Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1804–1807
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

LC/ESI-MS method for the determination of trimetazidine in human plasma: Application to a bioequivalence study on Chinese volunteers

Yang Jiao [a], Mingming Su [a], Minjun Chen [a,*], Wei Jia [a], Yiqun Chou [b], Zhongyi Huang [b], Nanlin Yang [c], Weida Tong [d]

[a] *School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200030, China*
[b] *Shanghai Jing An Central Hospital, Shanghai 200040, China*
[c] *JiangSu WuZhong Chinese Traditional Medicine R&D Co., Ltd., Suzhou 215000, China*
[d] *Division of Systems Toxicology, National Center for Toxicological Research (NCTR), FDA, 3900 NCTR Road, Jefferson, AR 72079, United States*

Received 11 July 2006; received in revised form 23 November 2006; accepted 29 November 2006
Available online 8 January 2007

Abstract

A rapid liquid chromatography electrospray ionization mass spectrometry (LC/ESI-MS) method with good sensitivity and specificity has been developed and validated for the identification and quantification of trimetazidine in human plasma. Trimetazidine and lidocaine (internal standard) were isolated from plasma samples by protein precipitation with methanol. The chromatographic separation was accomplished on a Xterra MS C_{18} Column (150 mm × 4.6 mm, 5 μm particle size) with the mobile phase consisting of methanol and water (40:60, v/v) (pH 2.0, adjusted with trifluoroacetic acid), and the flow rate was set at 0.6 mL/min. Detection was performed on a single quadruple mass spectrometer by selected ion monitoring (SIM) mode (*m/z* 267.0 for trimetazidine and *m/z* 235.0 for lidocaine) with the retention time at about 3.47 and 5.05 min, respectively. The calibration curve for trimetazidine was satisfactory with regression coefficient 0.9995 over the range of 2.5–100 ng/mL in the plasma. The LOQ (S/N = 10) was accordingly 2.5 ng/mL. The intra-day and inter-day precision expressed as relative standard deviation was 2.83–6.10% and 4.83–5.82%. The method was successfully applied to investigate the bioequivalence between two kinds of tablets (test versus reference product) in 19 healthy male Chinese volunteers. After a single 20 mg dose for the test and reference product, the resulting mean of major pharmacokinetic parameters such as AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, T_{max} and $t_{1/2}$ of trimetazidine were (673.1 ± 117.6 ng h mL^{-1} versus 652.3 ± 121.9 ng h mL^{-1}), (717.1 ± 120.9 ng h mL^{-1} versus 692 ± 128.6 ng h mL^{-1}), (74.85 ± 12.13 ng mL^{-1} versus 71.93 ± 14.32 ng mL^{-1}), (2.312 ± 0.663 h versus 2.211 ± 0.608 h) and (4.785 ± 0.919 h versus 4.740 ± 0.823 h), respectively, indicating that these two kinds of tablets were bioequivalent in the Chinese population.

Keywords: Trimetazidine; LC/ESI-MS; Pharmacokinetics; Bioequivalence; Chinese population

1. Introduction

Trimetazidine [1-(2,3,4-trimethoxybenzyl) piperazine dihydrochloride] is a clinically effective anti-anginal agent. By switching energy substrate preference from fatty acid oxidation to glucose oxidation [1,2], it has a direct anti-ischaemic effect on the myocardium without affecting myocardial oxygen consumption and coronary blood flow [3].

Several methods have been investigated for the determination of trimetazidine. For example, Courte and Bromet [4] developed a quantitative method using HPLC coupled with fluorescence detection and Fay et al. [5] determined trimetazidine with GC/MS. Both methods were very labor-intensive and time-consuming because of the required derivatization procedures. Min et al. [6] reported a HPLC method coupled with a liquid–liquid extraction procedure for sample clean-up, which, unfortunately, was obviously not suitable for the study of bioequivalence due to the relatively higher LOQ (10 ng/mL). De Jager et al. [7] developed a LC/MS-MS method, despite the claim of relative short turn-round time, avoiding the use of internal standard made some concern of reproducibility. Recently, Medvedovici et al. [8] developed a LC/APCI-MS-MS method for the determination of trimetazidine, however, the application of tandem mass is relative high-cost and operationally complex.

