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LC–MS determination and relative bioavailability of doxazosin mesylate tablets in healthy Chinese male volunteers

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Abstract

This study aims to develop a standard protocol for the relative bioavailability testing of doxazosin mesylate tablets. For this purpose, a simple rapid and selective LC–MS method using a single quadrupole mass spectrometer was developed and validated to determine the concentration of doxazosin mesylate in human plasma. Using this method, we carried out a study of relative bioavailability. *N*-Hexylane-tertiary butyl methyl ether (1:1, v/v) was used to extract doxazosin mesylate and terazosin (internal standard, I.S.) from an alkaline plasma sample. LC separation was performed on a Thermo Hypersil-Hypurity C18 (5 μm, 150 mm × 2.1 mm) using aqueous solution (20 mmol/l ammonium acetate, pH 4.28), methanol and acetonitrile (55:10:35, v/v/v) as the mobile phase. The retention time of doxazosin and the internal standard was 2.7 and 1.8 min, respectively. Quadrupole MS detection was done by monitoring at *m/z* 388 (M + 1) corresponding to doxazosin mesylate and at *m/z* 452 (M + 1) for I.S. The assay method described above showed acceptable precision, accuracy, linearity, stability, and specificity. The bioavailability of doxazosin mesylate was evaluated in 12 healthy Chinese male volunteers. The following pharmacokinetic parameters were elucidated after administering a single dose of 4 mg doxazosin. The area under the plasma concentration versus time curve from time 0 to 72 h ($AUC_{0–72\,h}$) 743.4 ± 149.5 ng h/ml; peak plasma concentration (C_{max}) 47.66 ng/ml; time to C_{max} (T_{max}) 3.0 ± 1.0 h; and elimination half-life ($t_{1/2}$) 18–20 h. The method was successfully used to determine the relative bioavailability of doxazosin mesylate.

Keywords: Doxazosin mesylate; Quadrupole mass spectrometer; Bioavailability

1. Introduction

Doxazosin mesylate[(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-yl-carbonyl) piperazine monomethansulphonate is a postsynaptic α-1 adrenoreceptor antagonist used either alone or in combination with diuretics or β-adrenergic-receptor-antagonist [1]. It is structurally similar to prazosin and terazosin, whose chemical structures are demonstrated in Fig. 1. Doxazosin is a potent antihypertensive agent and is very effective when administered orally or intravenously. It is slowly eliminated in man and its long half-life provides the basis for once-daily dose [2,3].

Doxazosin is a quinazoline derivative and presents similar clinical effects to prazosin [4], but its slow onset of hypotensive activity minimizes the first dose hypotensive effect seen with prazosin [5]. To prevent adverse effects due to its pharmacological activity, efficient screening procedures and methods for its quantitative determination at very low concentrations in biological samples are necessary.

Several methods have been reported on the determination of doxazosin mesylate, including the use of HPLC for its determination in the plasma and pharmaceutical formulations [6–8], differential-pulse-polarography [9–11], cathodic-stripping voltametry [12], adsorptive stripping voltametry [13], UV spectrophotometry and square-wave voltametry [14], and HPTLC [15]. These methods present some disadvantages such as low sensitivity, poor reproducibility, complicated testing procedures, and requirement of special testing equipment. So, most of them are not fit for the bioavailability analysis.

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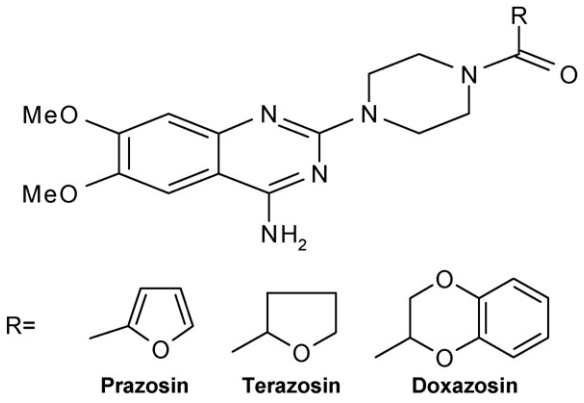

Fig. 1. Structures of prazosin, terazosin and doxazosin.

