Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Journal of Chromatography B, 850 (2007) 59–67
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of captopril in human plasma, using solid phase extraction and high-performance liquid chromatography, coupled to mass spectrometry: Application to bioequivalence study

Kênnia R. Rezende [b,*], Iram M. Mundim [a], Leonardo S. Teixeira [a], Weidson C. Souza [a], Douglas R. Ramos [a], Cláudio R.F. Cardoso [a], Inácia C. Souza [a], Mário Z. Gratão [a], Karini B. Bellório [a]

[a] *Pharmaceutical Sciences Institute, ICF, Goiânia, Goiás, Brazil*
[b] *Faculty of Pharmacy, Federal University of Goiás, UFG, Pça Universitária com 1ª, Avenida S/N Setor Universitário, Goiânia-GO, Goiás CEP 74605-220, Brazil*

Received 14 April 2006; accepted 1 November 2006
Available online 4 December 2006

Abstract

A specific high performance liquid chromatography–mass spectrometric (LC–MS/MS) assay was developed for the determination of captopryl in plasma. The retention time was 1.45 and 1.37 min for captopril and enalapril, respectively. The overall mean recovery, using SPE extraction with OASIS® HLB cartridges, was found to be 107.2 ± 9.5 and 100.04 ± 2%, respectively. Calibration curves were linear in the concentration range of 10.00–2000.00 ng/ml, and the lower limit of quantification (LLOQ) was 10.00 ng/ml. The LLOQ was sensitive enough for detecting terminal phase concentrations of the drug. Inter-batch precision of the method ranged from 0.88 to 1.95%. Intra-batch accuracy ranged from 97.15 to 105.77%, while intra-batch precision ranged from 2.49 to 5.66% at concentrations of 30.00, 760.00 and 1500.00 ng/ml. The developed method was applied to study bioequivalence of captopril in a group of 25 human subjects at a single oral dose of a 50 mg tablet.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Captopril; LC–MS; SPE; Bioequivalence; Pharmacokinetics; Stability validation

1. Introduction

Captopril (CPT) is the first orally active angiotensin-converting enzyme inhibitor widely used in the treatment of hypertension and congestive heart failure. It contains a sulphydryl group and binds readily to albumin and other plasma proteins. It also forms disulphides and endogeneous thiol-containing compounds (cysteine, glutathione), as well as disulphide dimmer of parent compound [1]. The measurement of free or unchanged captopril concentration needs to be preceded by chemical stabilizer addition and molecule derivatization of biological samples in order to prevent captopril disulphide formation [2].

Stabilizer agents often used include *N*-(1-pyrenyl)maleimide (NPM) and [3,4], *p*-bromophenacyl bromide (*p*-BPB) [5], as suitable to the applied (fluorescence or UV) detection methods. According to previously cited studies, oxidation reaction forming captopril disulphide can also be delayed by lowering the pH of the solution, adding chelating agents, increasing captopril concentration, using a nitrogen or low-oxygen headspace, or incorporating antioxidants and anti-irritants such as clobetasol [6]. However, recent stability studies show improved results with an EDTA chelating agent associated to working a pH range below 4.0 and deionized water solutions [5]. Even better protection of captopril oxidation is found with dithiothreitol (DTT), compared to NEM, by increasing free thiol content from human serum albumin.

Several analytical methods have been applied for captopril determination in plasma. The majority of published articles describe the use of HPLC [1,5,7–11], GC [12] or GC–MS [4] techniques, often involving CPT derivatives. Concerning extraction procedures, they are based on several evaporation/concentration steps, as in liquid–liquid extraction [13],

* Corresponding author. Fax: +55 62 3261 3999.
E-mail address: kennia@farmacia.ufg.br (K.R. Rezende).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.11.007

consisting of a very time consuming work. Aiming at a more practical method development, while using less organic solvent, an improved validated LC–MS/MS method, using fast solid phase extraction (SPE) cartridges with small amounts of sample plasma volume, is presented here. The method should be successfully applied to accurately measure total captopril concentration on a large number of human plasma samples from bioequivalence studies.

The aim of this work was to develop and validate an analytical method, using SPE cartridges and LC–MS/MS applied to bioequivalence studies, in accordance to FDA and Brazilian Governmental guidelines. In addition, the LC–MS/MS method must be validated by a short run time, in order to be able to analyze a large number of samples.

2. Experimental methods

2.1. Chemicals and materials

Captopril and enalapril maleate were provided by the Brazilian Pharmacopeia Standards, ANVISA (Rio de Janeiro, RJ, Brazil) as Chemical Reference Standards of batches 1001 and 1029, respectively. All organic solvents used for the mobile phase were HPLC grade, and were purchased from Tedia (Fairfield, USA). Trifluoracetic acid and dithiothreitol were purchased from Sigma (St. Louis, MO), Human Plasma samples (normal, hyperlipemic and hemolyzed) came from distinct drug free subjects (six different lots) and were obtained from INGOH Laboratories (Goiânia Institute of Hemotherapy, Brazil). Solid phase extraction (SPE) cartridges (Oasis HBL 30 mg 1 cm^3) were purchased from Waters (Milford, MA).