* Corresponding author. Tel.: +86 21 34204829; fax: +86 21 34204829.
E-mail address: minjunchen@gmail.com (M. Chen).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.11.040

In this study, a rapid LC/ESI-MS method coupled with a simple sample preparation was developed and validated, and it has been successfully applied to a bioequivalent study on Chinese population.

Although the pharmacokinetics and clinical pharmacology of trimetazidine have been thoroughly studied, there is little data on Chinese population. Therefore, a new HPLC/ESI-MS method for the determination of trimetazidine in plasma samples was developed, in order to carry out a pharmacokinetic and bioequivalence study of two immediate release trimetazidine formulations in healthy male Chinese volunteers.

2. Materials and methods

2.1. Reagents

The trimetazidine reference was supplied by JiangSu WuZhong Chinese Traditional Medicine R&D Co., Ltd. and lidocaine reference substances were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC grade) was commercially obtained from Merck (Germany). All the other chemicals were analytical grade. Ultra pure water was supplied by Millipore Simplicity^{TM} system (MA, USA). The drug-free human plasma was obtained from Shanghai Blood Center (Shanghai, China).

2.2. Subjects

A total of 19 healthy male Chinese volunteers were enrolled in this study in the Shanghai Jing-An Central Hospital, Shanghai. The mean age was 22.5 ± 2.1 year old with a range of 20–26 years and mean body weight was 64.5 ± 7.7 kg with a range of 52–80 kg. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurologic, gastrointestinal and hematologic disease, as assessed by physical examination, electrocardiography and the laboratory tests including hematology, biochemistry, electrolytes and urinalysis. None had a history or evidence of hepatic, renal, gastrointestinal or hematologic abnormality or any acute or chronic diseases or allergy to any drug. All the subjects were instructed to abstain from taking any medication for 2 weeks before and during the overall study period and signed the informed consents. The study protocols were approved by the relevant Ethical Review Committee in accordance with the principles of the Declaration of Helsinki, and the recommendations of the State Food and Drug Administration of China.

2.3. Formulations

The test preparation was trimetazidine tablets (Batch No. 000323), which was supplied by Jiangsu WuZhong Pharmaceutical Co., China. The reference preparation was trimetazidine tablets (Batch No. 4K4514), which was commercially obtained from Servier Pharmaceutical Co., France. Both preparations were labeled to contain 20 mg of trimetazidine.

2.4. Study design

The 19 volunteers participated in a single dose crossover bioequivalence study with a 1-week interval between each administration. Each subject received one trimetazidine test tablet or reference tablet randomly. After an overnight fasting (over 10 h), volunteers were given a single dose of either formulation (reference or test) of trimetazidine with 250 mL of water. Water intake was permitted 2 h after treatment while food was not allowed until 4 h after treatment. In addition, water, lunch and dinner were given to all the volunteers according to the specially designed schedule. About 4 mL venous blood samples were collected into heparinized polypropylene tubes at pre-dose (0 h), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after treatment. Blood samples were immediately centrifuged at $3000 \times g$ for 10 min at 4 °C, and the separated plasma was stored frozen at −20 °C until assayed.

2.5. Equipment

The experiments were carried out with an Agilent 1100 system. The system consisted of a G1312A binary pump, a G1322A mobile phase vacuum degassing unit, a G1313A autosampler, a G 1316A temperature-controlled column compartment and a G1946A single quadruple mass spectrometric (MS) detector equipped with an ESI ion source. Data were acquired and integrated by the ChemStation. A Waters Xterra MS C_{18} (150 mm × 4.6 mm, 5 μm) was used for the separation.

2.6. Chromatographic and MS conditions

Chromatographic separation was achieved using a mobile phase consisting of methanol and water (40:60, v/v; pH 2.0, adjusted with trifluoroacetic acid), and the elution rate was 0.6 mL/min. The analytical column was kept at 40 °C. Electrospray ionization was performed using nitrogen as nebulizing gas at 9.0 L/min flow rate, 40 psi nebulizing pressure and 350 °C drying gas temperature. Capillary voltage was set at 3000 V. Optimum fragment voltage of 70 V was selected after varying between 50 and 190 V. Positive-ion selected ion monitoring (SIM) mode was used to detect *m/z* 267.0 ([Trimetazidine +H]$^+$) and *m/z* 235.0 ([Lidocaine +H]$^+$).