During the last few years, mass spectrometry has repeatedly been proven to be a powerful technique for the rapid, quantitative determination of drugs and metabolites in physiologic fluids. To our best knowledge, the utilization of LC/MS or LC/MS/MS techniques for the determination of doxazosin has not been previously reported. In this study, a simple, rapid, and selective LC/MS method was developed and validated to determine doxazosin in human plasma, and a standard protocol for bioequivalence testing of doxazosin mesylate was devised.

2. Experimental

2.1. Materials and reagents

Doxazosin mesylate standard (100.6%, lot: 20050501) was kindly supplied by Europharm Laboratories Company Ltd. (12–14 Dai Wang Street, Tai Po Industrial Estate, Hong Kong). Terazosin hydrochloride standard (99.53%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (No. 2 Taintan Xili, Beijing); Ultra-pure water prepared by a Millipore Milli-Q purification system (Millipore Corp. Bedford, MA, USA) were used as the mobile phase of HPLC–MS, and all other chemicals and solvent were of the highest analytical grade available. Drug-free and drug-containing plasma was taken from the volunteers. Plasma was stored at −20 °C until further use for analysis.

2.2. Instrumentation

A Shimadzu LC–MS 2010 system (Japan) was used, equipped with LC-10AD *VP* low pressure gradient pump, CTO-10A *VP* column temperature oven, SCL-10AD *VP* system controller, and LC–MS chemstation. Separation was achieved on a Thermo Hypersil-Hypurity C18 column (150 mm × 2.1 mm, i.d., 5 μm, USA) at 40 °C Compounds were eluted up to a total retention time of 3 min using an isocratic mobile phase consisting of 20 mM ammonium acetate (pH 4.28) – methanol – acetonitrile (55:10:35, v/v/v) at 0.22 ml/min, and the injection volume was 5 μl. The operating parameters of ESI–MS (electrospray ionisation–mass spectrometry) were as follows: capillary voltage was 4.5 kV; nebulizer nitrogen gas flow-rate was 1.5 l/min; drying N_2 flow was 10 l/min; drying gas temperature was 250 °C, the gas used was of high purity, and system control and data evaluation were carried out using LC–MS chemstation (Japan). The mass selective detector (MSD) was operated in the positive ionization mode with selected-ion monitoring (SIM) at 452 for doxazosin (*m*/*z*) and *m*/*z* 388 for terazosin.

2.3. Preparation of stock solutions and calibration standards

All concentrations of the two standards refer to the free bases. Primary stock solutions of doxazosin (105 μg/ml) and standard solution terazosin (I.S. 200 ng/ml) were prepared in water. All stock solutions were stored at 4 °C before use. Calibration standards of doxazosin were prepared by spiking the appropriate amount of the stock solution into the blank plasma obtained from healthy, non-smoking volunteers who did not drink coffee, at 0.5, 1.25, 2.5, 5, 12.5, 25, 50, and 100 ng/ml, respectively, and it was mixed well. Prepared calibration curves covered the range 0.5–100 ng/ml. Quality control samples were weighed and prepared separately according to the same manner as the above described and the final concentrations were 1.25, 12.5, and 100 ng/ml.

2.4. Sample preparation and extraction procedures

Frozen human plasma samples were thawed at ambient temperature. A 50 μl aliquots of terazosin (I.S. 200 ng/ml) standard solution was added to 250 μl of each plasma sample and vortex-mixed. The plasma was then made alkaline by adding 100 μl saturated sodium carbonate solution. After a thorough vortex mixing for 30 s, the mixture was extracted with 1 ml *n*-hexylane-tertiary butyl methyl ether (1:1, v/v), vortex-mixed for 3 min, and centrifuged at 14,000 rpm for 5 min. The organic layer was removed and evaporated under a gentle stream nitrogen gas at 45 °C until it was completely dry. The dried residue was dissolved with 100 μl mobile phase. After centrifugation, 5 μl of the clear supernatant was injected into the LC–MS system.

2.5. Assay validation

2.5.1. Assay specificity and matrix effect

Specificity was assessed by extracting samples of six batches of blank plasma, and then comparing the results for plasma samples spiked with terazosin (I.S.) and doxazosin. The chromatograms were also inspected visually for interfering chromatographic peaks from endogenous substances.

The matrix effect was evaluated by referring the peak area of extracted sample to that of standard solution without matrix.

