Analysis of Captopril in Human Plasma Using Gas Chromatography-Mass Spectrometry (GCMS) with Solid-Phase Extraction (SPE)

Z. Chik, A.M. Mustafa, Z. Mohamed and T.C. Lee

1Shimadzu-UMMC Centre for Xenobiotics Studies (SUCXeS), 2University of Malaya Bioequivalence and Testing Centre (UBAT), Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; 3Info Kinetics Sdn. Bhd. Room 126, Kompleks Eureka, USAINS Holding, 11800 Penang, Malaysia

Abstract: A sensitive and simple Gas Chromatographic-Mass Spectrometric method was developed and validated for the determination of captopril in human plasma. Thiosalicylic acid was used as an internal standard, and plasma extraction was performed by solid phase extraction. The limit of quantification was 0.5 ng/mL with signal to noise ratio greater than 5. The calibration curve was linear from 1 to 160 ng/mL with r² greater than 0.99. The coefficient of variation for within and between assay imprecision of the standards and for the limit of quantification were ≤ 10 % and ≤ 7 %, respectively. The percentage of inaccuracy for within- and between-assay including lower and upper limits of quantitation were ≤ 8 % and ≤ 6 %, respectively. The absolute recovery of captopril and thiosalicylic acid in plasma were greater than 98 % and 99 %, respectively. The high sensitivity and accuracy of this method allowed us to measure low concentrations of captopril in plasma for bioequivalence studies in healthy subjects.

Keywords: Captopril, Gas chromatography-mass spectrometry, Solid phase extraction (SPE).

INTRODUCTION

Captopril, an angiotensin-converting enzyme inhibitor has been widely used for the treatment of patients with mild to moderate hypertension, severe hypertension not responsive to conventional diuretic or to β-blockers and also for patients with congestive heart failure. Captopril has four enantiomeric forms, namely R-R, R-S, S-R and S-S. Only the S-S (1-[3-mercapto-2-(S)-methyl-1-oxopropyl]-S (L)-proline) form is biologically active, while R-captopril possesses non-ACE inhibiting activity [1]. S-S captopril contains a sulphydryl (SH) group that distinguishes it from any other ACE inhibitors. The existence of this group makes captopril very unstable in biological fluid due to oxidation of captopril and the rapid formation of disulfides [2]. Quantitation of captopril from biological fluids has therefore been difficult and several approaches have been used to assay captopril accurately. Ascorbic acid and adetate have been used to prevent captopril from being oxidised upon blood collection [3, 4] while N-ethylmaleimide (NEM) has also been frequently used as a stabilising agent [5-7].

Several methods have been used for the measurement of captopril and their metabolites. All the methods have been performed largely using chromatographic techniques such as High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GCMS). These two methods consistently show high sensitivity and accuracy. A number of HPLC procedures have been described for the determination of captopril in biological samples. Pereira et al. [4] used N-(1-Pyrene)-maleimide to derivatise captopril and internal standard to the fluorescent adducts for the detection by HPLC with fluorescence detector. Bald et al. [8] used pre-column derivatisation of the drug via its sulphydryl group with 1-benzyl – 2 – chloropyridinium bromide followed by solid phase extraction and reversed-phase HPLC separation with ultraviolet detection. The present method minimised the oxidation of captopril by derivatising blood samples at the time of collection. This was performed by drawing blood directly into tubes containing solution of 1 – benzyl – 2 – chloropyridinium bromide. The limit of quantitation achieved by the above two methods was 10 ng/mL.

GCMS was found to be more sensitive than HPLC to detect low concentrations of captopril. The first method utilising GCMS was developed by Funke et al. [2] and involved the formation of a captopril–NEM (N- ethylmaleimide) derivative. This derivative was methylated and measured as the methyl ester. Drummer et al. [9] used hexafluoroisopropanol and perfluorobutyric anhydride to derivatise captopril to hexafluoroisopropyl ester agent prior to analysis by GCMS. The limit of quantitation achieved with this method was 1 ng/mL.

