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Short communication

Determination of tegaserod by LC–ESI-MS/MS and its application to a pharmacokinetic study in healthy Chinese volunteers

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Abstract

A simple, rapid and sensitive high performance liquid chromatography–electrospray ionization-tandem mass spectrometry (HPLC–ESI-MS/MS) assay for determination of tegaserod in human plasma using diazepam as internal standard (IS) was established. After adjustment to a basic pH with sodium hydroxide, plasma was extracted by ethyl acetate and separated by high performance liquid chromatography (HPLC) on a reversed-phase C_{18} column with a mobile phase of methanol: 5 mM ammonium acetate (75:25, v/v, adjusting the pH to 3.5 with glacial acetic acid). The quantification of target compounds was obtained by using multiple reaction monitoring (MRM) transitions; *m/z* 302.5, 173.2 and 285.4, 193.2 were measured in positive mode for tegaserod and internal standard (diazepam), respectively. The lower limit of quantification (LLOQ) was 0.05 ng/ml. The calibration curves were linear over the range 0.05–8.0 ng/ml ($r = 0.9996$) for tegaserod. The mean absolute recovery of tegaserod was more than 85.56%. Intra- and inter-day variability values were less than 9.21% and 10.02%, respectively. The samples were stable for 8 h under room temperature (25 °C, three freeze–thaw cycles in 30 days and for 30 days under −70 °C). After administration of a single dose of tegaserod maleate 4 mg, 6 mg and 12 mg, respectively, the area under the plasma concentration versus time curve from time 0 h to 12 h (AUC_{0-12}) were (2.89 ± 0.88), (5.32 ± 1.21) and (9.38 ± 3.42) ng h/ml, respectively; peak plasma concentration (C_{max}) were (1.25 ± 0.53), (2.21 ± 0.52) and (4.34 ± 1.66) ng/ml, respectively; apparent volume of distribution (V_d/F) were (6630.5 ± 2057.8), (7615.2 ± 2242.8) and (7163.7 ± 2057.2) l, respectively; clearance rate (CL/F) were (1851.4 ± 496.9), (1596.2 ± 378.5) and (1894.2 ± 459.3) l/h, respectively; time to C_{max} (T_{max}) were (1.00 ± 0.21), (1.05 ± 0.28) and (1.04 ± 0.16) h, respectively; and elimination half-life ($t_{1/2}$) were (3.11 ± 0.78), (3.93 ± 0.92) and (3.47 ± 0.53) h, respectively; MRT were (3.74 ± 0.85), (4.04 ± 0.56) and (3.28 ± 0.66) h, respectively. The essential pharmacokinetic parameters after oral multiple doses (6 mg, b.i.d) were as follows: C_{ssmax}, (2.72 ± 0.61) ng/ml; T_{max}, (1.10 ± 0.25) h; C_{ssmin}, (0.085 ± 0.01) ng/ml; C_{av}, (0.54 ± 0.12) ng/ml; DF, (4.84 ± 0.86); AUC_{ss}, (6.53 ± 1.5) ng h/ml. This developed and validated assay method had been successfully applied to a pharmacokinetic study after oral administration of tegaserod maleate in healthy Chinese volunteers at a single dose of 4 mg, 6 mg and 12 mg, respectively. The pharmacokinetic parameters can provide some information for clinical medication.

Keywords: Tegaserod; Pharmacokinetics; LC–ESI-MS/MS

1. Introduction

Tegaserod maleate (Fig. 1A) is an amino guanidine-indole with selective and partial 5-HT4-receptor agonist activity [1]. Both preclinical and clinical investigations have shown that tegaserod can stimulate motility throughout the gastrointestinal tract, approved in 2002 to cure patients suffering from constipation predominant irritable bowel syndrome (IBS), a complex gastrointestinal disorder [2–3]. Therefore, it is clinically important for a pharmacokinetic and pharmacodynamic study to detect tegaserod in human plasma.

So far, there are a few reports on the method of estimation of tegaserod. Plasma concentrations of tegaserod were analyzed using a gas chromatography/mass spectrometry method with negative chemical ionization (limit of detection, 0.1 ng/ml) [4].