2.5.2. Linearity

Calibration standards at eight doxazosin concentrations (ranging 0.5–100 ng/ml) were extracted and assayed. Least-squares linear regression was used to determine the plasma concentration from the peak area ratios (doxazosin versus terazosin).

2.5.3. Recovery, precision and accuracy

The doxazosin plasma working standards prepared above, at concentration of 0.5, 1.25, 2.5, 5, 12.5, 25, 50, and 100 ng/ml, were divided into two portions, one portion used for constructing calibration curves, the other used to determine the extraction recovery and intra- and inter-day precision and accuracy ($n = 5$) of the method. The recovery of the extraction procedure for doxazosin and the internal standard was calculated by comparing the peak area obtained after extraction with that of an aqueous drug solution of corresponding concentration without extraction.

2.5.4. Stability

The (1) short-term room temperature; (2) long-term storage; (3) stock solution; (4) post-preparative, and (5) freeze/thaw stabilities were tested. To test the stability of doxazosin in the plasma, QC samples were stored under different conditions. The freeze–thaw stability test was performed by freeze-thawing for 3 times; specifically, freezing was performed at −20 °C for 24 h and thawed at room temperature. During each cycle, triplicate of 250 μl aliquots were processed, analyzed, and the results averaged. Short-term stability testing was performed at room temperature over 6 h, and long-term stability was examined at −20 °C over 2 months. The results of the freeze–thaw, and short and long-term stability tests were compared with the Time-0 QC samples' averaged intra-day analysis. To test the stock solution stability of doxazosin and the I.S., stock standard (doxazosin, 105 μg/ml) and the I.S. (terazosin, 200 ng/ml) solution were left at −20 °C. Post-preparative stability testing was performed by comparing after-day analysis with the first intra-day analysis.

2.6. Bioavailability study design

The method was used to evaluate the bioequivalence of the two tablet formulations of doxazosin mesylate in healthy volunteers: DOXAZO® (test formulation: doxazosin mesylate tablets, 4 mg/tab, Lot: 511124, Hong Kong Registration No.: HK-52959) manufactured by Europharm Laboratories Company Ltd., HK and CARDURA® (standard reference formulation: Doxazosin mesylate tablets, 4 mg/tab, Lot: 0214822121) manufactured by Pfizer Pty. Limited, Australia.

2.6.1. Subjects

The bioequivalence protocol was approved by the State Food and Drug Administration (SFDA, Chinese) and Bioequivalence Test Regulation. Twelve healthy Chinese male volunteers, aged 21–25 years, were selected for this study after clinically assessment of their health status (physical examination, electrocardiograph) and hematology, biochemistry, electrolytes, and urinalysis testing. No subject had a history or evidence of a renal, gastrointestinal, hepatic, or hematologic abnormality or any acute or chronic disease, or allergies to any drugs. Subjects who had used drugs of any kind within 2 weeks before the study were excluded. All the subjects were non-drinkers and non-smokers. No tobacco, alcohol or drink with caffeine was allowed. Informed consent was obtained from all subjects and the nature and purpose of the study had been clearly explained.

The volunteers had the following clinical characteristics (expressed as means ± S.D. [range]): age, 22.3 ± 1.3 years [21–25]; height, 170.8 ± 4.0 cm [168–183]; body weight, 62.3 ± 4.6 kg [57–72].

2.6.2. Drug administration

The study was based on a single dose, randomized, two-treatment, and two-period crossover design. During phase 1, volunteers were hospitalized at 18:00 h and had a normal evening meal. After an overnight fast they were administered (at 08:00 h) a single dose of doxazosin mesylate (4 mg of either tablet formulation). Water (200 ml) was given immediately after drug administration and the volunteers were then fast for another 2 h. A bland lunch was served at 4 h after dosing, and an evening meal was permitted during the 'in-hours' period but liquid consumption was allowed ad libitum after lunch (except xanthine-containing drinks, such as tea, coffee, and cola). The research staff carefully recorded the sampling time and adverse reactions of the drug. After 14 days, the study was repeated in the same manner (phase 2) to complete the crossover design. The ordinary clinical dosage for the Doxazosin mesylate was 2–8 mg/day. Therefore, in this study a single oral dose of 4 mg (one tablet) was established.

