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ORIGINAL RESEARCH

Non-extractive procedure followed by LC/APCI MS/MS analysis of trimetazidine in plasma samples for assessing bioequivalence of immediate/modified release formulations

Andrei Medvedovici,[1,2]* Florin Albu,[2] Cristina Georgiţă[2] and Victor David[1,2]

[1]University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, Sos. Panduri, no. 90, Bucharest (5)-050663, Romania
[2]LaborMed Pharma, Splaiul Independentei no. 319, Bucharest (6)-060044, Romania

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ABSTRACT: Trimetazidine and internal standard [1-(2,4,5-trimethoxybenzyl)piperazine] were isolated from plasma by protein precipitation with trifluoroacetic acid. The neutralized supernatant was separated on a C_8 column with methanol–aqueous 0.11% triethylamine adjusted to pH 3.3 with formic acid (1:4, v/v) at a flow rate of 0.85 mL/min. The separation was achieved within 8 min and the column effluent was transferred into an ion trap analyzer via an atmospheric pressure chemical ionization interface. The mass analyzer was used in the selected reaction monitoring mode, to enhance detection selectivity. The method was fully validated with a quantitation limit for trimetazidine of 1.5 ng/mL. The method was successfully applied to assess bioequivalence of two immediate and two modified commercially available pharmaceutical formulations containing 20 and 35 mg of trimetazidine, respectively. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: trimetazidine; plasma; acid protein precipitation; bioequivalence; APCI MS/MS detection; immediate release; modified release formulations

INTRODUCTION

Trimetazidine [1-(2,3,4-trimethoxybenzyl)piperazine dihydrochloride] regulates ionic and extracellular exchanges, corrects the abnormal flow of ions across the cell membrane cause by ischemia and prevents cellular edema generated by anoxia. It ensures the functioning of ion pumps and the sodium/potassium cross-membrane flows and maintains cellular homeostasis.

Analytical procedures for determination of trimetazidine (TMZ) are relatively limited in number. Spectrometric methods have been proposed for the assay of TMZ in pharmaceutical formulations (Murthy *et al.*, 2002; Issa *et al.*, 2002). A qualitative LC/MS method for identification of TMZ and related metabolites in biological fluids has also been reported (Jackson *et al.*, 1996). HPTLC (Thoppil *et al.*, 2001a) and reversed-phase (RP)HPLC (Thoppil *et al.*, 2001b) stability-indicating methods for TMZ as a bulk active substance or in pharmaceutical formulations have been published. The first attempt to quantitatively assay TMZ in plasma samples was made using HPLC coupled to fluorescence detection (Courte and Bromet, 1981). TMZ was detected as a fluorescent dansyl derivative. Derivatization with *N*-(*t*-butylsilyl)-*N*-methyltrifluoroacetamide was used after liquid extraction of TMZ from plasma samples and subsequent separation by gas chromatography (Fay *et al.*, 1989). Low-wavelength UV detection (206 nm) successfully allowed quantitation of TMZ in blood and urine after isolation on Toxi-tubes and liquid chromatographic separation (Gaillard *et al.*, 1997). Electrochemical detection also represents an alternative for sensitive determination of TMZ in blood and plasma samples (Bari *et al.*, 1999). Recently, chemiluminogenic reaction of TMZ with potassium permanganate was proposed for its assay in biological fluids (Palilis and Calokerinos 2000). LC-MS was reported only once as a quantitative tool for assaying TMZ from plasma samples (de Jager *et al.*, 2001). The sample preparation procedure was based on liquid extraction of TMZ in hexane–dichloromethane followed by its derivatization with acetic anhydride. The acetyl derivative was back-extracted into aqueous media and separated by RP-HPLC. Detection was by mass spectrometry, using selected reaction monitoring (SRM) mode and ionization was realized by APCI. Despite the claim of the authors with respect to the method high throughput, the multiple step isolation procedure is somewhat cumbersome. Derivatization of TMZ to the acetylated compound, according to the

*Correspondence to: A. Medvedovici, University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, Sos. Panduri, no. 90, Bucharest (5)-050663, Romania.
E-mail: avmedved@yahoo.com

Abbreviations used: SRM, selected reaction monitoring; TFA, trifluoroacetic acid; TMZ, trimetazidine.