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The estimation of tegaserod has been carried out by HPLC at 1–10 μM concentration and its metabolites were identified by LC–MS [5]. Tegaserod has also been estimated in human serum by differential pulse voltammetry [6] with a detection limit of 3.0×10.10 M, Sonu [7] has developed an LC–MS/MS method for the bioequivalence study of tegaserod formulation on healthy male human subjects. The method is limited by time-consuming extraction procedure such as solid phase extraction (SPE) and the LLOQ of 0.20 ng/ml. In our study, because the clinical dosage of tegaserod is lower than Sonu [7], the LLOQ of 0.20 ng/ml is not sensitive enough. Present paper describes a simple, economic and yet sensitive LC–ESI-MS/MS method that can determine tegaserod plasma concentration in humans as low as 0.05 ng/ml using liquid–liquid extraction and can be used to evaluate the pharmacokinetics of tegaserod in humans.

2. Experimental

2.1. Materials

Tegaserod maleate standard reference (purity: 99.3%, lot no. 20050812) and tegaserod maleate tablets (2 mg/Tab, lot no. 20050603) were kindly provided by Hua Xin Pharmaceutical Limited (Nanjing, China). The internal standard (diazepam, IS, Fig. 1B) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (purity: 99.7%, lot no. 100374-200416; NICBP, Beijing, China); HPLC grade methanol was purchased from Tedia Company, Inc. (Fairfield, OH, USA). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA) and was used to prepare all aqueous solutions. Other chemicals and reagents were of analytical grade. Drug-free and drug-containing plasma were taken from the volunteers. Plasma was stored at −70 °C until assayed.

2.2. Instrumentation

A Waters (Milford, MA, USA) Alliance 2695 liquid chromatographic system interfaced to a 2996 dual wavelength UV detector, a Micromass Quattro ESI mass spectrometer and a Masslynx™ 4.0 data system were equipped with an Agilent Zorbax SB C_{18} column (100 mm × 2.1 mm ID, dp 3.5 μm) at a column temperature of 25 °C.

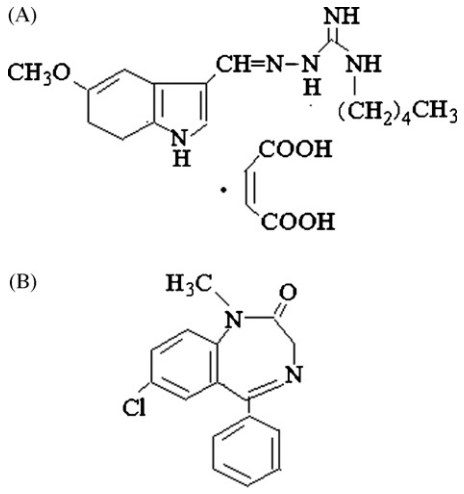

Fig. 1. Chemical structures of tegaserod maleate (A) and diazepam (B).

2.3. HPLC–ESI-MS/MS conditions

The mobile phase, methanol–5 mM ammonium acetate (75:25, v/v, adjusting the pH to 3.5 with glacial acetic acid), was run at a flow rate 0.20 ml/min, and the injection volume was 10 μl. Separation was conducted under isocratic conditions and the total running time was not more than 7 min. The autosampler was controlled at 8 °C. After establishing the final conditions for the chromatographic analysis of tegaserod, the detector interface and mass spectrometer were systematically optimized to maximize the response for the tegaserod $[M+H]^+$ ion with detection in the multiple reaction monitoring (MRM) mode. ESI-MS/MS was performed in the positive mode: capillary and cone voltage were 3000 and 32 V, respectively; drying-gas (N_2) flow rate was 500 l/h; the ionization sources were worked at 110 °C; the desolvation temperature was 450 °C; ion energy was 12.0. The gas used was of high purity. Multiple reaction monitoring (MRM) was used to quantify tegaserod *m/z* 302.5 $[M+H]^+$, daughter ion *m/z* 173.2, Collision was 23 V; and diazepam (IS) *m/z* 286.4 $[M+H]^+$, daughter ion *m/z* 193.2, collision was 30 V, as were shown in Fig. 2. The two pairs of ion were monitored simultaneously within the analytical procedure.

2.4. Preparation of standard solutions

All concentrations of tegaserod and diazepam refer to the free bases. Stock solutions of tegaserod and diazepam were prepared in a mixture of water:methanol (20:80, v/v). All stock solutions were stored at −70 °C when not in use. The stock solutions were further individually diluted with the same diluents to give working standard solutions of tegaserod and diazepam.