2.7. Standards

Stock solutions of trimetazidine (400 μg/mL) and the I.S. (lidocaine, 1 mg/mL) were prepared using the mobile phase. The working I.S. solution (1000 ng/mL) was freshly prepared using mobile phase prior to each batch assay.

2.8. Sample preparations

Each 500 μL of aliquot plasma and 50 μL of lidocaine solution (1000 ng/mL) were added into a 5.0 mL glass tube. The sample was vortexed for 30 s, and then deproteinated by addition of 1.0 mL methanol. The resulting mixture was centrifuged at $4000 \times g$ for 8 min. The supernatant was acidified with 30 μL

of trifluoroacetic acid (1:5, v/v), and subsequently dried in a rotational-vacuum-concentrator at 50.0 ± 0.5 °C. The residue was re-dissolved in 200 μL of mobile phase, and a 90 μL aliquot was injected into the LC/MS system.

2.9. Validation and calibration

The calibration curves were constructed routinely for spiked plasma containing 2.5, 5, 10, 20, 40, 80 and 100 ng/mL of trimetazidine during the process of validation and the study as well. Similarly, quality control samples were prepared in spiked plasma containing 5, 20 and 80 ng/mL of trimetazidine. Accuracy was assessed as follow: Bias (%) = ((concentration added − concentration detected)/concentration added) × 100.

2.10. Pharmacokinetics and statistical analysis

Pharmacokinetic analysis was performed using a non-compartmental method. Peak of the ratio of trimetazidine concentrations to lidocaine 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 (AUC) from the time of drug administration to the last plasma sampling time (AUC_{0-t}) was calculated according to the linear trapezoidal rule. The AUC from 0 to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-\infty} = AUC + C_t/\lambda_z$ (where C_t is the last plasma concentration measured). The elimination rate constant (λ_z) was determined by linear regression analysis of the log-linear part of the plasma concentration–time curve. The half-life ($t_{1/2}$) of trimetazidine was obtained by $t_{1/2} = \ln 2/\lambda_z$.

Bioequivalence of the two trimetazidine products was assessed by calculating individual AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and T_{max} values. Their ratios (test versus reference) of log-transformed data, together with their means and 90% confidence intervals, were analyzed with analysis of variance (ANOVA) using a commercially software package named Drug And Statistics (DAS, Version 1.0, recommended by Mathematical Pharmacology Professional Committee of China). If the parameters between the two preparations was not statistically different with each other ($P \geq 0.05$), and the 90% confidence interval for the parameters located within 80–125%, the two drugs would be considered as the bioequivalent preparations.

3. Results and discussion

Typical chromatograms of test plasma samples were shown (Fig. 1). The retention time for trimetazidine and lidocaine (I.S.) were 3.47 and 5.05 min, respectively. No endogenous interference was observed with either trimetazidine or lidocaine.

The mean recovery of the extraction was determined by comparing the peak area obtained from the plasma sample with peak area obtained by the direct injection of pure drug standard solution at four different concentration levels. The mean recovery of trimetazidine was over 72% (Table 1).

The calibration curve was established by plotting the peak area ratio versus concentration, which was linear over the range of 2.5–100 ng/mL with the regression equation: $Y = 0.0028X - 0.0015$ ($r = 0.9995$, $n = 7$). The limit of quantification (LOQ) for trimetazidine was 2.5 ng/mL. In addition, the intra-day and inter-day precision was about 2.84–6.10% ($n = 5$) and 4.83–5.82%, respectively. Accuracy expressed as bias ranged from −3.7% to +3.2% (Table 2).

The mean plasma concentration–time curves of two formulations of trimetazidine products on 19 healthy male Chinese volunteers with a single 20 mg oral dose are shown (Fig. 2). The calibration curve is $Y = (0.0021 \pm 0.0005)X - (0.0010 \pm 0.0008)$ ($r = 0.9991$, $n = 10$) and the R.S.D. of quality control calculated by these calibration curves is less than 15%. The primary pharmacokinetic parameters for both drugs are listed in Table 3.