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cited authors, was necessary to eliminate important carry over effects and chromatographic elution problems (peak splitting, peak symmetry). Recommendation to avoid the use of internal standards was also made, for reproducibility concerns.

The present work describes a single step isolation method for TMZ and the internal standard (I.S.) from plasma samples, using protein precipitation with trifluoroacetic acid (TFA). After neutralization of the supernatant, the sample was directly injected and separated on an RP-HPLC column and eluted with an acidic mobile phase without problems related to peak efficiency, symmetry or focusing phenomena. APCI ionization and MS^2 detection with an ion trap mass analyzer operated in the SRM mode provided quantitation limit in the 1.5 ng/mL range. The method was validated and successfully used to assess bioequivalence of immediate and modified release pharmaceutical formulations containing TMZ, under single/multiple dose administration designs.

EXPERIMENTAL

Instrument. Experiments were performed on an Agilent 1100 series LC/MSD system composed of the following modules: degasser (G1379A), quaternary pump (G1311A), thermostated autosampler (G1329A), column thermostat (G1316A), APCI (G1947A), ion trap mass spectrometric detector SL series (G24450) and nitrogen generator (5183-2003). System control and data acquisition were carried out using the Agilent LC/MSD trap software version 4.2, incorporating the MSD Trap Control software version 5.1, from Brucker Daltronics. The system was operationally qualified before and after the bioequivalence study. The repeatability (n = 6) of the MSD ion trap SL determined for 5 pg of reserpine loaded onto the APCI interface was characterized during the study by relative standard deviations (RSD%) of 11% (before) and 9.7% (after).

Chromatographic conditions. A Zorbax Eclipse XDB-C_8 column (Agilent), 150 × 4.6 mm internal diameter and 5 µm particle size fitted with a Chromolith Guard cartridge RP-18e (10 × 4.6 mm; Merck) was used. The column was thermostated at 45°C and was validated before and after completion of the bioequivalence studies, by computing the reduced height plate (h) for the fluoranthene peak. This parameter remained practically unchanged (1.96 before and 2.0 after injection of more than 2500 processed plasma samples).

Elution was isocratic, using methanol and aqueous 0.11% triethylamine (v/v) brought with formic acid to pH 3.3, mixed in the ratio of 1:4 (v/v). The flow rate was 0.85 mL/min and injection volume was set at 200 µL.

Interface parameters. The parameters controlling the APCI interface were: drying gas flow, 10 L/min; drying gas temperature, 350°C; vaporizer temperature, 350°C; pressure of the nebulizer gas, 60 psi; capillary voltage, 4500 V; high voltage end plate offset, –500 V; corona, 5000 V.

MSD ion trap SL operational parameters. Ion polarity was positive for both trimetazidine and IS. The SRM working mode was used. The trap parameters were identical for target compounds: chromatogram segment, 2.4–8 min; scanning interval, m/z 150–280; accumulation time, 100 ms; ion current control, 15,000; eight averaged spectra per data point; isolated parent ion mass, 267.1; width, 4 a.m.u.; fragmentation amplitude, 1.6 V; fragmentation time, 40 ms; product ion, 181.

Chemicals. All solvents were HPLC grade from Merck (Darmstadt, Germany). Formic acid (98–100%) reagent Ph. Eur. grade, trifluoroacetic acid (minimum 99.8%) Uvasol grade and triethylamine (TEA; minimum 99%) synthesis grade were also from Merck. Water for chromatography (minimum resistivity 18 MΩ and maximum content of Total Organic Carbon 30 ppb) was produced within the laboratory by means of a TKA Lab HP 6UV/UF instrument and used during experiments. Trimetazidine working standard (batch R-2440-84/201), alcalimetric assay 100.1% and the IS [1-(2,4,5-trimethoxy-benzyl)piperazine] working standard (batch RD-99/173; 98.4% assayed by HPLC) were obtained from Sochinaz S.A. (Switzerland).