2.5. Sample preparation

After frozen human plasma samples were thawed at ambient temperature, a 1 ml aliquot plasma sample was added with 50 μl IS (500 ng/ml) solution and 0.1 ml of 0.1 M sodium hydroxide solution. After a thorough vortex mixing for 30 s, mixtures were extracted with 5 ml of ethyl acetate, vortex-mixed for 3 min, and centrifuged at $1072 \times g$ ($r = 0.03$ m) for 10 min. The 4 ml of organic layer was removed and evaporated under a stream of nitrogen gas in the thermostatically controlled water-bath maintained at 50 °C until completely dry. The dried residue obtained was dissolved in 100 μl of mobile phase, vortex-mixed for 1 min, centrifuged at $11{,}411 \times g$ ($r = 0.02$ m) for 3 min, and 10 μl of the supernatant liquid was then injected into the LC–ESI-MS/MS system.

2.6. Preparation of calibration curves and quality control samples

Calibration standards of tegaserod were prepared by spiking appropriate amount of the working standards solutions

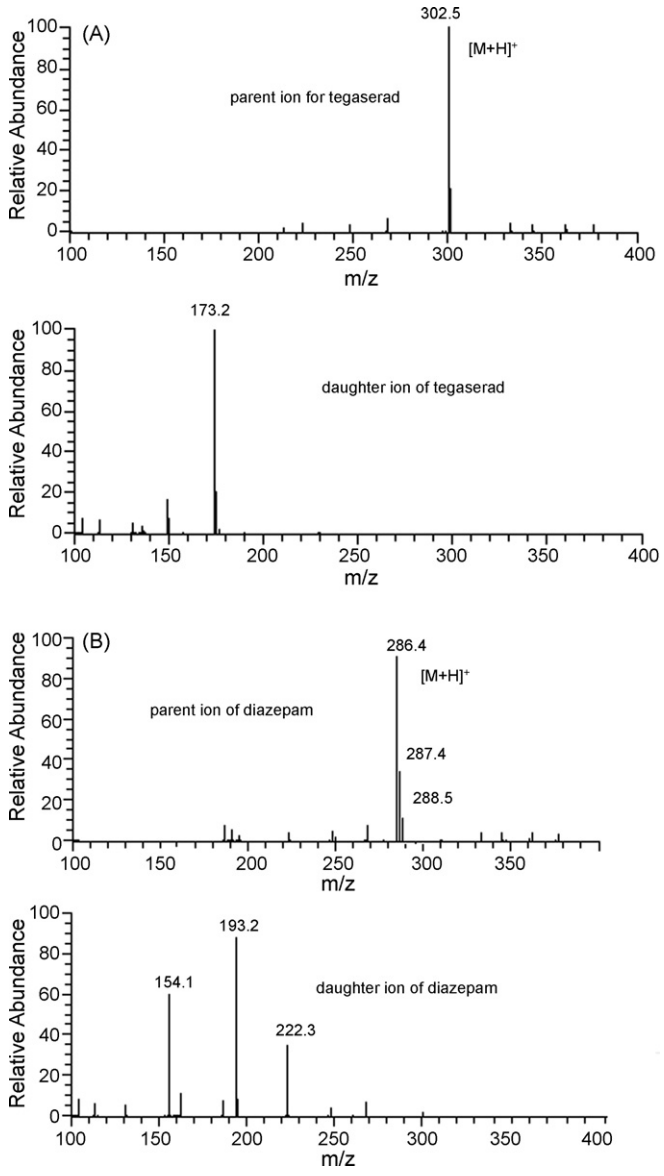

Fig. 2. (A) Mass spectrum plot of parent and daughter ions acquired at apex of the chromatographic peak for tegaserod by continuous scanning over a mass range of 100–400 a.m.u. (B) Mass spectrum plot of parent and daughter ions acquired at the apex of the chromatographic peak for diazepam by continuous scanning over a mass range of 100–400 a.m.u.

of tegaserod in screw-capped glass tubes, then, they were evaporated to dryness under a stream of nitrogen in the thermostatically controlled water-bath maintained at 50 °C for about 5 min, respectively, and 1.0 ml drug-free plasma obtained from healthy volunteers was added and mixed well. Standard curves were prepared in the range of 0.05–8.0 ng/ml for tegaserod at concentrations of 0.05, 0.1, 0.25, 0.5, 1.0, 5.0 and 8.0 ng/ml. The calibration curve was prepared and assayed along with quality control (QC) samples and each batch of clinical plasma samples. QC samples were prepared in drug-free plasma at concentrations of 0.1, 0.5 and 5.0 ng/ml for tegaserod in the same manner as standard curves. Then, the calibration standards and QC samples were treated following the sample preparation procedure, as indicated in Section 2.4. The QC samples were prepared independently of the calibration standards, and analyzed with processed test samples at intervals in each run. The results of the QC samples provided the basis for accepting or rejecting the run.

