linearity over the concentration range of 1–2500 ng/mL with correlation coefficients from inter-day analysis greater than 0.9976 in all cases. A typical equation of the calibration curve was: $y = 0.0135x + 0.0051$ ($r = 0.9986$), where y is the peak-area ratio of Y101 to IS and x is the plasma concentration of Y101.

The LLOQ for Y101 was established at 1 ng/mL, which was sensitive enough for pharmacokinetic study of Y101 in rats. The precision and accuracy at this concentration level were acceptable, with 5.3% of the precision and −6.8% of the accuracy as shown in Table 1.

No carry-over peaks were observed at the retention times and the ion channels of either Y101 or IS.

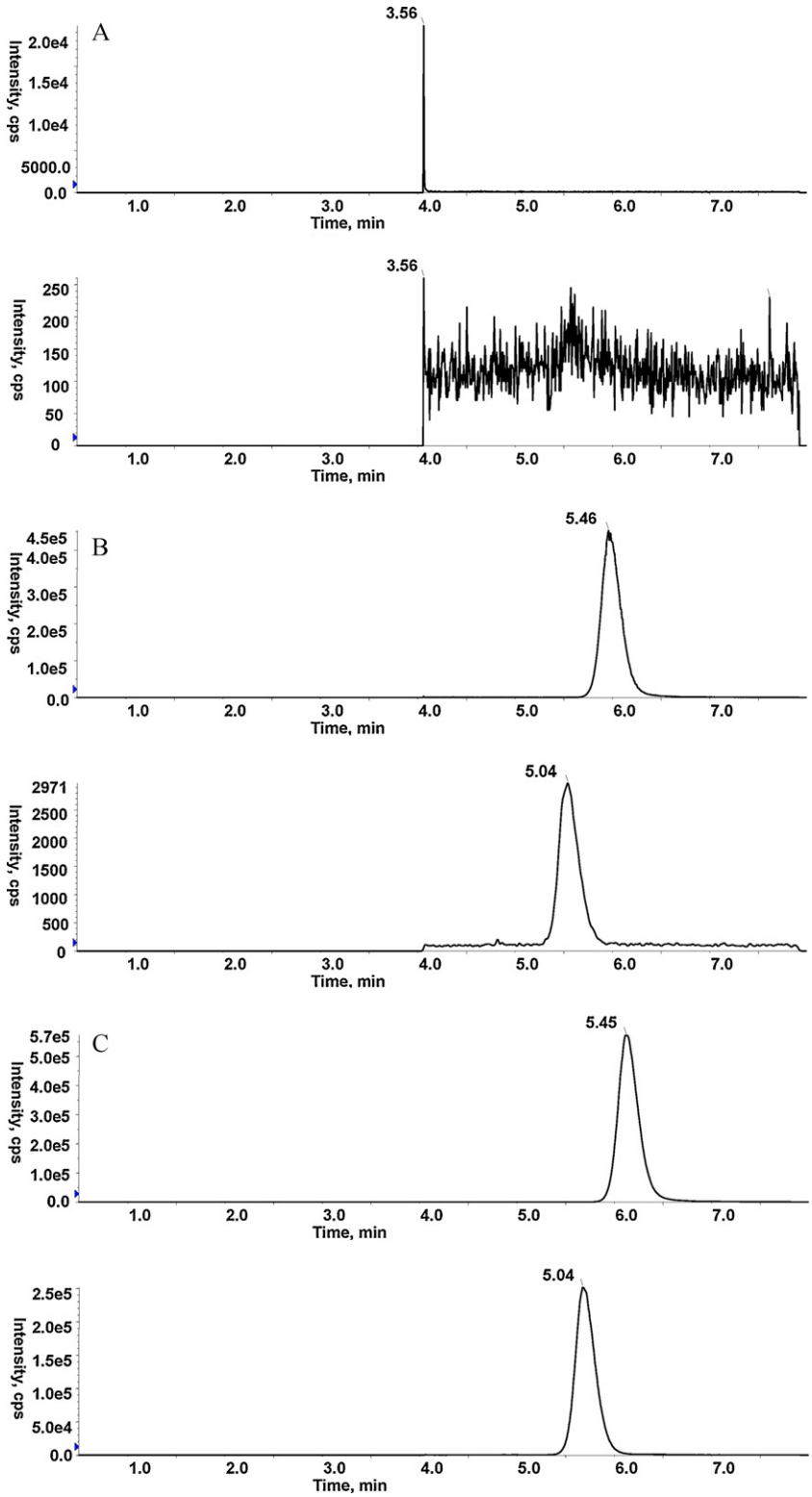

Fig. 3. Representative MRM chromatograms of Y101 (A) a blank rat plasma sample; (B) a blank rat plasma sample spiked with Y101 (LLOQ, 1 ng/mL) and IS (200 ng/mL); and (C) a rat plasma sample obtained from 5 h after an intra-gastric administration of Y101 25 mg/kg spiked with IS.