Sample preparation procedure. A 1 mL aliquot of plasma sample was mixed with 50 µL of a 1000 ng/mL IS solution in methanol. After addition of 100 µL TFA, sample was vortexed for 3 min, then centrifuged for 10 min at 4000 rpm and 4°C. The supernatant was separated and neutralized with 80 µL of TEA followed by homogenization on vortex for 3 min at 2000 rpm.

Methodology and pharmacokinetic parameters. During the first open-label, randomized, two-period, two-sequence, crossover bioequivalence study, 24 healthy male and female volunteers received two doses of 20 mg TMZ from the tested (T1) and reference (R1) immediate-release products, with a 7 day wash-out period. The study medication was orally administered after overnight fasting. Blood samples were collected before dosing (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h post-dose. The pharmacokinetic parameters considered for evaluation of the bioequivalence between tested and reference products were: C_{max}, observed maximum TMZ plasma concentration; T_{max}, sampling time of the maximum plasma concentration; $t_{1/2}$, terminal elimination half life time; AUD, area under plasma concentration–time curve until last quantifiable value; $AUC_{0-\infty}$, AUD extrapolated to infinity.

During the second open-label, randomized, two-period, two-sequence, crossover bioequivalence study, 24 healthy male and female volunteers received nine doses of 35 mg TMZ from the tested (T2) and reference (R2) modified release products, at 12 h interval, with a 22 day wash-out period. Blood samples were taken before the first administration, at 1, 2, 3, 4, 5, 5.5, 6, 6.5, 7, 8, 9, 10 and 11 h after the first and the ninth administration, 12 h after the first, second, fourth, sixth, eighth and ninth administrations and 24 and 48 h after the ninth administration (35 samples per volunteer and per phase). The pharmacokinetic parameters used as primary evaluation criteria for bioequivalence assessment were: C_{max} during 12 h after the first administration; corresponding T_{max}; area under plasma concentration–time curve up to 12 h

after the first dose administration (AUD); C_{max} at steady state ($C_{max,SS}$); and area under the curve at steady state (AUD_{SS}).

Pharmacokinetic parameters were determined by means of the Kinetica software (version 3.1./2000) from Innaphase Corp., USA.

RESULTS AND DISCUSSION

Method development

Choice of the IS. Trimetazidine and the IS are positional isomers. Computed logarithms of the partition coefficient between *n*-octanol and water (log *P*) as a descriptor of the molecular hydrophobicity were 0.63 for TMZ and 0.84 for the IS. Acid–base dissociation constants (pK_a) for TMZ were 4.32 and 8.95. In acidic media, both analyte and IS should exist in double protonated forms. Computed log *P* for these forms are –8.22 for TMZ and –8.01 for the IS. It is worthwhile to note that calculated values are in good agreement with some experimental data found by Reymond *et al.* (1999). We can further assume that during protein precipitation water solubility for both target analytes is important. Chromatographic behavior also sustains the previous observation, as long as TMZ and IS are reciprocally separated with moderate resolution (~2.4) and relatively low retention. The experimental reversed elution order (theoretically RP mechanism achieves separation in the increased order of log *P* values) could be explained by a higher solubility of IS in methanol compared with TMZ.

Both analytes have the same exact molecular mass (266.16306 a.m.u.). Under APCI ionization conditions, the protonated molecule, $[M+H]^+$, at *m*/*z* 267.1 was formed. In the case of the IS, the fragment ion resulting after the loss of the piperazine ring also appeared with low intensity (<10%) in APCI. Under collision-induced dissociation, the precursor ion at *m*/*z* 267 gave a product ion at *m*/*z* 181 by the loss of the piperazine moiety. For TMZ, the subsequent loss of a methyl radical is possible, leading to a minor product ion at *m*/*z* 166 (<30%).