2.7. Assay validation

2.7.1. Specificity and selectivity

The specificity and selectivity of the method were checked by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Each blank plasma sample was tested using the proposed extraction procedure and LC–ESI-MS/MS conditions to ensure no interference of tegaserod and IS from plasma.

2.7.2. Linearity of calibration curves and lower limits of quantification

Validation runs were conducted on five separate days. Each validation run consisted of a set of the spiked calibration standards at seven concentration levels at 0.05, 0.1, 0.25, 0.5, 1.0, 5.0 and 8.0 ng/ml ($n = 5$ at each concentration). Calibration curves ($y = ax + b$) were represented by plotting the peak area ratios (y) of tegaserod to IS versus the concentrations (x) of the calibration standards. Calibration curves were obtained from weighted ($1/x$) least-squares linear regression analysis of the data. The linearity was also assessed for five consecutive days for the standard solutions of the same range of concentrations prepared from the stock solutions.

The LLOQ was defined as the lowest concentration on the calibration curve at which precision was within 20% and accuracy was within ±20% [8], and it was established using five samples independent of standards.

2.7.3. Precision and accuracy

The precision of the assay was determined from low, medium and high QC plasma samples by replicate analysis of three different concentrations (0.1, 0.5 and 5.0 ng/ml). Validation samples were prepared and analyzed on three separate runs to evaluate the accuracy, intra-run and inter-run precision of the analytical method. The precision was defined as the relative standard deviation (R.S.D.) from mean (M) using the formula R.S.D.% = S.D./$M \times 100$. The accuracy is the degree of closeness of the determined value to the nominal true value under prescribed conditions. Accuracy is defined as the relative deviation in the calculated value (E) of a standard from that of its true value (T) expressed as a percentage (RE%). It was calculated by using the formula: RE% = $(E - T)/T \times 100$.

2.7.4. Extraction recovery

The extraction recoveries of tegaserod were determined at low, medium and high concentrations by comparing the responses of tegaserod extracted from plasma samples with standard solutions without extraction. The recovery is calculated by the formula: recovery (%) = (detector response of extracted analyte/detector response for non-extracted analyte) × 100, where detector response is the area of the chromatographic peak for extracted or non-extracted analyte divided by the

area of the chromatographic peak for the internal standard added.

2.7.5. Stability

The stability experiments aimed at testing all possible conditions that the samples might be exposed to during sample shipping and handling. The stock solutions were investigated by storing under refrigeration at −70 °C. To evaluate tegaserod stability in human plasma, drug-free plasma samples were spiked at 0.1, 0.5 and 5.0 ng/ml (QC) ($n = 3$ per test and each concentration). After extraction, samples were arranged in the autosampler at 8 °C and were analyzed. The short-term temperature stability was assessed by analyzing QC samples that were kept at ambient temperature (25 °C) for 8 h. Freeze–thaw stability (−20 °C in plasma) was checked through three cycles in 30 days. The long-term stability was performed at −70 °C in plasma for 30 days. The stability of extracted and dried residues of plasma samples containing tegaserod were also evaluated.

2.8. Pharmacokinetic study design

The developed method was used to study the pharmacokinetic characteristics of tegaserod maleate tablets in healthy Chinese volunteers.