The study was conducted at the Clinic Pharmacy Research Laboratory, Second Xiangya Hospital of Central South University, which was equipped with standard emergency drugs and equipment. A doctor with GCP training and two experienced nurses were recruited as part of the research staff to monitor the changes of vital signs and adverse reactions during the study.

2.6.3. Blood sampling

Heparinized blood samples (3 ml) were collected from a suitable forearm vein using an indwelling catheter into heparin containing tubes before (0 h) and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, and 72 h after dosing. The blood samples were centrifuged at 3000 rpm for 10 min, and plasma samples were separated and stored at −20 °C until analysis. Two weeks later, the two groups were given reverse drugs and the same amount of blood samples were taken at the same time points.

2.6.4. Pharmacokinetic analysis

Plasma concentrations of doxazosin were analyzed using the developed LC–MS method. The lower limit of quantification (LOQ) of the present assay was 0.5 ng/ml. Pharmacokinetic parameters including $AUC_{0–72\,h}$ (the area under the plasma concentration), T_{max} (time to C_{max}), K_e (terminal rate constant) and $t_{1/2}$ (terminal rate constant), and $t_{1/2}$ (elimination half-life) were calculated using Drug and statistics (Version 2.0; Chinese).

2.6.5. Statistical analysis

ANOVA was used to check the difference of the means of the pharmacokinetic parameters between the two preparations at a significant level of 0.05. Bioequivalence was determined by two one-sided *t*-tests. After logarithmic conversion, $AUC_{0–72\,h}$ and C_{max} underwent the analysis of variance to obtain the standard deviation of different groups. Then, two one-sided *t*-tests were carried out to determine the bioequivalence. If the 90%

confidence limit of the trial preparation $AUC_{0-72\,h}$ and C_{max} falls within 80–125% of the reference preparation $AUC_{0-72\,h}$ and C_{max}, we may conclude that the trial preparation and the reference preparation are bioequivalent [16]. T_{max} also underwent the analysis of variance.

3. Results and discussion

3.1. Chromatography and specificity

To develop this LC–MS-based method to quantify doxazosin mesylate in human plasma, electrospray ionization (ESI) sources were evaluated in positive ion mode. In general, ESI produced greater sensitivity and exhibited less interference than atmospheric pressure chemical ionization (APCI) sources. ESI positive MS spectra for doxazosin and terazosin were dominated by the $[M+1]^+$ ions, i.e., *m/z* 452 for doxazosin and *m/z* 388 for terazosin. A typical SIM spectra Doxazosin and Terazosin was shown in Fig. 2. The composition of the mobile phase was found to be the critical factor for achieving good chromatographic peak shape and resolution. In the present study, 20 mmol/l ammonium acetate (pH 4.28), methanol and acetonitrile (55:10:35, v/v/v) was selected as an isocratic mobile phase. The retention time of doxazosin and terazosin was less than 3 min. The selection of terazosin as the I.S. was based on its chemical structure and its chromatographic and extraction behavior.

A plasma blank (free of analyte and the I.S.), a blank sample spiked with doxazosin and the I.S., and a plasma sample collected 4.0 h after a single oral administration of doxazosin mesylate tablet (4 mg) were used to check the interference. For all plasma sample, the regions of the analyte and the I.S. were found to be free of interference. The method showed good specificity. (Fig. 3), also the matrix effects were minimal and no co-eluting "unseen" endogenous species interfered with the ionization of the analyte and internal standard. Thus, the ratio of the peak area was not influenced by the endogenous components via sample matrix.

3.2. Linearity and lower limit of quantification

The standard curve range used was 0.5–100 ng/ml for doxazosin calculated based on the response/concentration curve was established in human plasma over 0.5–100 ng/ml by the goodness-of-fit test. The regression equation was obtained as:

$$y = 0.0869x + 0.1726, \quad r^2 = 0.9977$$

The lower detection limit (LOD), defined at a S/N > 3, was 0.1 ng/ml (Fig. 4) and the lower limit of quantification (LOD) of doxazosin, defined at a S/N > 10, was 0.5 ng/ml (Fig. 5).