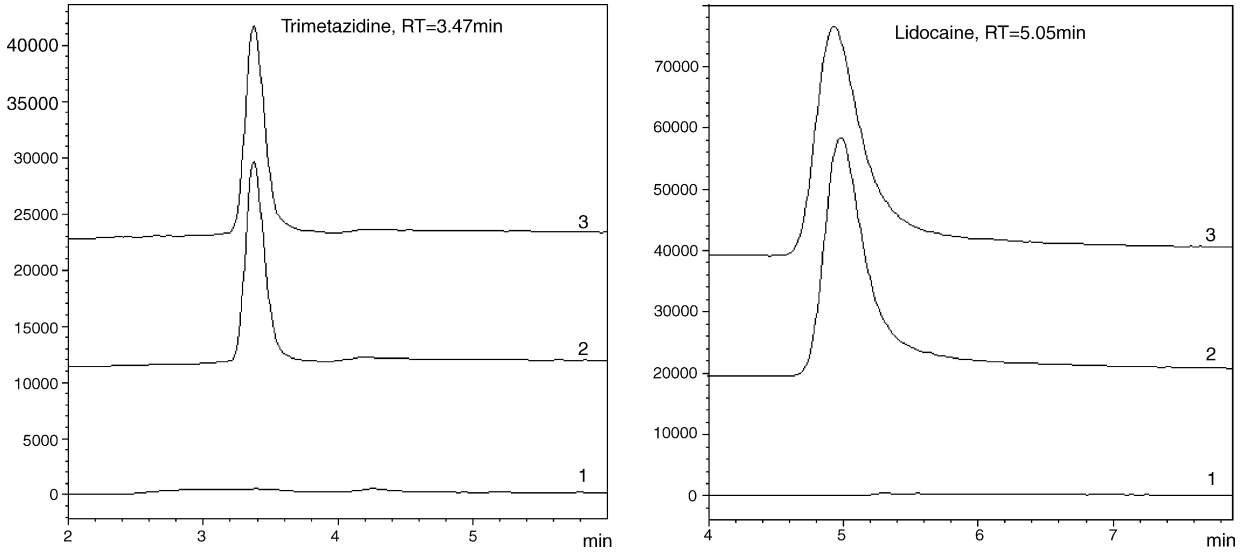

Fig. 1. Chromatograms of trimetazidine and lidocaine in plasma at SIM mode; (1) blank plasma; (2) a spiked plasma sample with trimetazidine (40 ng/mL) and lidocaine(1000 ng/mL); (3) test plasma sample (containing 42 ng/mL trimetazidine); retention time was 3.47 min for trimetazidine and 5.05 min for lidocaine, respectively.

Table 1
Recovery of trimetazidine in plasma

Plasma concentration (ng/mL)	Recovery (%)					Mean (%)	R.S.D. (%)
2.5	74.1	70.5	76.1	73.3	73.7	73.5	2.71
5	70.1	74.3	71.6	73.0	72.3	72.2	2.17
20	77.4	75.8	67.7	75.6	77.0	74.7	5.34
80	75.3	81.9	83.4	78.4	83.5	80.5	4.42

Table 2
Intra/inter-day accuracy and bias of trimetazidine in human plasma

Theoretical concentration (ng/mL)	Intra-day ($n=5$)			Inter-day ($n=5$)		
	Mean concentration Calculated (ng/mL)	R.S.D. (%)	Bias (%)	Mean concentration calculated (ng/mL)	R.S.D. (%)	Bias (%)
2.5	2.55	4.18	+2.0	2.53	4.91	+1.2
5	5.15	2.84	+3.0	5.16	4.83	+3.2
20	19.27	6.10	−3.7	20.00	5.82	0
80	82.35	3.35	+2.9	79.75	5.77	−0.3