2.2. Chromatographic conditions

LC–MS/MS experiments were performed on a reverse-phase Chromolith C18 column (Merck, Darmstadt, Germany) 50 mm × 4.6 mm, 5 μm, attached to a LC system, comprising a LC-10AV DP pump, a DGU-14 A degasser, an autosampler SIL-10 AD VP (Shimadzu, Kyoto, Japan) maintained at controlled room temperature (22 °C) and a UV/vis detector SPD-10 AP VP (Shimadzu, Kyoto, Japan). The compounds were eluted with methanol and water (65:35, v/v) using formic

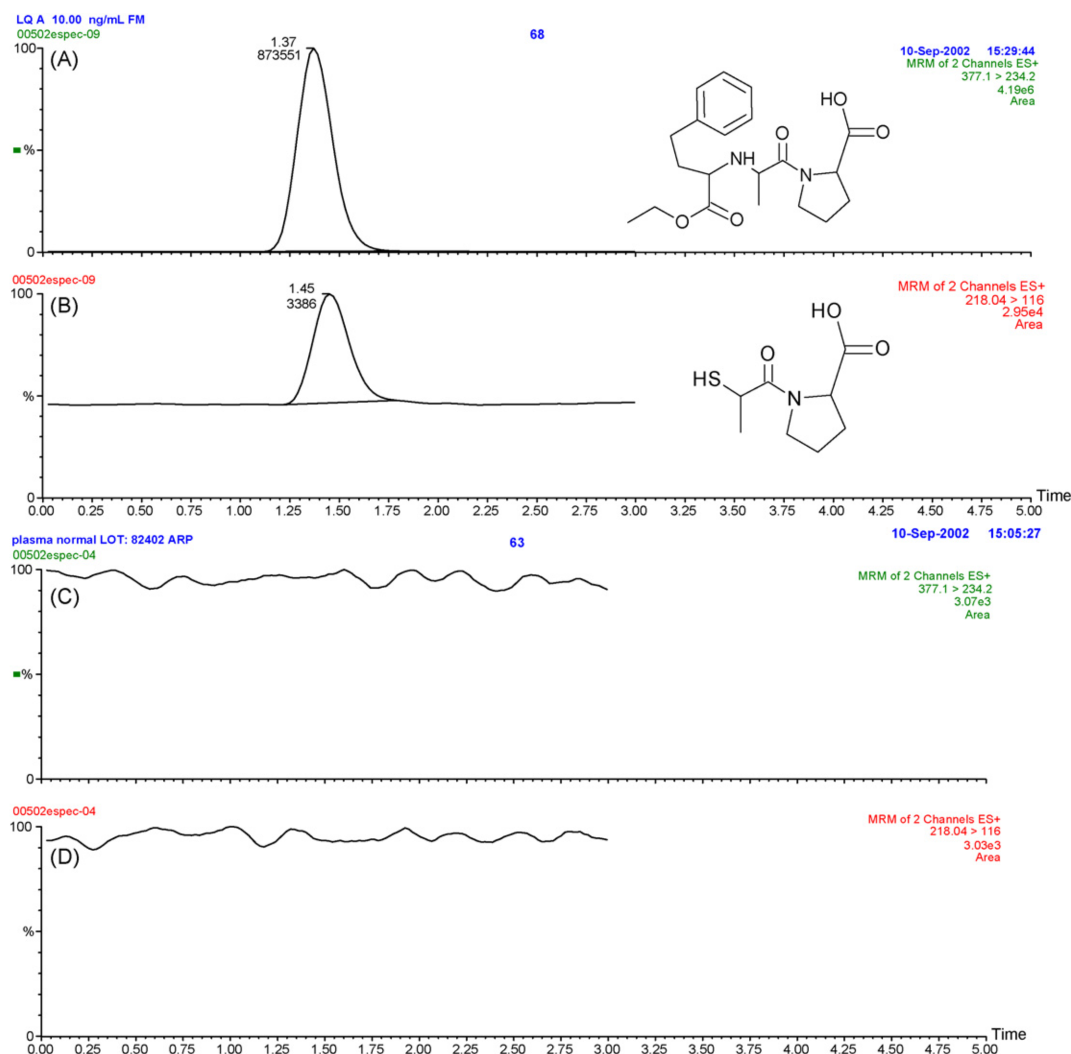

Fig. 1. LC–MS/MS chromatograms of human plasma samples: (A) IS (enalapril) at channel 1 (377.1); (B) Captopril at LLOQ channel 2 (218.04); (C) blank plasma in both channels.

acid or ammonium acetate to adjust to pH 3.1. The total run time was 3.0 min with captopril and enalapril retention time of 1.45 and 1.37 min, respectively (Fig. 1).