Atmospheric-pressure electro-spray interface (AP-ESI) gave a practically identical ionization pattern to APCI for both analytes of interest. However, under AP-ESI conditions, the presence of trifluoroacetate ions seriously reduced the ionization yields and consequently had a major influence on sensitivity. In APCI the ionization yields were practically unaffected by the presence of trifluoroacetate ions.

One can conclude that TMZ and the IS form high-yield protonated molecules under APCI conditions, characterized by the *m*/*z* 267 peak. The precursor ion was readily fragmented to the major product ion at *m*/*z* 181 during CID. This product ion was further monitored to obtain chromatograms.

Protein precipitation procedure. Addition of trifluoroacetic acid is one of the most efficient and quantitative ways of precipitating protein from plasma. Precipitation efficacy was proven by the conservation of the chromatographic column efficiency after more than 3000 injections of samples (note that injection volume is also high). Addition of TEA to the supernatant is explained by the need to reduce sample acidity. However, neutral or alkaline pH in sample solution should be avoided. Moderately acidic pH values preserve column integrity and eliminate the risk of focusing phenomena on injection and the carry-over effect.

Recovery from spiked plasma samples at concentration levels of 1, 5, 50, 100 and 200 ng/mL was 82.2 ± 9.8% ($n = 15$) for TMZ. For the IS, a recovery of 79.2 ± 8.2% ($n = 15$) was obtained at 50 ng/mL concentration. One can conclude that the extraction procedure is simple, precise and robust.

The chromatographic method. Reciprocal variations of ±0.2% of the components of the mobile phase produced retention data within the normal variation interval (3.6–3.9 min for TMZ and 5.2–5.6 for the IS). An increase of 5% in the methanol content of the mobile phase still generated acceptable separation of the target compounds, while the same variation of the aqueous component led to a 30% longer chromatographic run. Replacing formic acid by acetic acid did not influence the retention behavior of the analytes. When formic acid was replaced by TFA, the same chromatographic resolution was obtained with approximately 30% increase of the retention for both compounds. Lowering the pH of the aqueous component of the mobile phase resulted in a serious degradation of selectivity and a small increase of the ionization yield. Increasing pH also generated selectivity loss. The pH of the mobile phase is therefore important for reproducible results and should be kept in the 3.3 ± 0.1 range. The increase of TEA concentration slightly reduced retention but increased selectivity. Concerns related to column life motivated the choice of the TEA concentration at around 0.1%. Below such a limit, resolution between compounds was affected. Variations of column temperature within a ±4°C interval produced retention data within normal variation intervals. According to these results, the present method should be considered as robust.

Method validation

Linearity, limit of quantitation (LOQ), limit of detection (LOD). Linearity was tested at 10 concentration points (0, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100 and 250 ng/mL, respectively) for TMZ and at 50.0 ng/mL concentration of the IS. Calibration was done by plotting the ratio of the peak area corresponding to TMZ

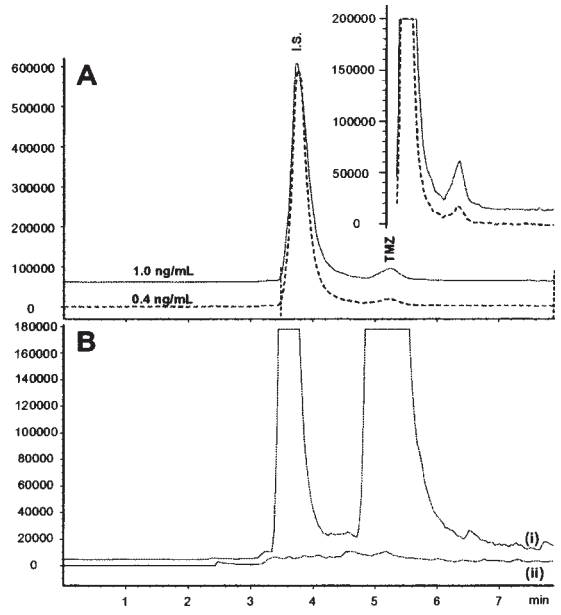

Figure 1. Extracted ion chromatograms resulting after processing spiked plasma samples: (A) trimetazidine spiked at LOD and LOQ levels and IS at 50 ng/mL; (B) illustrating the fact that no carry-over effect could be observed [blank sample (ii) injected immediately after a spiked sample (i) with TMZ 200 ng/mL and IS 50 ng/mL].