3.3. Precision, accuracy, and recovery

The precision of the assay was determined from plasma samples of eight concentrations of doxazosin (ranging 0.5–100 ng/ml). Intra-day precision was determined by repeating the analysis of standard 5 times a day, and inter-day precision was determined by repeating the analysis on 3 consecutive days. Sample concentrations were determined using calibration standards prepared on the same day. Assay precision was defined as the relative standard deviation (S.D.) from the mean (*M*), as calculated with the equation:

$$\text{R.S.D.}\,(\%) = \left(\frac{\text{S.D.}}{M}\right) \times 100$$

Accuracy was defined as the ratio of the mean computed value (*E*) to the true value (*T*) expressed as a percentage (accuracy,

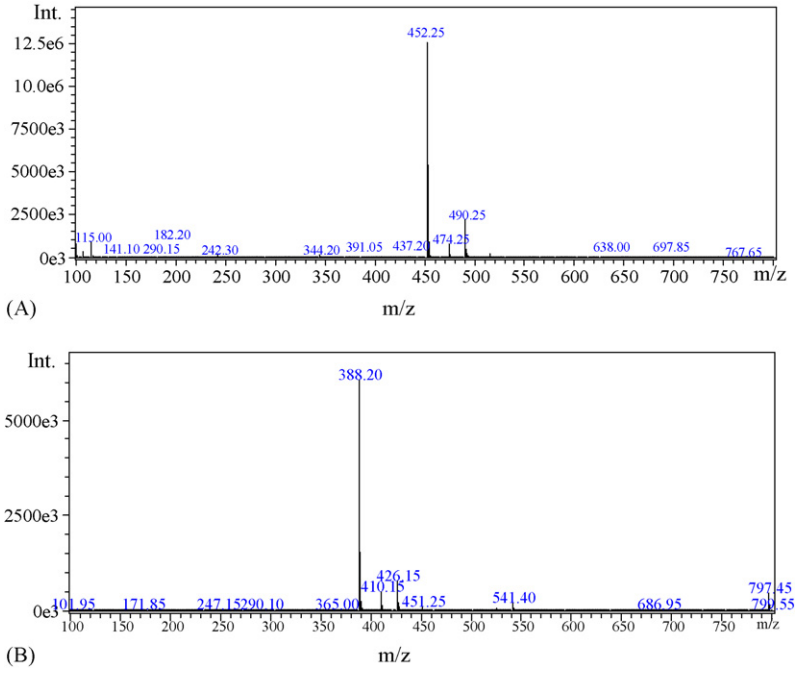

Fig. 2. Full scan mass spectra of doxazosin (A) and terazosin (B).