2.8.1. Subjects

The pharmacokinetic study protocol used was approved by the Stated Food and Drug Administration (SFDA, China). The volunteers had the following clinical characteristics (expressed as mean ± S.D. [range]): age, 22.4 ± 2.8 [20–25]; height, 173.4 ± 5.6 cm [168.2–180.2]; body weight, 66.2 ± 5.0 kg [50–76]. Thirty healthy volunteers including 18 males and 18 females are randomly divided into three groups, such as Groups A–C. Each group was made up of six males and six females. They were selected after passing a clinical screening procedure including a physical examination and laboratory tests, which included hematology, blood biochemistry, and urine analysis. No volunteers had a history or evidence of a renal, gastrointestinal, hepatic, or hematologic abnormality or any acute or chronic disease, or an allergy to any drugs. This was done to ensure that the existing degree of variation would not be due to an influence of illness or other medications. All volunteers avoided using other drugs for at least 2 weeks prior to the study and until after its completion. This study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of good clinical practice (GCP). The protocol of this study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (Nanjing, China). All participants signed a written informed consent after they had been informed of the nature and details of the study. Volunteers were hospitalized at 9:00 p.m. 1 day before this study and fasted 10 h before each drug administration. At 8:00 a.m., Groups A–C were administered a single dose of tegaserod maleate 4 mg, 6 mg and 12 mg with 250 ml water, respectively. A standard lunch was served after 4 h, and an evening meal was provided 12 h after administration. During the 24 h period after drug administration, no strenuous physical or mental activity was permitted. No other food was permitted during the 'in-house' period but liquid consumption was allowed ad libitum after lunch (with the exception of alcohol, soda, and coffee drinks, as well as juices). Heparinized blood samples (5 ml) were collected from a suitable forearm vein using an indwelling catheter into heparin containing tubes before (0 h) and 0.25 h, 0.5 h, 0.75 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 16 h, 24 h, 36 h, 48 h after dosing. The blood samples were centrifuged at 1072 × g ($r = 0.03$ m) for 10 min, and plasma samples were separated and stored at −70 °C until required for analysis. In the design of multiple doses, Group B received tegaserod maleate 6 mg with 250 ml water at 8:00 a.m. and 8:00 p.m. for 13 consecutive oral doses. In days 5, 6 and 7, 5 ml of venous blood before every dosing at 8:00 a.m. was drawn to observe minimum value of steady plasma-drug concentration. In day 7, the procedure was as that of single dose mentioned above.

2.8.2. Pharmacokinetic analysis

The data analysis of pharmacokinetic parameters was performed by using Drug and Statistics software (Version. 2.0, Chinese). Two analysis results of compartment and non-compartment were described. The types of the compartmental model were simulated by orally administrated open one- or two-compartmental model according to Akaike's information criterion (AIC) method, respectively. The maximum tegaserod plasma concentration (C_{max}) and the time to C_{max} (T_{max}) were determined by inspection of the individual plasma concentration–time profiles of the drug. The area under the plasma concentration–time curve from time zero to the last measurable concentration (AUC_{0-t}) was calculated using the linear trapezoidal rule and was extrapolated to infinity ($AUC_{0-\infty}$) according to the relationship: $AUC = (AUC_{0-t} + C_t)/k_e$. C_t is the last concentration evaluated in plasma greater than the lower limit of quantification (LLOQ) and the elimination rate (k_e) was obtained as the slope of the linear regression of the log-transformed concentration–time curve data in the terminal phase. The half-life ($t_{1/2}$) and CL/F were calculated based on the following equations: $t_{1/2} = 0.693/k_e$; $CL/F = k_e \times V_c$. V_c was the apparent volume of distribution of the center compartment, which was estimated by the model after calculation. V_d/F was the apparent volume of distribution, MRT was mean residence time.

3. Results and discussion

3.1. Method development

Sample preparation is usually required for the determination of pharmaceuticals in biological samples owing to complex matrices in order to remove possibly interfering matrix components and increase the selectivity and sensitivity. Liquid–liquid extraction (LLE) was a widely adopted method and often achieved satisfactory extraction recoveries of analytes from biological samples. In that assay [3], the test compounds were extracted from 0.5 ml of plasma using the SPE method and