and the IS against the concentration of TMZ in spiked plasma samples, expressed in ng/mL. Calibration was repeated during three different days. The slope (B) of the linear regression was 0.0204 ± 0.0027 and the intercept (A) −0.0003 ± 0.0062. The mean correlation coefficient (r_{xy}) was 0.9993. LOQ was computed according the relationship LOQ = 10 * s_A/B, where s_A is the standard deviation of the intercept and B is the mean slope of the linear regression. Calculations on experimental data gave a LOQ for TMZ of 1.52 ng/mL and an LOD of 0.46 ng/mL (LOD ≈ LOQ/3.33). Note that peak areas of the IS during experiments were characterized by an RSD% of 8.22% (n = 30). In Fig. 1(A) chromatograms of spiked plasma samples containing 0.4 ng/mL (close to LOD), 1.0 ng/mL (close to LOQ) of TMZ and 50 ng/mL IS are presented. Figure 1(B) illustrates the fact that carry-over effects were not experienced (overlaid chromatograms of a blank plasma sample injected immediately after a spiked plasma sample containing 250 ng/mL TMZ and 50 ng/mL IS are presented—note that no needle wash procedure between injections was used).

Precision. Repeatability was studied at three concentration levels (1, 100 and 250 ng/mL) for TMZ and the 50 ng/mL level for the IS. Ten aliquots were prepared from the stock solution at each concentration level. The relation standard deviations of the ratios between indapamide and IS peak areas were 9.5% at 1 ng/mL, 2.6% at 100 ng/mL and 3.3% at 250 ng/mL. The RSD% calculated for the values of the peak area corresponding to the IS during the study was 5.2% (n = 30). The RSD% for retention time values characterizing chromatographic peaks was 0.89% for TMZ and 1.19% for the IS.

The intermediate reproducibility was carried out on five different days at the same concentration levels for spiked plasma samples freshly prepared daily. The RSD% for ratios between indapamide and IS peak areas were 13.0% at 1 ng/mL level, 4.9% at 100 ng/mL level and 4.6% at 250 ng/mL level, respectively. The RSD% calculated for peak area values characterizing IS during the study was 7.5% (n = 15). The trend in variation of the peak areas of the IS obtained during the whole bioequivalence study of TMZ immediate release formulations (720 samples) is presented in Fig. 2 (RSD% = 14.6%).

Accuracy. Accuracy was determining by measuring quality control plasma samples spiked at 1, 5, 8, 10, 20, 25, 40, 50, 80 and 100 ng/mL levels during 22 different days. IS was always spiked at 50.0 ng/mL. The values of the ratio between TMZ and IS peak areas were then interpolated in the linear regression equation found during the linearity study, to calculate experimental concentration values. The results are given in Table 1. It can be observed that recovery outside the ±20% interval from the theoretical value was obtained only for the 1 ng/mL level, which is below the determined LOQ value.

Freeze–thaw stability. Stock TMZ spiked plasma samples having concentrations of 1, 25 and 100 ng/mL, respectively, and 50.0 ng/mL IS were stored at −40°C for 24 h, then thawed unassisted at room temperature. An aliquot from each stock plasma sample was then processed. Stock plasma samples underwent five successive freeze and thaw cycles. The RSD% calculated for TMZ recovered concentration at each level during five cycles were 7.6% (1.12 ± 0.18 ng/mL), 3.8% (26.6 ± 2 ng/mL), and 3.6% (98.2 ± 7 ng/mL), respectively. During such studies, RSD% of 20% at LOQ and 15% at higher concentration were considered as acceptable for proving stability.