3.4.3. *Precision and accuracy*

The intra-day and inter-day precision and accuracy of QC samples (2, 200 and 2000 ng/mL) is summarized in Table 1. The intra-day precision ranged from 2.5% to 8.3% and the inter-day precision ranged from 1.7% to 14.1% with the accuracy ranged from −4.0% to 2.8%. These data demonstrated that the current method had satisfactory accuracy, precision and reproducibility for the quantification of Y101 throughout a wide dynamic range.

To assess the accuracy and precision of dilution, six replicated samples of dilution were prepared at the concentration of 10,000 ng/mL, and diluted ten-fold. The precision and the accuracy was 2.7% and 7.2% respectively, indicating that samples can be

Table 1
Precision and accuracy of the assay method for Y101 in rat plasma (n = 3 days, six replicates per day).

Concentration (ng/mL)		Precision (RSD(%))		Accuracy
Spiked	Measured (mean ± SD)	Intra-day	Inter-day	(RE(%))
2	1.9 ± 0.2	8.3	14.1	−4.0
200	207 ± 5.1	2.5	2.5	2.5
2000	1961 ± 53.5	2.8	1.7	2.8

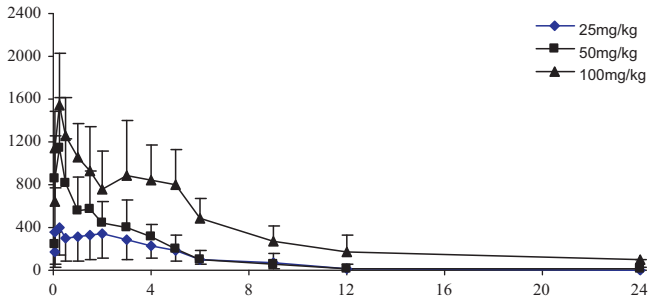

Fig. 4. Mean plasma concentration–time curves of Y101 following single intra-gastric administration of Y101 at doses of 25, 50 and 100 mg/kg to rats (n = 8, mean ± SD).

accurately determined after dilution when the measured concentration of a sample was above the upper limit of quantitation.

3.4.4. Recovery and matrix effect

In the study, the clean-up of the plasma samples was achieved through a simple and fast single-step protein precipitation procedure and acquired accepted and repeated extraction recoveries for Y101 and IS. The extraction recoveries of Y101 were (111 ± 5.3)%, (108 ± 2.2)% and (106 ± 2.8)% at three concentrations of 2, 200 and 2000 ng/mL, respectively, while the recovery of the IS was (102 ± 2.4)%. These results suggested that the recovery of Y101 was consistent and not concentration-dependent.

Matrix effect can affect on the reproducibility from the analyte or the internal standard of the assay [15–18]. The method developed in this study yielded satisfactory results for the determination of Y101 and IS in biological matrix. The matrix effects of Y101 were (108 ± 5.5)%, (100 ± 1.3)% and (101 ± 0.9)% at three concentrations of 2, 200 and 2000 ng/mL, respectively, while the matrix effect of the IS was (100 ± 0.7)%, suggesting that the conditions had limited effect by any signal of plasma after simple protein precipitation clean-up step.

Table 3
Main pharmacokinetic parameters of Y101 following intragastric administration at doses of 25, 50 and 100 mg/kg to rats (n = 8).