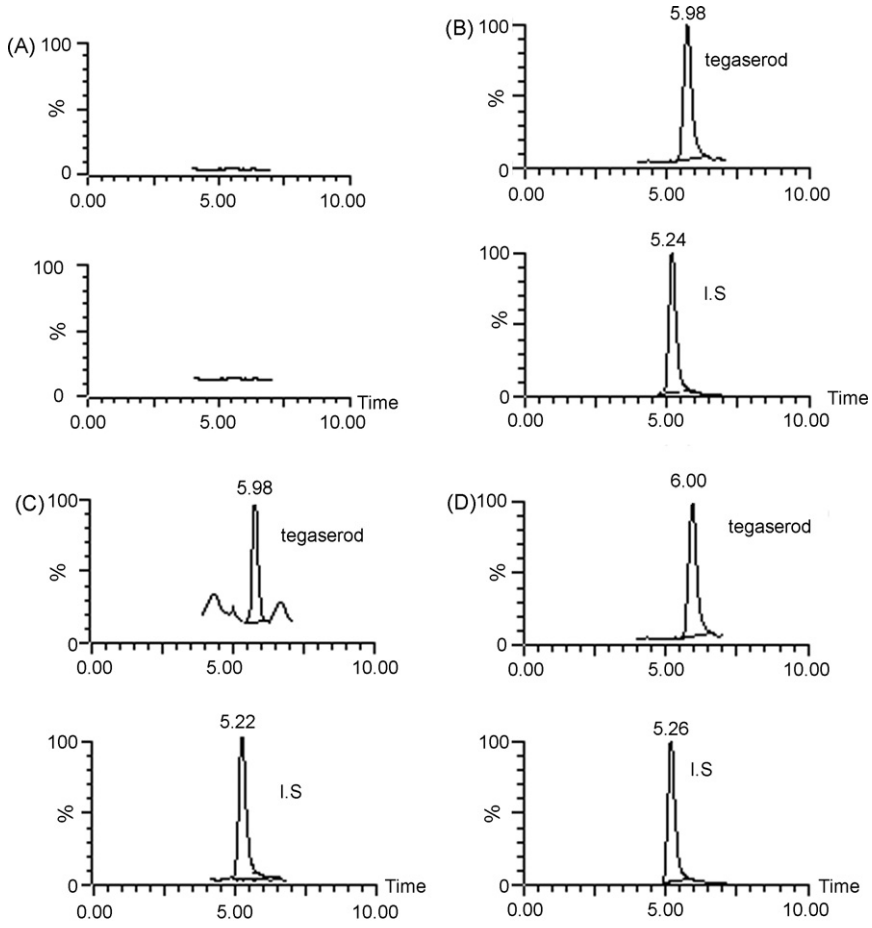

Fig. 3. Typical MRM chromatograms of blank plasma (A), plasma spiked with tegaserod (1.0 ng/ml) and the IS. (B), LLOQ for tegaserod in plasma (0.05 ng/ml) and IS (C), plasma obtained from a volunteer at 0.75 h after oral administration of 6 mg tegaserod (D).

the extract was injected into the LC system. In the present work, ethyl acetate was used for the extraction of tegaserod from human plasma, which produced a clean chromatogram for a drug-free plasma sample and offered satisfactory extraction recoveries for the analytes more than 85.56%. An Agilent Zorbax SB C_{18} column (100 mm × 2.1 mm ID, dp 3.5 μm) was used for the chromatographic separation. In optimizing the chromatographic conditions, the concentration and pH of the ammonium acetate buffer were investigated. The mobile phase of 10 mM ammonium acetate buffer yielded tailing peaks. We decreased the concentration of ammonium acetate buffer to 5 mM, and the chromatographic peaks became sharp and symmetric. For mobile phase, a mix of methanol:5 mM ammonium acetate (75:25, v/v, adjusting the pH to 3.5 with glacial acetic acid) was found to be optimal for the study, which provided symmetric peak shapes of the analytes and the internal standard as well as a short run time. For the selection of the internal standard, the similar extracted recovery and the retention time become the significant choice. Several compounds (i.e. trimetazidine, pseudoephedrine) were tried and diazepam was finally used as the internal standard in this work. A preliminary series of experiments indicated that tegaserod was very responsive to ESI-MS/MS with positive ion detection.

3.2. Method validation

3.2.1. Specificity and selectivity

Selectivity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. The product ion chromatograms extracted from plasma are depicted in Fig. 3. As shown, the retention times of tegaserod and diazepam (IS) using our system were (5.98 ± 0.05) min and (5.24 ± 0.05) min, respectively. No interference was observed at the retention times of either tegaserod or internal standard in six different batches of drug-free human plasma samples used for analysis. It showed that the method exhibited good specificity and selectivity and was applicable to clinical use.

3.2.2. Calibration curve and sensitivity

Five calibration analyses were performed on five consecutive days and the back-calculated values for each level were recorded. The regression equations for calibration curves at the range 0.05–8.0 ng/ml were $y = 0.0386x + 0.00152$ for tegaserod. The correlation coefficient was 0.9996, indicating a good linearity. Standard curves were prepared daily and were checked using the QC samples (Tables 1 and 2). The calibration curves did not exhibit any non-linearity within the chosen range.

Table 1
Individual and mean values for slope, intercepts and correlation coefficients of five calibration curves for tegaserod

Analyte	Curve	Slope	Intercept	Correlation (r)
Tegaserod	1	0.0378	0.00190	0.9995
	2	0.039	0.00110	0.9993
	3	0.0403	0.00140	0.9999
	4	0.0385	0.00220	0.9999
	5	0.0375	0.00100	0.9993
	Mean	0.0386	0.00152	0.9996
	S.D.	0.00111	0.00052	

The back-calculated results showed good day-to-day accuracy and precision. For this method, the LLOQ of tegaserod was 0.05 ng/ml, R.S.D. = 11.4% (S/N = 10).