A method by Ito et al. [6] also used NEM as the stabilising agent while S-benzylcaptopril was used as an internal standard. The derivatisation of captopril and internal standard were performed using pentafluorobenzylbromide (PFB-B) while the captopril and internal standard was converted into a bis-PFB derivative. The quantitation limit with this method was 10 ng/mL. Franklin et al. [10] developed a rapid and sensitive method for the quantitation of captopril by GCMS. The stabilising and derivatising agent was the same as that used by Ito et al [6] but the internal standard used was thiosalicylic acid (TSA). Extraction of captopril and TSA from plasma have been achieved by liquid-liquid extraction. Captopril and TSA were detected as bis-pentafluorobenzyl derivatives. The quantitation limit was 10 ng/mL. A recent
method by Rezende et al. [11] used solid-phase extraction (SPE) without derivatisation of captopril and internal standard, followed by quantitation by LC-MS/MS. The quantification limit achieved by this method was also 10 ng/mL. In this study, we have improved a GCMS analysis of captopril as mentioned above by using a simple SPE method. The amount of plasma used and incubation time for the derivatisation of captopril also has been reduced accordingly whilst the sensitivity of captopril’s detection was increased to 1 ng/mL.

EXPERIMENTAL

Chemicals

Captopril (USP Reference standard) was obtained from (Hubert Lendo, USA); Thiosalicylic acid (TSA), N-ethylmaleimide and pentafluorobenzylbromide were obtained from Across Organics, Geel, Belgium. Potassium phosphate (for the preparation of buffer) was obtained from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from SamplTech, Malaysia. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA. Sodium hydroxide was purchased from Sigma Chemical, St Louise USA.

Apparatus

A Shimadzu QP5000 gas chromatograph–mass spectrometer was used for the quantification of captopril and the internal standard TSA. The ISOLUTE® ENV+ from International Sorbent Technology, UK was used for the extraction of captopril and internal standard from plasma.

Chromatographic System

The analytical column was a capillary column BPX35MS (SGE, Australia) with 25 m (length), 0.25 mm (internal diameter) and 0.22 μm film thickness. Initial oven temperature was set at 160 °C, then ramped at 35 °C/min. to 330 °C and held for 5 minutes. Injector and interface temperature were set at 320 °C and 330 °C, respectively. Splitless injection mode was used with injection volume of 1 μL. Helium was used as the carrier gas at a rate of 0.9 mL/min. Detection was done using a quadrupole detector with electron impact source. Selected ion monitoring (SIM) for the pentafluorobenzylated derivatives of the analyte and the internal standard, TSA were as follows: Captopril, m/z 294, 396; TSA, m/z 333, 514. The retention times for captopril and TSA during method validation were 6.4 ± 0.1 min and 5.2 ± 0.1 min, respectively.

Assay Procedures

Preparation of Stock Solution

A stock solution of captopril and TSA were prepared in phosphate buffer (0.2M, pH 7.0). Since captopril is unstable in blood and plasma ex vivo due to rapid formation of disulfides, N-ethylmaleimide (NEM) was used to prevent the oxidation. A 0.5% (w/v) of powdered NEM was put into a phosphate buffer solution when preparing the standard. All the sub-stock solutions were stored frozen at approximately -20 °C. All the calibrators and QC samples were prepared by appropriate dilution of sub-stock dilution.

Extraction Procedure

A 200 μL volume of plasma sample containing captopril and 100 μL internal standard solution was put into the ENV+ column at a solid phase extraction manifold. The plasma was allowed to flow under vacuum condition at 1 inHg until finished. To clean out any endogenous compounds which are trapped at the packing material, a 1 mL volume of phosphate buffer solution (0.01 M, pH 2.5) was added to the column twice under the same vacuum condition until finished. Following that, the column was kept under vacuum at 10 inHg for two minutes. A 2 mL volume of acetone:ethyl acetate (3:1) was added to the column to elute out captopril and the internal standard, which are then collected in labeled glass tubes. The contents of the glass tubes were then evaporated to dryness under nitrogen stream at 40 °C. The residue was then derivatised by the addition of 0.1 mL pentafluorobenzylbromide (2% in acetone) in the presence of 0.1 mL of 0.5 M sodium hydroxide in methanol. The tube was capped and incubated at 60 °C for 30 minutes. Excess derivatising agent was evaporated to dryness under nitrogen stream at 40 °C. The residue was finally reconstituted in 0.2 mL chloroform for analysis by GCMS, as described previously.

VALIDATION PROCEDURES AND RESULTS

Development of MS Method

Derivatisation procedures carried out for captopril (MW:217) and TSA (MW: 154) in this study resulted in the formation of bis-pentafluorobenzyl derivative without the artificial formation of diastereoisomers with the M+• m/z 577 for captopril derivative and M+• of 514 for TSA derivative.