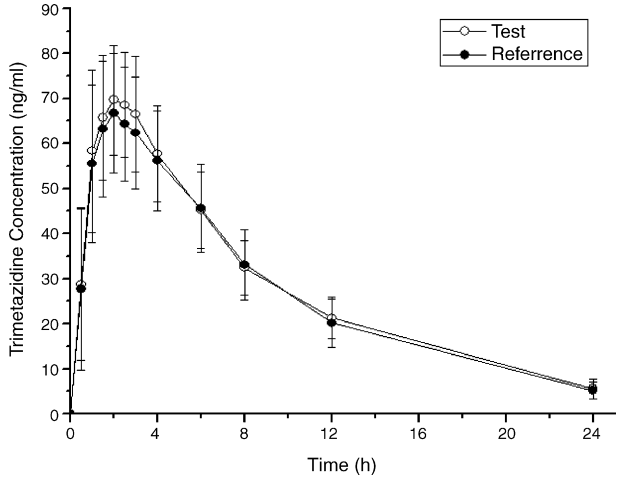

Fig. 2. Mean plasma concentration–time curve for trimetazidine after oral administration of 20 mg trimetazidine as test and reference formulations in 19 healthy volunteers.

According to the current study, the relative bioavailability of the test formulation was 102.6% (Mean AUC_{0-24}) and 102.2% (mean $AUC_{0-\infty}$), respectively. There were no significant differences between the two formulations on the basis of assessment by a two one-sided *t*-test. The 90% confidence intervals of test to reference ratio of the AUC_{0-24} were within the bioequivalence criteria range of 80–125%, and that of C_{max} was within 70–143%. Therefore, the two products were bioequivalent.

Table 3
Main pharmacokinetic parameters after administration of a single oral 20 mg dose of trimetazidine in the healthy Chinese volunteers ($n=19$)

Parameters	Test tablet	Reference tablet
AUC_{0-24} (ng h mL^{-1})	673.1 ± 117.6	652.3 ± 121.9
$AUC_{0-\infty}$ (ng h mL^{-1})	717.1 ± 120.9	692 ± 128.6
C_{max} (ng mL^{-1})	74.85 ± 12.13	71.93 ± 14.32
T_{max} (h)	2.312 ± 0.663	2.211 ± 0.608
$t_{1/2}$ (h)	4.785 ± 0.919	4.74 ± 0.823
Relative bioavailability of test tablet duo to AUC_{0-24} (%)	103.9 ± 8.8	

C_{max}, peak concentration; AUC, area under concentration–time curve and T_{max}, time to C_{max}.

4. Conclusion

The proposed LC/ESI-MS method provided a simple assay for the determination of trimetazidine in human plasma with good sensitivity and specificity. In the bioequivalence study on Chinese volunteers, two 20 mg trimetazidine formulations in the form of tablets were assessed, and the statistical comparison of AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} obviously showed no significant difference between the two formulations. About 90% of log-transformed data for the mean ratio (test/reference) of parameters AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} for the two formulations were 80–125%, in the acceptance range of bioequivalence. The present study indicates that these two products are bioequivalent in the Chinese population, and the pharmacokinetics of trimetazidine in Chinese population does not significantly differ from data obtained from Romania population [8].

References

[1] P.F. Kantor, A. Lucien, R. Kozak, G.D. Lopaschuk, Circ. Res. 86 (2000) 580–588.
[2] L. Argaud, L. Gomez, O. Gateau-Roesch, E. Couture-Lepetit, J. Loufouat, D. Robert, M. Ovize, J. Mol. Cell. Cardiol. 39 (2005) 893–899.
[3] J.M. Detry, P. Sellier, S. Pennaforte, D. Cokkinos, H. Dargie, P. Mathes, Br. J. Clin. Pharmacol. 37 (1994) 279–288.
[4] S. Courte, N. Bromet, J. Chromatogr. 224 (1981) 162–167.
[5] L. Fay, G. Michel, P. Goupit, C. Harpey, M. Prost, J. Chromatogr. Biomed. Appl. 490 (1989) 198–205.
[6] K.J. Min, S.K. Kyoung, S.K. Chang, H.K. Nam, Y.B. Chung, T.H. Jin, D.C. Moon, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 1299–1309.
[7] A.D. De Jager, F.C.W. Sutherland, D. Badenhorst, H.K.L. Hundt, K.J. Swart, T. Scanes, A.F. Hundt, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 2121–2132.
[8] A. Medvedovici, F. Albu, C. Georgita, V. David, Biomed. Chromatogr. 19 (2005) 549–555.