Other analytical conditions were tested, using different chromatographic and mass conditions as nebulizer gas source and dessolvation probe temperature for analyte and internal standard. Nevertheless, we had a better combination of chromatographic performance, selectivity, run time, peak size and shape when using a short high performance column, a pH controlled elution at 3.1 and a flow rate of 0.6 ml/min.

2.3. Mass spectrometric conditions

The LC equipment was connected to a Micromass Quattro LC system (Milford, USA) with a positive electrospray ionization (ESI^+) interface source, using a crossflow counter electrode. The multiple reaction monitoring (MRM) detection mode was employed to captopril (*m/z* 218.04/116.0 Da) and enalapril (*m/z* 377.10/234.2 Da) parent and daughter ion fragments (Fig. 1A and B), respectively, with dwell cell set at 0.5 s for each transition. Drug and IS were run in positive ion mode. The operating cone conditions were set at 15/30 V and collision energy set at 15/18 eV, respectively. Data acquisition and analysis were performed, using the software MassLynx (v 3.5) and Windows NT (v 4.0).

LC–MS–MS operating in electrospray ion positive (ESI^+) mode produces a gentle ionization with minimal fragmentation of the analyte, yielding high mass-to-charge precursor $[M+1]^+$ and daughter ion. The triple quadrupole, combined to ESI and the multiple reaction monitoring (MRM) operating mode lead to short retention time and yields both high selectivity and sensitivity.

2.4. Drug standards solutions

Standard stock solutions (25 ml) of captopril and enalapril maleate (internal standard) were prepared, from separate weighting, in methanol–water (50:50, v/v) at a concentration of 0.3 and 1.0 mg/ml, respectively, transferred to polypropylene screw cap Falcon™ tubes from Becton Dickinson (Mountainview, CA) and kept at −20 °C. Intermediary solutions of captopril were prepared in 50% methanol, by appropriate dilution of stock solutions. The internal standard solution (enalapril maleate) was also diluted in 50% methanol to obtain the working solution of 1800.0 ng/ml. Formic acid and dithiothreitol (200 mmol/l) solutions were prepared at concentrations of 30 µl/ml (3%, v/v) and 30.84 g/l, respectively.

All calibration curve samples (non-zero samples), except blank plasma, were prepared by spiking four different blank plasma batches aliquots of 300 µl each, with 50 µl of the intermediary captopril solutions, to yield final plasma concentrations of 2000.0; 1500.0; 1000.0; 760.0; 400.0; 100.0; 30.0, and 10.0 ng/ml. All zero calibration curve samples were spiked with 50 µl of methanol/water (50:50, v/v). All these solutions were fractionated in aliquots sufficient for one workday and were stored at −20 °C. No change in stability over a period of 64 days was observed.

2.5. Quality control samples

Quality control (QC) samples were prepared at low level (30.0 ng/ml), middle level (760.0 ng/ml) and high level (1500.0 ng/ml). Low limit of quantification was set at 13.0 times the lower limit of quantification, LLOQ), instead of three times the LLOQ as often published, because the aim of the validation process had been already reached. QCs were prepared by spiking different blank plasma aliquots (300 µl) with the corresponding captopril intermediary standard solution (50 µl) to produce a final concentration equivalent to 30.0, 760.0 and 1500.0 ng/ml of captopril.

2.6. Sample extraction

Drug was extracted from plasma samples, using solid phase extraction (SPE) technique. Each human plasma sample gave satisfactory values for recovery with a single extraction with an OASIS® HLB solid-phase extraction cartridge (1 cm^3, 30 mg,Waters Corporation). The cartridge was conditioned by rinsing with 1 ml methanol and 1 ml water. For sample preparation, 300 µl aliquot of plasma samples (calibration curves and QCs) were transferred to polypropylene tubes (Falcon), then 50 µl of 200 mM 1,4-dithiothreitol solution were added to each sample, vortexed (5 s) and left for 10 min at room temperature. Samples were then spiked with IS working solution (1800 ng/ml, 50 µl), acidified (3% formic acid, 200 µl) and vortexed (5 s). After sample load, the cartridge-polypropylene tube set was centrifuged (3400 rpm/2 min). The first eluate was discarded and, captopril/IS elution followed methanol addition (600 µl). Tube solutions were vortexed, transferred to polypropylene vials and distributed on a rack auto-sampler kept at 22 °C, following sample injection (20 µl) and analysis into the LC–MS/MS system. No solvent evaporation was needed.

2.7. Method validation

2.7.1. Specificity

Four randomly selected normal plasma, one hyperlipemic and other hemolyzed plasma samples from distinct healthy subjects were donated by Hemotherapy Institute (INGOH), processed by the solid–liquid extraction procedure and chromatographed to determine the extent to which endogenous plasma components may contribute to the interference at retention time of analyte and internal standard. On the day of the study, all volunteers had a blank plasma sample collected before drug administration. Any interference at the analyte and IS retention time should not account for more than 20% of LLOQ peak response area.