Long-term stability. Stock TMZ spiked plasma samples having concentrations of 1, 25 and 100 ng/mL, respectively, and 50.0 ng/mL IS, were divided into separate vials and stored at −40°C. At the beginning of each daily session, one vial for each concentration level was thawed unassisted at room temperature and then processed. The RSD% calculated for recovered TMZ concentration at each level for samples processed during 22 days were 12.7% (1.07 ± 0.28 ng/mL), 4.4%

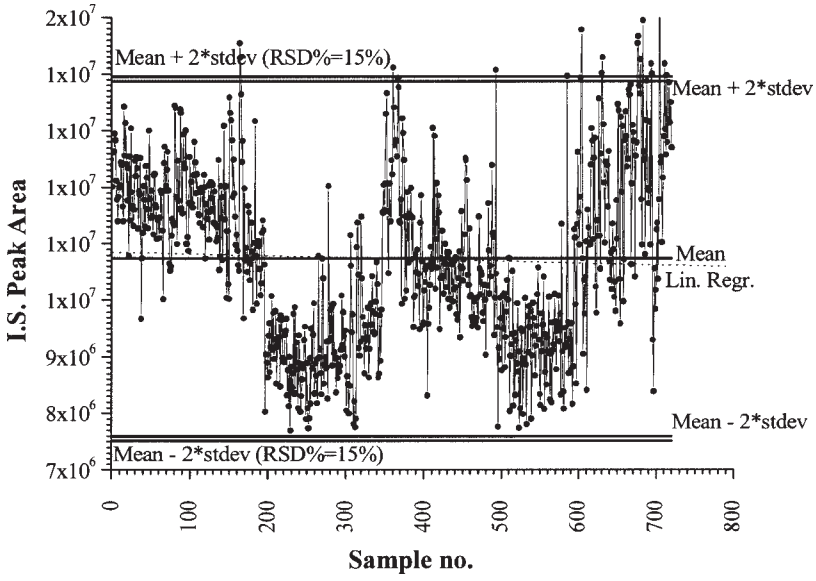

Figure 2. Trend in variation of the IS peak area values integrated in the chromatograms of the real plasma samples processed during the single dose bioequivalence study.

(25.6 ± 2.28 ng/mL) and 6.8% (104.5 ± 14 ng/mL), respectively.

Short-term stability. Stock TMZ spiked plasma samples having concentrations of 1, 25 and 100 ng/mL, respectively, and 50.0 ng/mL IS, were stored for 24 h at −40°C, then thawed unassisted at room temperature and kept at this temperature for 72 h. Aliquots from each stock plasma sample were processed immediately after thawing, and 4, 12, 24 and 72 h later. The RSD% calculated for recovered TMZ concentration at each level for processed samples were 6.0% (1.13 ± 0.14 ng/mL), 6.5% (26.4 ± 3.4 ng/mL) and 7.7% (101.0 ± 15.6 ng/mL), respectively.

Stock solution stability. Stock solution of the IS (0.01 mg/mL) in methanol was stored at room temperature for 12 days. From this solution, spiked plasma samples at 50 ng/mL level were made during the first, third, fifth, ninth and twelfth day. The resulting samples were processed according to the above procedure. The RSD% calculated for IS peak areas was 11.6%.

Post-preparative stability. Processed stock samples obtained from 1, 25 and 100 ng/mL TMZ spiked plasma samples were stored at room temperature, on the bench top or in the autosampler. The samples were assayed immediately and 1, 3, 6, 12, 18 and 24 h after processing. RSD% calculated for recovered TMZ concentration at each level for processed samples kept at room temperature for 24 h were 7.9% (1.03 ± 0.16 ng/mL), 4.4% (25.8 ± 2.3 ng/mL) and 7.3% (96.3 ± 14.0 ng/mL), respectively.

Pharmacokinetic parameters

The present method was used to assess bioequivalence of two TMZ 20 mg immediate release and two TMZ 35 mg modified release pharmaceutical formulations (coated tablets) found on the Romanian market. For both sets, one product was considered as the reference (R), the other one as the tested (T) sample. The pharmacokinetic parameters obtained for the studies are shown in Table 2.