3.2.3. Assay precision and accuracy

Table 3 summarizes the intra- and inter-run precision and accuracy for tegaserod. The precision was calculated by using one-way ANOVA. The evaluation of precision was based on the criteria [4] that the relative standard deviation for each concentration level should not be more than ±15% except for the LLOQ, for which it should not be more than ±20%. Similarly, for accuracy, the mean value should not deviate by ±15% of the actual concentration except for the LLOQ where it should not deviate by more than ±20% of the actual concentration. The results in Table 3 demonstrate that the precision and accuracy of this assay are within the acceptable range and the method is accurate and precise.

3.2.4. Extraction recovery

The ethyl acetate was chosen as the extraction solvent for its higher extraction efficiency to the two target compounds. The extraction recoveries of tegaserod and IS could be promoted by raising the pH of the plasma samples with sodium hydroxide, and so 0.1 ml of 0.1 M sodium hydroxide solution was added to 1.0 ml plasma sample before extraction. The extraction recoveries of tegaserod from the human plasma were (85.56 ± 7.31) %, (87.59 ± 4.28) % and (88.46 ± 3.70) % at concentration levels of 0.1, 0.5 and 5.0 ng/ml, and the R.S.D. were 8.54%, 4.89% and 4.18% (n = 5), respectively.

3.2.5. Stability

The stability of tegaserod was studied under a variety of storage and handling conditions. The stock solutions were investigated by storing under refrigeration at −70 °C and were discovered to be stable for at least 45 days with the R.S.D. below 3.31%. In the short-term stability study, the QC plasma samples were found to be stable for 8 h at ambient temperature (25 °C).

Table 3
Accuracy and precision for the analysis of tegaserod in human plasma (in prestudy validation, three runs, five replicates per run)

Measurement	Added concentration (ng/ml)	Tegaserod	
		Precision[a] R.S.D. (%)	Mean accuracy[b] (%) (n = 15)
Intra-run	0.1	9.2	99.3
	0.5	4.7	108.3
	5.0	1.8	109.2
Inter-run	0.1	10.0	100.2
	0.5	5.5	106.6
	5.0	2.5	99.0

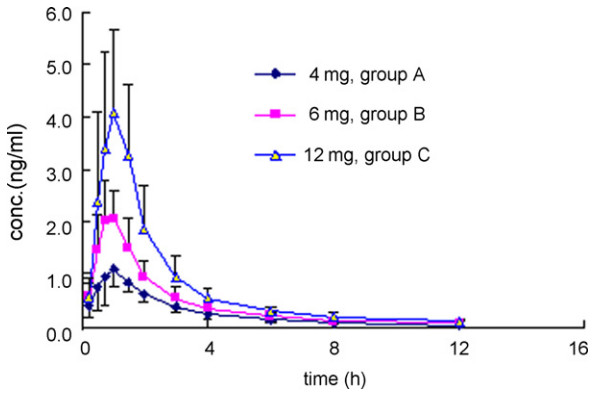

Fig. 4. Plasma concentration–time profile of tegaserod after an oral administration of 4 mg, 6 mg and 12 mg tegaserod maleate tablets to 36 healthy volunteers (Groups A–C). Each point represents a mean ± S.D. (n = 12).

In the long-term stability study, the plasma samples spiked with the QC plasma samples also showed no loss of analytes when they were stored for 30 days at −70 °C. Extracted and dried residues of plasma samples containing tegaserod were stable at −70 ± 5 °C. The final stability test was demonstrated after three freeze–thaw cycles in 30 days. No significant degradation of tegaserod was observed under any of these conditions (Table 4). The results were obtained by a comparison with freshly prepared solutions, and the percentage concentration deviations were within ±5%.

3.3. Pharmacokinetic study

Sonu Sundd Singh et al. [7] described the pharmacokinetic parameters in 24 healthy volunteers receiving 6 mg of test and reference tegaserod formulations. The developed method in this paper was successfully used for a pharmacokinetic study in which plasma concentration of tegaserod in 36 healthy Chinese volunteers (Groups A–C)

Table 2
Validation from QC samples of human plasma extracts (n = 5)

Added C (ng/ml)	Detected C (ng/ml)					Mean	±S.D.	R.S.D. (%)	RE (%)
0.1	0.0999	0.0911	0.1050	0.0930	0.1075	0.0993	0.0072	7.2	−0.7
0.5	0.4882	0.5093	0.5141	0.5227	0.4703	0.5009	0.0213	4.3	0.2
5.0	5.1561	5.2369	5.0554	5.2000	4.9311	5.1159	0.1236	2.4	2.3