2.7.2. Linearity

Calibration curves were constructed using eight non-zero standard points covering the range of 10.0–2000.0 ng/ml. In addition, a blank (non-spiked sample) and a zero plasma sample (only spiked with IS) were run to discard the presence of interferences. Plasma samples were spiked in duplicates at concentrations of 10.0, 30.0, 100.0, 400.0, 760.0, 1000.0, 1500.0 and 2000.0 ng/ml. The samples were extracted as described in

item 2.6. The standard calibration curves for captopril were constructed using the analyte/IS peak–area ratios versus nominal concentrations of the analytes. Linear least-square regression analysis, with weighting factor of 1/x, was performed to assess the linearity, as well as to generate the standard calibration equation: $y = ax + b$, where y is the peak–area ratio, x the concentration, a the slope and b is the intercept of the regression line.

2.7.3. Recovery

Spiked plasma samples were assayed using five replicates at three concentration levels of 30.0, 760.0 and 1500.0 ng/ml of captopril, and extracted as already described (item 2.6). Recovery (extraction efficacy) was calculated by comparing the peak-area of the extracted sample to that of the unextracted pure authentic standard solutions.

2.7.4. Precision and accuracy

Precision and accuracy of this method were evaluated using three different batches of quality control samples at concentrations of 30.0, 760.0 and 1500.0 ng/ml of captopril, also including the lowest limit of quantification, LLOQ, 10.0 ng/ml (last data not shown). For intra-batch assay precision and accuracy, *eight* replicates of quality control samples at the three concentration levels were assayed all at once within a day to obtain CV(%) and accuracy values. The inter-batch assay precision and accuracy were determined by analyzing mean values of quality control samples from three plasma batches, yielding the corresponding inter-batches CV(%) and accuracy values (Table 1).

2.7.5. Sensitivity

The limit of detection (LOD) was determined as the lowest concentration, which gives a signal-to-noise ratio of three

Table 1
Intra and inter-batch accuracy and precision for captopril determination in spiked plasma samples

	Captopril concentration in human plasma		
	Low QC (30 ng/ml)	Medium QC (760 ng/mL)	High QC (1500 ng/ml)
Batch of analysis			
Batch 1	32.58	760.51	1565.26
	31.19	736.28	1463.73
	30.59	760.84	1439.61
	29.21	760.09	1558.87
	28.91	714.23	1577.17
	30.92	702.90	1594.66
	28.03	745.67	1552.12
	30.51	725.93	1566.21
Mean 1 (N = 8)	30.24	738.31	1539.70
Precision (CV%)	4.80	3.04	3.65
Accuracy	100.81	97.15	102.65
Batch 2	31.51	738.18	1587.31
	29.45	742.88	1550.16
	32.00	799.50	1518.86
	31.14	804.68	1592.81
	27.89	761.91	1667.10
	31.05	713.06	1602.36
	28.55	782.29	1606.83
	27.85	749.69	1567.21
Mean 2 (N = 8)	29.93	767.15	1586.58
Precision (CV%)	5.66	4.06	2.76
Accuracy	99.77	100.94	105.77
Batch 3	31.19	762.38	1598.25
	32.07	721.89	1508.79
	32.01	754.27	1503.53
	30.54	779.82	1570.63
	29.03	740.24	1601.74
	31.69	708.84	1592.00
	27.29	777.12	1581.17
	29.85	742.93	1551.64
Mean 3 (N = 8)	30.46	748.44	1563.47
Precision (CV%)	5.48	3.35	2.49
Accuracy	101.53	98.48	104.23
Inter-batch assay			
Mean (N = 3)	30.21	751.30	1563.25
Precision (CV%)	0.88	1.95	1.50
Accuracy	100.70	98.85	104.22

times. The lower limit of quantification (LLOQ) was determined for captopril, based on two criteria: (a) the analyte response at LLOQ had to be at least five times baseline noise; (b) the analyte response at LLOQ c being determined with sufficient precision and accuracy, i.e., precision of 20% and accuracy of 80–120%. Calculations were based on eight replicates of three blank plasma batches.

2.7.6. Stability of analytes

2.7.6.1. Freeze–thaw stability. Stability of captopril was assessed in plasma samples subjected to three freeze–thaw cycles of −20 °C during 24 h. Five replicates of plasma spiked with captopril at 30.0 and 1500.0 ng/ml, underwent three freeze–thaw cycles: frozen samples were allowed to thaw at controlled ambient temperature (22 °C) and were subsequently refrozen for 12 h. Aliquots of all samples were quantified at the end of the third freeze–thaw cycle. Analysis of captopril concentrations were compared to fresh samples not subjected to the freeze–thaw cycles and expressed in percentage of degradation.

2.7.6.2. Short term storage stability. Five replicates of low and high QCs (30.0 and 1500.0 ng/ml) were subjected to a natural thaw process, at room temperature (∼25 °C). All samples remained on the benchtop for a time exceeding the maximum period of time expected for routine sample preparation (12 h). Samples were extracted and further compared to fresh prepared ones at equivalent concentration.