The mean TMZ plasma concentration–time plots, obtained for tested and reference pharmaceutical formulation, on 24 healthy volunteers, during the open-label, randomized, two-period, two-sequence, crossover, single/multiple dose bioequivalence studies are given in Fig. 3.

CONCLUSIONS

A method for determination of trimetazidine in plasma samples at the low ng/mL level is presented. 1-(2,4,5-Trimethoxybenzyl)piperazine, a positional isomer of TMZ, was used as internal standard. The sample preparation was based on protein precipitation with TFA. After centrifugation, the pH of the supernatant was controlled by addition of triethylamine. The sample preparation procedure was simple and robust. The chromatographic method was based on a reversed-phase mechanism carried out under isocratic conditions. Tandem mass spectrometric detection was used; ionization was realized with an atmospheric pressure chemical ionization interface. The ion trap mass ana-

Table 1. Illustration of the method accuracy at 10 trimetazidine concentration levels during 22 separate experimental sessions

Theoretical concentration (ng/mL)	Accuracy experiment (day no.)																						Recovered (ng/mL)		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Mean concentration	SD	RSD%
1	1.2	1.2	1.4	2.0	1.1	1.1	1.0	1.0	1.1	1.1	0.9	1.2	1.0	1.3	0.9	1.0	1.3	0.9	1.0	1.3	0.9	1.0	1.2	0.27	23.4
5	5.0	5.3	4.7	5.3	5.2	5.3	6.0	4.8	4.9	5.4	4.5	4.9	4.8	4.4	4.7	4.8	4.4	4.7	4.8	4.4	4.7	4.8	5.0	0.45	8.9
8	8.5	8.6	7.7	7.9	7.1	7.8	7.6	8.5	7.0	6.5	7.8	7.0	7.8	7.6	7.1	7.8	7.6	7.1	7.8	7.6	7.1	7.8	7.3	0.73	9.9
10	9.8	9.6	9.4	10.0	10.4	10.6	9.7	9.5	8.4	9.6	9.9	8.7	10.6	10.5	10.3	10.6	10.5	10.3	10.6	10.5	10.3	10.6	9.6	0.64	6.6
20	18.9	18.0	19.9	22.5	19.4	18.7	19.7	18.9	21.1	16.2	18.9	18.4	20.3	23.4	19.9	20.3	23.4	19.9	20.3	23.4	19.9	20.3	19.3	1.93	10.0
25	25.6	25.6	26.6	25.3	25.2	26.4	26.2	26.2	26.2	25.9	26.5	26.6	25.2	26.1	26.5	25.2	26.1	26.5	25.2	26.1	26.5	25.2	25.6	1.14	4.4
40	42.6	39.2	39.8	40.7	39.1	37.4	43.0	35.9	39.5	35.1	43.0	41.3	42.9	41.2	41.2	42.9	41.2	41.2	42.9	41.2	41.2	42.9	39.4	2.54	6.5
50	50.1	53.4	53.1	51.5	48.7	50.4	52.6	55.2	47.1	57.2	48.2	49.3	44.7	50.1	51.7	44.7	50.1	51.7	44.7	50.1	51.7	44.7	50.6	2.88	5.7
80	79.0	83.0	89.5	90.3	72.6	84.5	85.7	82.3	86.8	79.6	83.0	77.9	89.9	78.4	86.7	89.9	78.4	86.7	89.9	78.4	86.7	89.9	83.2	5.59	6.7
100	106.8	110.9	109.8	108.5	94.5	107.6	106.9	108.4	100.1	116.6	99.1	102.4	95.6	102.0	109.2	95.6	102.0	109.2	95.6	102.0	109.2	95.6	103.7	6.54	6.3

Table 2. Pharmacokinetic parameters determined during bioequivalence assessment of two immediate release formulations containing 20 mg trimetazidine and of two modified release formulations containing 35 mg of the same active substance