Parameters	Mean ± SD		
	25 mg/kg	50 mg/kg	100 mg/kg
AUC_{0-t} (μg/L h)	2088 ± 904	3067 ± 761	6500 ± 1183
$AUC_{0-\infty}$ (μg/L h)	2104 ± 900	3085 ± 787	6554 ± 1210
$t_{1/2}$ (h)	3.1 ± 1.9	2.5 ± 1.6	3.0 ± 1.0
T_{max} (h)	0.9 ± 0.8	0.6 ± 0.5	0.9 ± 1.7
CL (L/h/kg)	14.4 ± 7.7	17.1 ± 3.9	15.7 ± 2.5
C_{max} (μg/L)	467 ± 255	1271 ± 371	1522 ± 375

3.4.5. Stability

The stability of Y101 was investigated to cover expected conditions during all of the sample storage and process periods, including the stability data from freeze/thaw, short-term, autosampler and long-term stability tests. These data are summarized in Table 2. The results indicated that Y101 were stable after being placed in rat plasma for three cycles when stored at −2 °C and thawed to ambient temperature, under repeated exposure to ambient temperature for 24 h, in the autosampler tray at 4 °C over 24 h and stored at −20 °C for 30 days. Y101 and IS were also stable in stock solutions at room temperature for 24 h and in refrigerator (4 °C) for 1 month (data not shown). Taking all the points into consideration, Y101 can be stored and processed under routine laboratory conditions without special attention.

3.4.6. Application to pharmacokinetic study

After single intra-gastric administration of Y101 at doses of 25, 50 and 100 mg/kg, Y101 concentrations in rat plasma were successfully determined by using the LC–MS/MS method described above. The mean plasma concentration *vs* time profiles for Y101 are presented in Fig. 4. The major pharmacokinetic parameters were calculated by non-compartmental method and are listed in Table 3. The area under the plasma concentration (AUC_{0-t}) of Y101 were (2088 ± 904), (3067 ± 761) and (6500 ± 1183) μg/L h, respectively. The concentration maximum (C_{max}) were (467 ± 255), (1271 ± 371) and (1522 ± 375) μg/L, respectively. Over the Y101 dose range investigated, mean AUC_{0-t} and C_{max} increased proportionally to the doses given. The values of $t_{1/2}$ and CL appeared to be consistent with the doses increased. The results indicated dose linearity for doses between 25 and 100 mg/kg in the rat after intra-gastric administration, and the pharmacokinetic parameters obtained from this study can give some useful information for further research of Y101.

The concentration–time curve of Y101 rat plasma after intragastric administration showed the second peak phenomenon. This

Table 2
Stability of Y101 in rat plasma samples under various conditions (n = 6).

Storage conditions	Concentration (ng/mL)		Precision	Accuracy
	Spiked	Measured (mean ± SD)	RSD (%)	RE (%)
Three free–thaw cycles	2	2.0 ± 0.2	7.6	1.3
	200	200 ± 2.9	1.5	0.2
	2000	1912 ± 44.3	2.3	−4.4
At room temperature for 24 h	2	2.1 ± 0.2	9.6	2.3
	200	195 ± 2.6	1.4	−2.7
	2000	1912 ± 40.0	2.1	−4.4
At −20 °C for 30 days	2	1.8 ± 0.9	4.6	−8.0
	200	199 ± 8.8	4.4	−0.5
	2000	2003 ± 5.0	0.3	0.1
At 4 °C in the autosampler for 24 h	2	2.1 ± 0.1	3.6	3.4
	200	209 ± 5.1	2.5	4.4
	2000	1998 ± 31.8	1.6	−0.1

phenomenon might be caused by the following reasons: (1) the absorption varied within different regions of the gut; (2) enterohepatic circulation might exist, and the identification of enterohepatic recirculation may be required by a comparison of AUC obtained after oral administration of the compound in normal and bile duct cannulated rats. Further studies are needed to investigate the exact mechanism underlying the second-peak phenomenon for Y101.

4. Conclusion

The optimized LC–MS/MS method was validated to guarantee a reliable determination of Y101 in rat plasma. The method has been proved to be very simple, sensitive, and the major advantage of this method is the simple one-step precipitation for sample preparation which can be easily implemented into related routine practice; The method also produces accurate and precise measurements of Y101 plasma concentrations with a small plasma volume of 100 μL and in a short run time of 7.5 min which satisfied the requirements of high sensitivity, specificity and rapid sample throughput. The results for parameter validation such as recovery, matrix effect and stability were within the acceptable limits. Plasma concentrations of Y101 can be quantified from 1 to 2500 ng/mL, making it possible to analyze samples up to 24 h or longer after single intra-gastric administration of Y101 at three doses of 25, 50 and 100 mg/kg. The LC–MS/MS assay was successfully applied to determine Y101 in rat plasma, and the pharmacokinetic profiles of Y101 were investigated for the first time in rats.

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