2.7.6.3. Long term storage stability. The storage time of long term stability was assessed by five replicates of low and high QCs (30.0 and 1500.0 ng/ml). Samples were subjected to freeze storage (−20 °C) during the entire period covered by the bioequivalence study, i.e., from the first day of volunteer sample collection up to the last day of sample analysis. Storage stability was defined, comparing sample concentration to the mean values obtained at first-day analysis.

2.7.6.4. Stock solution stability. Internal standard and stock solutions in plasma were prepared and stored at −20 °C. Sample aliquots of five replicates of all three QCs levels were evaluated after sitting 24 h at room temperature, and also after freeze storage for 7 days. Results were compared to fresh prepared solutions at corresponding concentrations.

3. Results and discussion

3.1. Validation results

All sample analysis were carried out in a GLP-compliant manner and therefore the LC–MS–MS methods need to be carried out according to the current Brazilian Regulatory Agency (ANVISA)[14], yet in accordance to US Food and Drug Administration Bioanalytical method validation guidance [15].

3.2. Assessment of linearity and specificity

Linearity was tested for the range of concentrations 10.0–2000.0 ng/ml, showing good linear response to the method. Correlation coefficient ranged from 0.9972 to 0.9982, while calculated inter-batch accuracy and precision between three batches at the LLOQ (10.0 ng/ml) were found to be 110.9 and 3.1%, respectively (data not shown). The chromatograms obtained from LLOQ (10.0 ng/ml) and extracted blank are depicted in Fig. 1. The captopril and enalapril retention times were 1.45 and 1.37 min, respectively.

Specificity of the response for the interfering peaks at the same retention time of the drug were less than 20% of the LLOQ response, when analyzing the four batches of blank normal plasma, and the two other batches of hemolysed and hyperlipidemic plasma (Fig. 2). The response for the interfering peaks at the retention time of the drug and the internal standard were less than 20 and 5%, respectively, from the response in the concentration used. Furthermore, blank plasma samples from all 25 volunteers were run before unknown sample quantification, showing a clear chromatogram. The main reason was the improvement of clean-up SPE procedure, compared to liquid–liquid extraction, besides the high selectivity of the MRM mode on LC–MS–MS spectrometer. Therefore, the high selectivity of the method was confirmed by both drug and IS, as no endogenous peaks were seen at analytical conditions previously described (Figs. 1C and 2).

3.3. Recovery of captopril

Absolute recoveries for both captopril and IS were evaluated, according to Section 2.7.3. Results of sample extraction procedure showed an overall mean value of 107.20%. At different QCs levels (Low, Medium and High) it was as follows: 117.48; 105.37, and 98.76%, respectively, showing a very selective extraction procedure.

3.4. Accuracy and precision measurement

Intra-batch precision and accuracy of the assay was measured for captopril and IS (enalapril) at each QC level (30.0, 760.0 and 1500.0 ng/ml), also including LLOQ (10.0 ng/ml). Method intra-batch precision and accuracy (% CV) ranged from 2.49 to 5.66%, and 97.15 to 105.77%, respectively. Method inter-batch precision (% CV) and accuracy ranged from 0.88 to 1.95%, and 98.85 to 104.22%, respectively, as presented in Table 1. These results were within the acceptance criteria for precision and accuracy, i.e., deviation values were within ±15% of the authentic values, except for LLOQ, which could show a ±20% deviation [14,15].

For sensitivity determination, the lower limit of quantification (LLOQ) for captopril was found to be 10.0 ng/ml, with precision and accuracy of 3.10% (% CV) and 110.90%, respectively. In this work, the LLOQ signal-to-noise ratio was 13.03 units, which means that it could be brought to a lower level of detection, although the purpose of this study had already been reached.