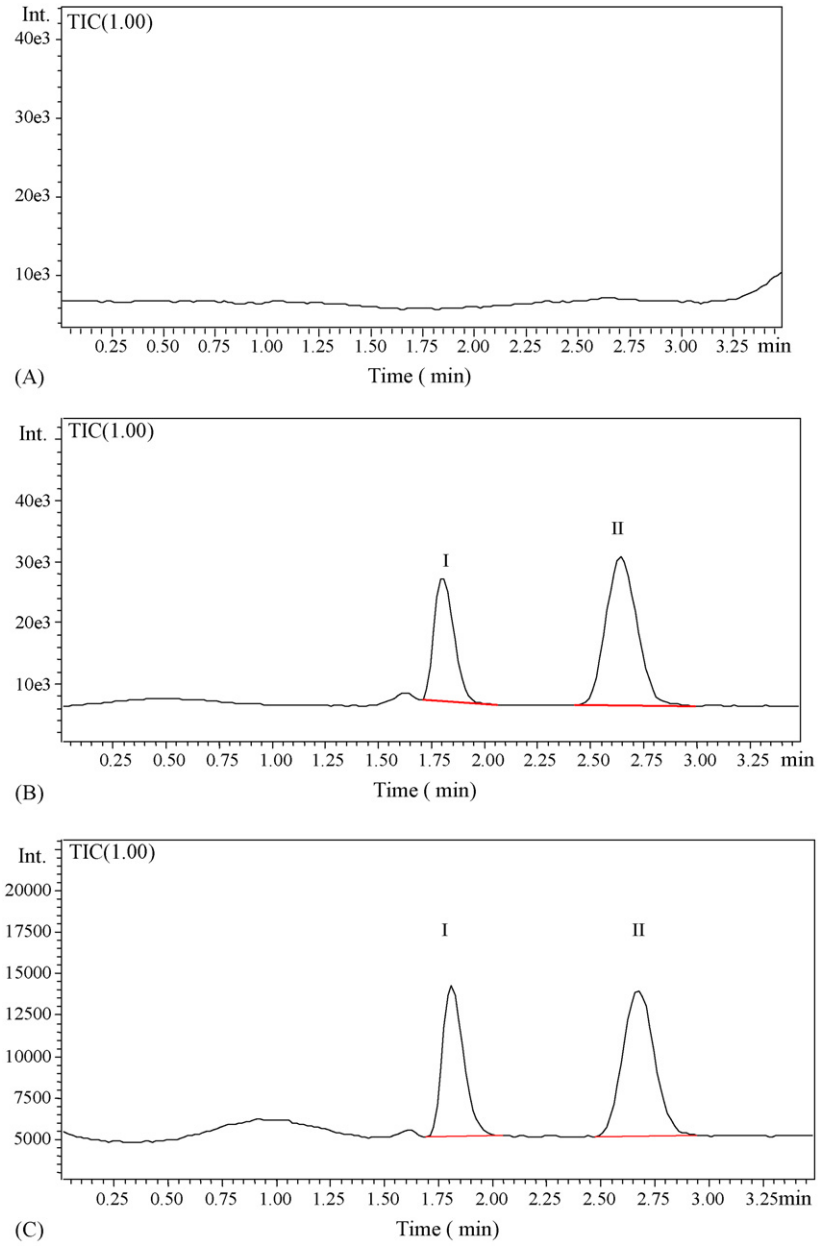

Fig. 3. Representative total ion chromatograms (TIC) of (A) blank plasma, and (B) plasma spiked with doxazosin (II) and terazosin (I, I.S.), and (C) a plasma sample 4 h after a single oral dose of doxazosin mesylate tablet (4 mg) to a healthy volunteer. The retention time of doxazosin and terazosin (I.S.) were 1.8 and 2.7 min, respectively.

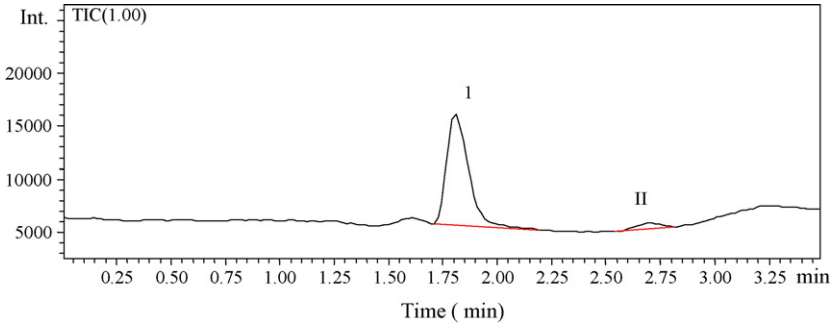

Fig. 4. Representative total ion chromatogram (TIC) of the lower detection limit (LOD) of doxazosin (II, 0.1 ng/ml) (S/N > 3).

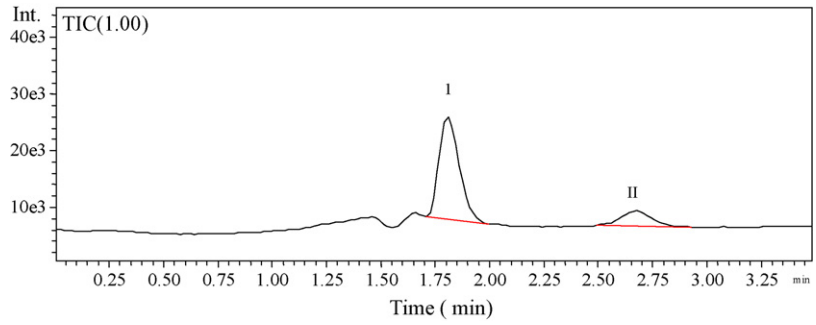

Fig. 5. Representative total ion chromatogram (TIC) of the lower limit of quantification (LOD) of doxazosin (II, 0.5 ng/ml) (S/N > 10).