Table 4
Stability data for tegaserod ($n = 3$ per test and each concentration)

Stability conditions	Added concentration (ng/ml)		
	0.1	0.5	5.0
The stock solution (45 days, −70 °C)	0.0945 ± 0.0024	0.4685 ± 0.0130	0.4840 ± 0.0160
Long-term (30 days, −70 °C)	0.1035 ± 0.0014	0.5020 ± 0.0140	0.4905 ± 0.0085
Short-term (8 h, 25 °C)	0.0955 ± 0.0026	0.5055 ± 0.0115	0.5165 ± 0.0125
Auto sampler (24 h, 8 °C)	0.0938 ± 0.0026	0.4815 ± 0.0100	0.4935 ± 0.0135
Three freeze–thaw cycles, (30 days, −70 °C)	0.1018 ± 0.0019	0.5170 ± 0.0075	0.4875 ± 0.0080
Died-extract (48 h, −70 °C)	0.0985 ± 0.0018	0.5085 ± 0.0145	0.5110 ± 0.0120

Note. R.S.D., relative standard deviation; RE, relative error.

Table 5
Pharmacokinetic parameters of tegaserod in 36 healthy Chinese volunteers ($n = 12$, mean ± S.D.)

Parameters (unit)	4 mg	6 mg	12 mg
$t_{1/2\beta}$ (h)	3.11 ± 0.78	3.93 ± 0.92	3.47 ± 0.53
T_{max} (h)	1.00 ± 0.21	1.05 ± 0.28	1.04 ± 0.16
C_{max} (ng/ml)	1.25 ± 0.53	2.21 ± 0.52	4.34 ± 1.66
V/F (l)	6630.5 ± 2057.8	7615.2 ± 2242.8	7163.7 ± 2057.2
CL/*F* (l/h)	1851.4 ± 496.9	1596.2 ± 378.5	1894.2 ± 459.3
$AUC_{(0-12)}$ (ng h/ml)	2.89 ± 0.88	5.32 ± 1.21	9.38 ± 3.42
MRT (h)	3.74 ± 0.85	4.04 ± 0.56	3.28 ± 0.66

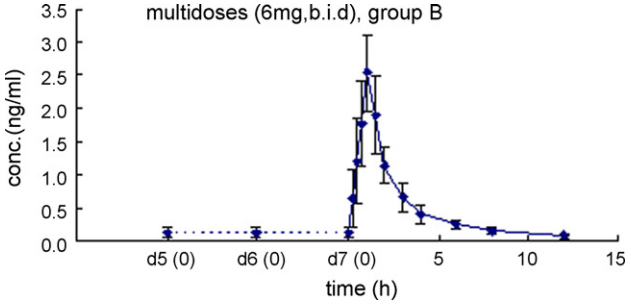

Fig. 5. Mean plasma concentration–time profile of tegaserod in 12 healthy volunteers (Group B) after oral multiple doses of tegaserod maleate tablets (6 mg, b.i.d). Each point represents a mean ± S.D. ($n = 12$).

were determined up to 12 h after the oral administration of 4 mg, 6 mg and 12 mg tegaserod maleate tablets. The mean plasma concentration–time curves were shown in Fig. 4. The concentration was below the limit of the quantification after 12 h. The mean plasma concentration–time curves of multiple doses are shown in Fig. 5. By using Drug and Statistics software (Version 2.0, Chinese) analysis, the compartmental and non-compartmental analysis pharmacokinetic parameters were listed in Table 5. The main pharmacokinetic parameters of multiple doses were as follows: C_{ssmax}, (2.72 ± 0.61) ng/ml; T_{max}, (1.10 ± 0.25) h; C_{ssmin}, (0.085 ± 0.01) ng/ml; C_{av}, (0.54 ± 0.12) ng/ml; DF, (4.84 ± 0.86); AUC_{ss}, (6.53 ± 1.5) ng h/ml.

4. Conclusion

There were no adverse events during the conduct of the study. The developed method adopted a simple preparation, offered sufficient sensitivity, satisfactory selectivity and good reproducibility. So, it can be successfully applied to the pharmacokinetic study. The pharmacokinetic characteristics of the healthy Chinese volunteers were fitted for two-compartmental mode, moreover, the data of T_{max}, V_d/F, CL/*F* and $t_{1/2\beta}$ illustrated that tegaserod was rapidly absorbed, widely distributed and fastly eliminated in healthy Chinese body.

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