Pharmacokinetic parameter	Immediate-release TMZ 20 mg products										Modified-release TMZ 35 mg products									
	C_{max} (ng/mL)		T_{max} (h)		$t_{1/2}$ (h)		AUD (ng/mL h)		$AUC_{0-\infty}$ (ng/mL h)		C_{max} (ng/mL)		T_{max} (h)		AUD (ng/mL h)		$C_{max, SS}$ (ng/mL)		AUD_{SS} (ng/mL h)	
Product	R1	T1	R1	T1	R1	T1	R1	T1	R1	T1	R2	T2	R2	T2	R2	T2	R2	T2	R2	T2
Mean value	164.5	160.8	1.60	1.65	7.04	7.13	1471.4	1395.9	1724.7	1635.9	84.5	87.6	4.7	4.5	622.9	652.0	126.7	131.2	1001	1038.4
Minimum value	94.6	89.7	1.00	0.50	4.1	4.4	855.6	912.4	944.2	997.9	29.8	36.1	3.0	3.0	216.0	250.9	43.3	62.7	245.9	395.2
Maximum value	265.2	264.1	3.00	3.00	16.6	16.8	3080.2	3021.7	4685.7	4297.9	133.6	136.9	7.0	6.5	929.0	974.2	222.0	217.3	1618.3	1685.5
RSD%	22.1	20.8	37.9	40.8	48.2	48.3	37.1	57.3	35.6	55.7	28.3	28.6	28.3	20.7	27.8	28.5	36.2	32.2	36.5	36.4

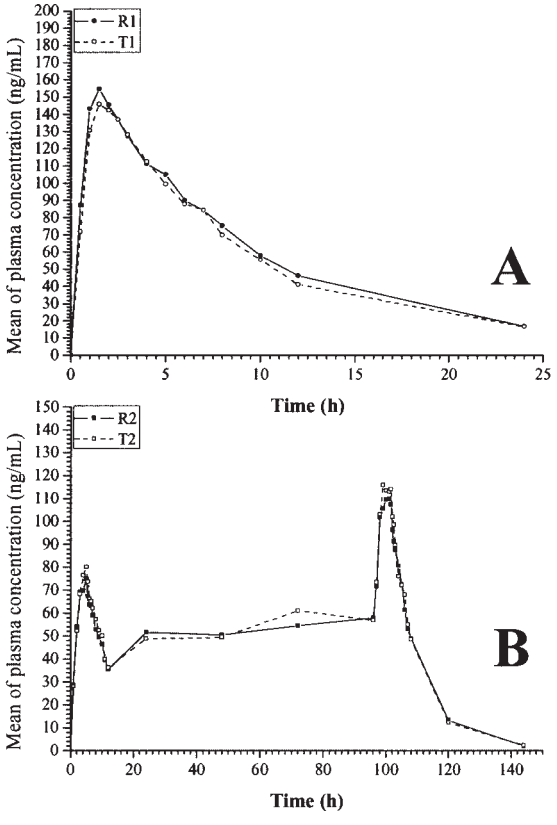

Figure 3. Mean plasma concentration–time plots obtained during assessment of the bioequivalence of: (A) two marketed immediate-release pharmaceutical formulations containing 20 mg of trimetazidine carried out on 24 healthy volunteers, in a randomized, two-period, two-sequence, crossover, single dose design; (B) two marketed modified-release pharmaceutical formulations containing 35 mg of trimetazidine carried out on 24 healthy volunteers, in a randomized, two-period, two-sequence, crossover, multiple-dose design.

lyzer isolated first the protonated molecules of the target compounds as precursor ions while, after CID, the product ion at *m*/*z* 181 was monitored. The method was validated and used to assess the bioequivalence of two marketed immediate-release formulations containing 20 mg of trimetazidine and two marketed modified-release formulations containing 35 mg of trimetazidine, respectively. During the study, 2400 plasma samples were processed and analyzed within 22 working days, demonstrating the high-throughput characteristics of the method.

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