Table 1
Extraction recovery, inter- and intra-day precision and accuracy ($n = 5$)

Concentration (ng/ml)	Recovery		Inter-day		Intra-day	
	Mean (%)	R.S.D.%	Precision (R.S.D.%)	Accuracy (%)	Precision (R.S.D.%)	Accuracy (%)
0.5	102.3	9.6	8.3	101.4	5.7	99.5
1.25	97.8	10.2	10.5	98.6	7.5	97.5
2.5	98.6	6.9	7.1	97.8	6.1	102.3
5	97.9	7.7	6.8	102.3	5.9	98.6
12.5	99.5	5.2	9.9	97.9	4.6	97.5
25	96.9	7.0	5.3	98.5	3.9	100.3
50	101.5	4.1	6.1	102.5	4.8	98.6
100	99.5	7.8	8.1	99.5	4.3	102.4

Date obtained from five replicates at each concentration.

%). The recovery is calculated by the formula:

$$\text{Recovery}\,(\%) = \left(\frac{\text{detector response of extracted analyte}}{\text{detector response for non-extracted analyte}}\right) \times 100$$

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.

The extraction recovery, inter- and intra-day precision and accuracy values are presented in Table 1.

The recovery rates of the method ranged 97.8–102.3%, and both the intra- and inter-day R.S.D.s were smaller than 11%, which met the methodological requirements of biological sample analysis.

3.4. Stability studies

The stability experiments aimed at testing all possible conditions in which the samples might be exposed to during sample shipping and handling. To test the short-term and long-term stability of the extracted analytes, the QC plasma samples of 1.25, 12.5, and 100 ng/ml in the plasma were determined after several freezing and thawing cycles. The long-term storage stability at −20 °C was determined after 2 months. The stability of the stock solutions was investigated by storing in the refrigerator at −20 °C. Moreover, the short-term stability of the extracted samples during storage for 24 h at 4 °C and at room temperature was also determined.

In the short-term stability study, the QC plasma samples were stable for 24 h at 4 °C and at room temperature (Table 2.). In

Table 3
Freeze/thaw stability data for doxazosin ($n = 3$ per test and each concentration)

Drug	Added concentration (ng/ml)	Average (%)	R.S.D. (%)
Doxazosin	1.25	98.5	5.3
	12.5	99.2	4.3
	100	101.4	5.2

Date obtained from five replicates at each concentration.

Table 2
Stability data for doxazosin ($n = 3$ per test and each concentration)

Drug	Added (ng/ml)	2 Months, −20 °C		24 h, room temperature		24 h, 4 °C	
		Average (%)	R.S.D. (%)	Average (%)	R.S.D. (%)	Average (%)	R.S.D. (%)
Doxazosin	1.25	97.8	4.5	100.3	4.8	99.8	5.6
	12.5	101.3	5.1	98.6	5.3	97.2	4.7
	100	100.1	6.5	97.5	4.1	101.3	5.6

Date obtained from five replicates at each concentration.

Table 4
Mean pharmacokinetic parameters and 90.0% confidence interval for doxazosin, after the administration of an oral dose of 4 mg of test (DOXAZO®) and reference (CARDURA®) formulations to healthy Chinese male volunteers

Pharmacokinetic parameters	DOXAZO (mean ± S.D.)	CARDURA (mean ± S.D.)	Confidence limit 90.0%
T_{max} (h)	3.0 ± 1.0	2.6 ± 1.1	–
C_{max} (ng/ml)	56.6 ± 7.7	55.9 ± 7.3	97.7–104.7
AUC_{0-t} (ng h/ml)	743.4 ± 149.5	726.9 ± 159.8	97.4–108.1
$AUC_{0-\infty}$ (ng h/ml)	796.2 ± 145.7	781.1 ± 172.7	100.2–115.0
$t_{1/2}$ (h)	20.9 ± 5.9	18.8 ± 5.5	–
K_e (h^{-1})	0.0336 ± 0.0134	0.0408 ± 0.0150	–

Table 5
ANOVA of $AUC_{0\rightarrow 72\,h}$ after a single oral dose of 4 mg doxazosin mesylate tablets in the subjects

ANOVA	*f*	SS	MS	*F*	*P*
Total variation	23	0.900466			
Variation between preparations	1	0.00393	0.00393	0.7338115	0.41171
Variation between periods	1	0.003081	0.003081	0.5753114	0.46566
Variation between subjects	11	0.839904	0.076355	14.25815	0.00011
Deviation	10	0.053552	0.005355		

Table 6
ANOVA of C_{max} after a single oral dose of 4 mg doxazosin mesylate tablets in the subjects