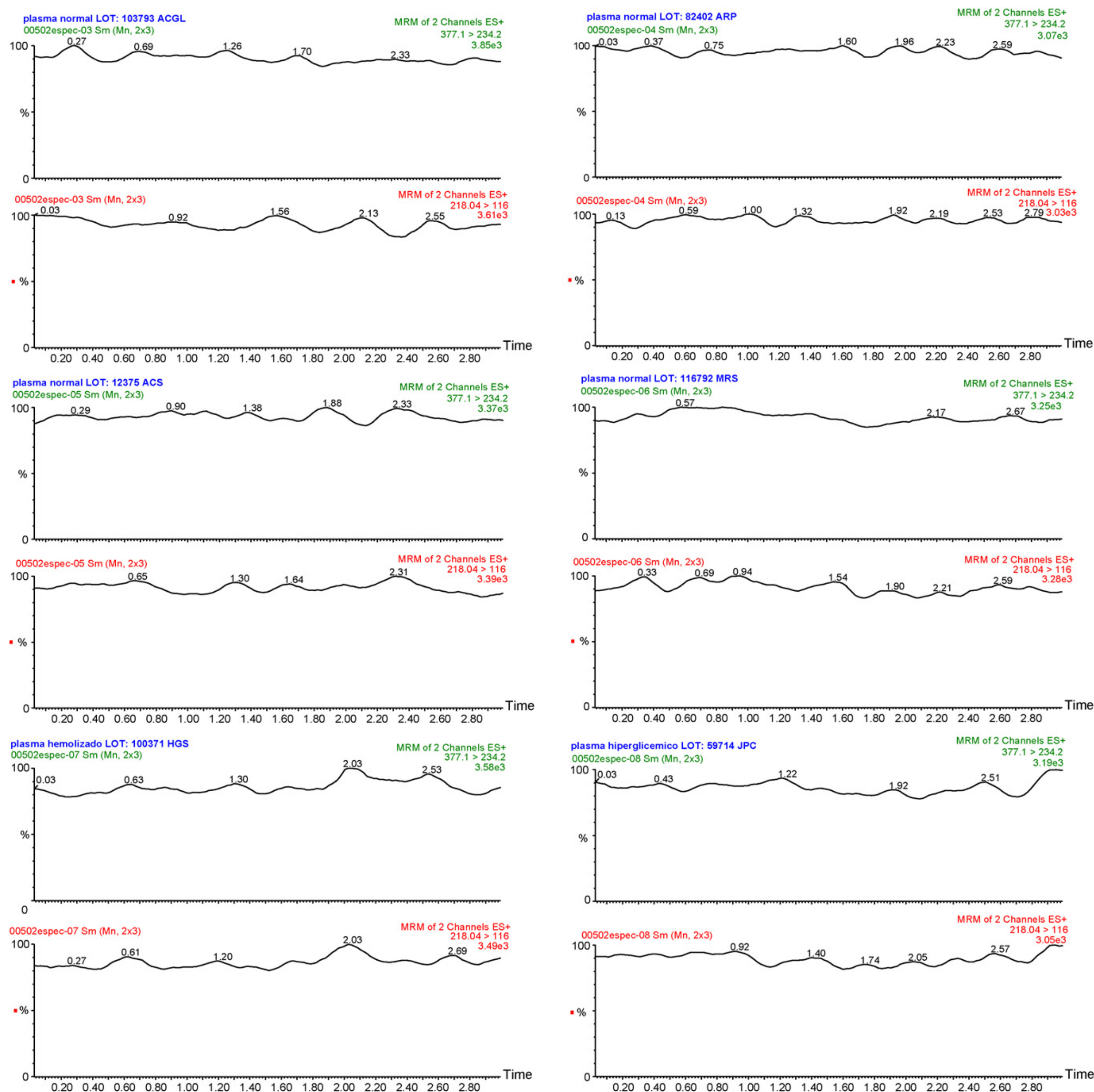

Fig. 2. Chromatograms of six batches of blank plasma samples at both LC–MS/MS channels. From top to bottom: four normal plasma, 01 hyperlipemic and 01 hemolyzed plasma sample.

3.5. Assessment of stability

3.5.1. Stock solution stability

Following seven days of captopril storage in mobile phase, differences in analysis of frozen and fresh stock solutions varied in accuracy (and precision) from 102.09% (% CV 8.59) to 110.64% (% CV 7.45) for low QC, and from 97.61% (% CV 6.89) to 104.76% (% CV 3.21) for high QC samples. So, frozen and fresh solution accuracy differed in 8.38 and 7.32%, to low and high QC levels, respectively. IS samples showed a −4.51% accuracy deviation. All of them were within analytical method acceptance criteria, i.e., not higher than 15% of fresh solutions [14,15].

3.5.2. Short term stability (STS)

Twenty-four hour stock solution stability (in plasma) was assessed according to Section 2.7.6.2. After extraction procedure, drug recovery after 24 h varied from 101.65% (% CV 4.92) to 107.46% (% CV 11.21) for low QC and from 101.4% (% CV 4.22) to 109.19% (% CV 8.01) for high QC samples. Therefore, the difference between fresh and frozen sample accuracy was 5.71 and 7.69% to low and high QC,

Table 2
Data showing captopril stability under freeze and thaw conditions (UFTC)

Sample	Low QC		High QC	
	UFTC	Fresh	UFTC	Fresh
Mean ($n=5$)	28.86	30.50	1719.18	1520.93
Precision (% CV)	3.78	4.92	1.75	4.22
Accuracy	96.19	101.65	114.61	101.40
Difference (%)	−5.38		13.04	

Table 3
Data showing long-term storage stability (LTSS) of captopril in human plasma at low and high QC samples

Samples	Low QC		High QC	
	First day analysis	LTSS	First day analysis	ELD
Mean ($n=5$)	29.7826	29.8068	1500.343	1485.061
Precision (% CV)	5.13	8.08	9.53	8.98
Accuracy	99.28	99.36	100.02	99.00
Difference (%)	0.08		−1.02	

respectively, concluding that it was within allowed variability range.

3.5.3. Post-processing stability

After thaw and 12 h sitting in the autosampler, stability assessment of samples showed a reliable stability behavior under such conditions. After extraction procedure, drug recovery varied from 94.05% (% CV 2.32) to 101.65% (% CV 4.92) for low QC, and from 108.75% (% CV 2.69) to 101.04% (% CV 4.22) for high QC samples. Accuracy difference between fresh and frozen samples was −7.48 and 7.26% for low and high QC, respectively.