ANOVA	*f*	SS	MS	*F*	*P*
Total variation	23	0.377061			
Variation between preparations	1	0.000739	0.000739	0.21678	0.65148
Variation between periods	1	0.002294	0.002294	0.6730514	0.43111
Variation between subjects	11	0.339942	0.030904	9.0664797	0.00080
Deviation	10	0.034086	0.003409		

Table 7
ANOVA of T_{max} after a single oral dose of 4 mg doxazosin mesylate tablets in the subjects

ANOVA	*f*	SS	MS	*F*	*P*
Total variations	23	3.663454			
Variation between preparations	1	0.18017	0.18017	3.4615385	0.09244
Variation between periods	1	0.020019	0.020019	0.3846154	0.54901
Variation between subjects	11	2.942775	0.267525	5.1398601	0.00761
Deviation	10	0.520491	0.052049		

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 2 months at −20 °C (Table 2.). The stock solutions were stable for at least 1 month. The difference values between the Time-0 QC plasma samples and the test solution in stock solution stability were <5% for doxazosin and terazosin, respectively. The post-preparative samples were stable at room temperature for at least 6 days including the residence time in the autosampler. The final stability test was demonstrated after three freeze–thaw cycles. No significant deterioration of the analytes was observed under any of these conditions (Table 3).

3.5. Pharmacokinetic study

The developed method was successfully applied to the bioequivalence study of the two doxazosin mesylate tablet formulations. The mean (±S.D.) plasma concentrations–time profiles of doxazosin after a single oral dose of 4 mg of either formulation in tablet form were shown in Fig. 6. The pharmacokinetic parameters of the two doxazosin formulations were shown in Table 4.

For the bioequivalence test, AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were evaluated as primary parameters. The ANOVA's data were listed as Tables 5–7. The means and standard deviations of these

Table 8
Two one-sided *t*-tests of $AUC_{0\rightarrow 72\,h}$ and C_{max} after a single oral dose of 4 mg doxazosin mesylate tablets in the subjects

Parameters	T_L	T_H	$T_{0.05}$	*P*	90% confidence limit	
					90% CL–L	90% CL–H
$AUC_{0-72\,h}$	8.33	6.61	1.81	High side, <0.05	97.4	108.1
			1.81	Low side, <0.05		
C_{max}	9.83	8.90	1.81	High side, <0.05	97.7	104.7
			1.81	Low side, <0.05		

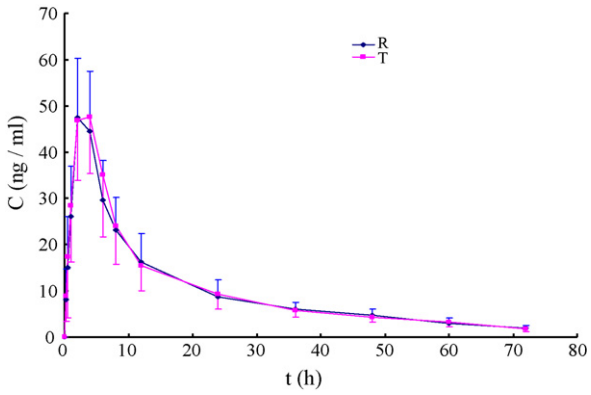

Fig. 6. Mean plasma concentration vs. time graph of doxazosin after the administration of the test (DOXAZO®; ■) and reference (CARDURA®; ⬟) formulations to healthy, adult, male subjects under fasting condition.

parameters for the two brands were similar, indicating that the pharmacokinetics of doxazosin in the two brands are similar. The 90.0% confidence intervals for the ratios of test drug to reference drug in terms of AUC_{0-t} and C_{max}, were within the range 80.0–125.0% (Table 8), which is the range accepted by the State Food and Drug Administration.

4. Conclusions

The purpose of the present study was to develop a standard protocol for the bioequivalence testing of doxazosin mesylate tablet. We devised and validated, a simple and rapid LC–MS method using a simple liquid–liquid extraction procedure and isocratic chromatography, to determine doxazosin levels in human plasma, and used this test to conduct a bioavailability study by administering 4 mg of doxazosin mesylate to healthy Chinese male volunteers. The developed assay showed acceptable precision, accuracy, linearity, stability, and specificity.

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