3.5.4. Under freeze/thaw conditions (UFTC)

Data representing captopril concentration at the end of the third thaw cycle are summarized in Table 2. It shows that both analyte and IS analysis are stable at such experimental conditions.

3.5.5. Long term storage stability (LTSS)

The performed tests are in agreement with Section 2.7.6.3. All analyzed samples were kept frozen over a period of 64 days, i.e., a larger period of time than volunteer samples. Results of tested samples were within acceptable criteria and no stability-related problems could be expected to occur for bioequivalence studies during daily routine. The results are presented in Table 3.

4. Application of the method

The analytical method developed here was applied to evaluate the bioequivalence of two tablets formulations of captopril in health volunteers: Captozen (test formulation from Vitapan 50 mg; lot no. 4450001) and Capoten (standard reference formulation from Capoten® 50 mg Bristol-Myers Squibb Brasil SA; lot no. 43066). The study was a single oral dose, two-way randomized crossover design with a 5-day washout period between the doses. Twenty-three healthy volunteers, as assessed by studying their clinical history, physical examination and laboratory tests, i.e., hematology, biochemistry serology and urine analysis were enrolled in the study. The study was conducted strictly in accordance with the current Good Clinical Practices (GCP). All subjects gave written informed consent and local ethics committee approved the protocol. During each period, the drugs were administrated with water (200 ml) and under fasting conditions. No other food was permitted during the ‘in-house” period and liquid consumption was allowed *ad libitum* after a standard lunch (with the exception of xanthine-containing drinks, including tea, coffee, and cola). The subjects were monitored throughout the study and the formulations were considered to be well tolerated. Blood samples were collected by indwelling catheter into EDTA containing tubes before and 0; 15; 30; 45; 60; 75; 90 min and also 2, 3, 4, 6, 8, 10, 12, and 24 h post-dosing. Samples were centrifuged at 2000 × *g* for 10 min at room

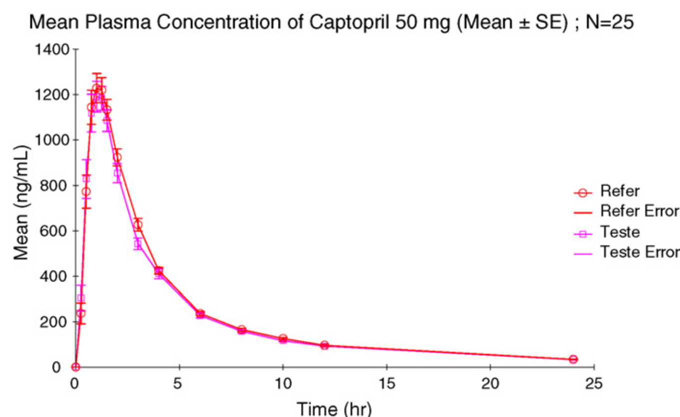

Fig. 3. Captopril mean plasma concentration after 50 mg tablet administration from 25 volunteers.

Table 4
Arithmetic mean (or median) pharmacokinetic parameters of captopril for test and reference preparations in 25 human volunteers after administration of a single 50 mg oral dose

	Capoten (reference)	Captozen (test)
C_{max} (ng/ml)		
Mean ± S.D.	1335.16 ± 302.61	1304.19 ± 323.95
90% CI	1231.61–1438.71	1193.35–1415.04
$AUC_{0–t}$ (ng h/ml)		
Mean ± S.D.	5463.40 ± 984.46	5208.29 ± 980.44
90% CI	5126.54–5800.26	4872.81–5543.78
$AUC_{0-\infty}$ (ng h/ml)		
Mean ± S.D.	5842.71 ± 1054.04	5591.74 ± 1046.75
90% CI	5482.05–6203.38	5233.57–5949.92
T_{max} (h)		
Median	1.00	1.00
90% CI	0.98–1.14	0.89–1.09
$T_{1/2}$ (h)		
Median	7.50	8.11
90% CI	7.36–7.90	7.71–8.25

T_{max}, time to maximum concentration; C_{max}, maximum concentration; $AUC_{0–t}$, area under the curve of plasma concentration until the last concentration observed; $AUC_{0-\infty}$, area under the curve between the first sample and infinitive; $T_{1/2}$, elimination half life.

Table 5
Geometric means of invididual pharmacokinetics parameters, LSM ratios (test/reference) (test/reference) and the respective 90% confidence intervals (CIs) and coeficient variation (intra subject) values

Parameter	Capoten (reference)	Captozen (test)	LSM ratio (T/R)	90% CI	CV_{intra} (%)
ln (C_{max})	1301.87	1265.06	97.17	90.08–104.82	15.72
ln (AUC_{last})	5377.50	5122.74	95.26	90.35–100.44	10.95
ln (AUC_{∞})	5750.63	5501.73	95.67	90.74–100.87	10.93

temperature and the plasma stored at 22 °C until analysed for captopril content. The mean plasma captopril concentration *vs.* time curves for both preparations is shown in Fig. 3. The maximum reached concentration time curve (C_{max}), area under the plasma concentration–time curve from 0 h to the last measurable ($AUC_{0–24h}$) and area under the plasma concentration–time curve from 0 h to infinity ($AUC_{0-\infty}$) were compared and pharmacokinetic parameters were computed using WinNonlin Professional Software—version 4.0.1 (Table 4). Statistical calculations were defined at the level of $P \leq 0.10$. Bioequivalence for Captozen Vitapan and Capoten® formulations was concluded as the 90.0% confidence interval for C_{max}, $AUC_{0–t}$ and $AUC_{0-\infty}$ felt within the range of 80.0–125.0% defined by both the Food and Drug Administration (FDA) and the National Sanitary Surveillance Agency (ANVISA). Data are summarized in Table 5.

5. Conclusion

In conclusion, our validated method was successfully applied to pharmacokinetic studies of captopril in plasma samples, and proved to be the most sensitive LC–MS–MS method for captopril determination ever published, with a short run time (3.0 min), specific as well as precise. It proved to be superior when compared to the previously reported LC–MS–MS method [13], in sensitivity (LLOQ 10.0 versus 25.0 ng/ml), accuracy and precision, especially as to inter-batch precision, which was five to ten times improved in addition to a less time consuming procedure for sample preparation. As a result, it could be feasible for preparing and analyzing 86 samples/6.92 h and around 350 unknown volunteer's samples up to the end of the bioequivalence study. Considering that solid phase extraction has been often used when a faster method with high accuracy and precision is designed [5], even better results were obtained coupling a triple quadrupole mass spectrometer and the MRM mode. Less interferences from the biologic matrix were observed resulting in a great method advantage. Furthermore, new analytical conditions (lower flow-rate, pH controlled mobile phase, column temperature adjustment) were developed in order to improve method performance and also to spend less organic solvents during sample preparation and analysis. Hence, our method is more suitable for supporting environmental responsiveness and, altogether, very appropriate for quantitative high-throughput analysis, such as pharmacokinetic studies at therapeutic drug concentrations in human plasma.

Acknowledgement

The authors wish to acknowledge the Instituto de Ciências Farmacêuticas, Goiânia, GO, Brazil, and the CNPq Foundation, both having provided the financial support for running the above described study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2006.11.007.

References

[1] J. Klein, P. Colin, E. Scherer, M. Levy, G. Koren, Ther. Drug Monit. 12 (1990) 105.
[2] K.L. Duchin, D.N. McKinstry, A.I. Cohen, B.H. Migdalof, Clin. Pharmacokinet. 14 (1988) 241.
[3] C. Arroyo, C. Lopez-Calull, L. Garcia-Capdevila, I. Gich, M. Barbanoj, J. Bonal, J. Chromatogr. B Biomed. Sci. Appl. 688 (1997) 339.
[4] M.E. Franklin, R.S. Addison, P.V. Baker, W.D. Hooper, J. Chromatogr. B Biomed. Sci. Appl. 705 (1998) 47.
[5] M. Bahmaei, A. Khosravi, C. Zamiri, A. Massoumi, M. Mahmoudian, J. Pharm. Biomed. Anal. 15 (1997) 1181.
[6] Y.B. Huang, Y.H. Tsai, J.S. Chang, J.C. Liu, M.J. Tsai, P.C. Wu, Int. J. Pharm. 241 (2002) 345.
[7] T. Huang, Z. He, B. Yang, L. Shao, X. Zheng, G. Duan, J. Pharm. Biomed. Anal. (2006).
[8] A. Engalytcheff, R. Debuyst, G.C. Vanhaelewyn, F.J. Callens, B. Tilquin, Radiat. Res. 162 (2004) 616.
[9] F. Tache, A. Farca, A. Medvedovici, V. David, J. Pharm. Biomed. Anal. 28 (2002) 549.
[10] A. Medvedovici, C. Mircioiu, V. David, D.S. Miron, Eur. J. Drug Metab. Pharmacokinet. 25 (2000) 91.

[11] R.J. Kok, J. Visser, F. Moolenaar, D. de Zeeuw, D.K. Meijer, J. Chromatogr. B Biomed. Sci. Appl. 693 (1997) 181.
[12] M.S. Bathala, S.H. Weinstein, F.S. Meeker Jr., S.M. Singhvi, B.H. Migdalof, J. Pharm. Sci. 73 (1984) 340.
[13] I.I. Salem, W.A. Saif, Y. Jmeian, J.I. Al Tamimi, J. Pharm. Biomed. Anal. 37 (2005) 1073.
[14] ANVISA, D.O.U., Diário Oficial da União, Poder Executivo, 02 de junho de 2003. Guia para validação de métodos analíticos e bioanalíticos, 2003.
[15] FDA, Center for Drug Evaluation and Research, U S Department of Health and Human Services, Guidance for Industry, Bionalytical Method Validation, 2001.