The M+ for captopril derivative at M+• m/z 577 as observed by Ito et al. (1987) was not observed in our system. Only m/z 396, 294 and 250 were observed with the signals at 396 and 294 resulting from the respective losses of one pentafluorobromide (PFB) moiety (181 Da) and of the side chain following cleavage of the C-N bond, respectively. For bis-pentafluorobenzyl-TSA, an M+• of 514 was observed with another signal at m/z 333 representing loss of one PFB moiety. Therefore, the fragment ions at m/z 294 and 396 for captopril were selected for the assay whilst for TSA, the fragment ion at m/z 333 and molecular ion of M+• of 514 were selected. The chemical structures for the derivative of captopril and TSA are shown in Fig. (1). The mass spectrum scans for the above two compounds are shown in Fig. (2).
Specificity

No significant interfering peaks were found at the retention times at which captopril and TSA appears. Fig. (3) shows the chromatogram obtained from blank plasma spiked with 1 ng/mL captopril and blank plasma spiked with 20 ng/mL TSA. The signal to noise ratios for both drugs were greater than 5.

![Chemical structure for pentafluorobenzyl derivative of captopril (M⁺: m/z 577) and TSA (M⁺: m/z 514).](image1)

![Total ion chromatograms obtained from extracted blank plasma spiked with 1 ng/mL captopril (A) and 20 ng/mL TSA (B). The fragment ions for captopril and TSA are 294, 396, and 333, 514, respectively under selected ion monitoring (SIM) mode.](image2)

Calibration Curve

The calibration curve covered the range from 1 to 160 ng/mL of captopril in plasma with six calibrators. Five batches of calibration curves were prepared for validation purposes. The calibration curve was plotted using the area ratio of captopril to TSA versus known concentrations of captopril. All the results were calculated using the $y = Ax + B$ linear regression. The regression coefficient for all the calibration curves obtained were greater than 0.99. Mean regression parameters obtained from five curves are summarized in Table 1.

Imprecision and Inaccuracy

Imprecision and inaccuracy were assessed by replicate analysis using 6 determinations at 5 concentration levels which covers the calibration curve range: the lower limit of quantitation (LLOQ): 1 ng/mL, within three times the LLOQ (low QC); 1.5 ng/mL, around 50% of the calibration curve range (medium QC); 75 ng/mL, at about 75% of the upper calibration curve range (high QC); 150 ng/mL, and upper limit of quantitation (ULOQ):160 ng/mL [12].
Within-Assay Reproducibility

The three quality control samples (low QC, mid QC and high QC), LLOQ and ULOQ were initially each extracted five times in one batch. Subsequently, they were extracted five times in two additional batches. On each occasion, a separate calibration curve was prepared. The CV for imprecision and the percentage inaccuracy for QC samples including LLOQ and ULOQ were all within the accepted range, which was 2.0 to 9.4 % and 0 to 9 %, respectively.

Between-Assay Repeatability

For each of the three assays mentioned above, the mean concentration from each assay was used to calculate the between-assay reproducibility. The CV and the percentage for between assay precision and accuracy including LLOQ and ULOQ were all within the accepted range, which was 2.5 to 6.6 % and 1 to 6 %, respectively.

Tables 2 and 3 summarize the CV% for within and between-batch imprecision and the percentage inaccuracy obtained during validation assay. All the results were contained within the accepted range as outlined in the Bioanalytical Method Validation, U.S. Food and Drug Administration, 2001 [13].

Recovery

Absolute recovery of captopril was tested using human plasma spiked with captopril at the same nominal concentrations as the quality control samples. Absolute recovery of TSA was tested at a nominal concentration of 20 ng/mL. Peak area measurements obtained from the extracted samples were compared to the peak area measurements obtained from direct solvent injection of the test compounds. Mean and standard deviation were calculated from at least three measurements at each level. The absolute recovery of captopril and TSA ranged from 98 to 104 % and for TSA was 99 %.

APPLICATION OF THE METHOD

The analytical method developed here was applied to the analysis of captopril in plasma obtained from bioequivalence study of various captopril products. All the studies were a

<table>
<thead>
<tr>
<th>No. of Batches</th>
<th>Nominal conc. (ng/mL)</th>
<th>Mean n = 6</th>
<th>SD</th>
<th>CV (%)</th>
<th>Mean Inaccuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>1.08</td>
<td>0.1</td>
<td>503</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.47</td>
<td>0.1</td>
<td>80.0</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>74.20</td>
<td>7.0</td>
<td>9.4</td>
<td>-1</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>143.00</td>
<td>7.8</td>
<td>5.4</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>160.40</td>
<td>3.8</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>1.01</td>
<td>0.0</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.49</td>
<td>0.0</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>74.80</td>
<td>4.6</td>
<td>6.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>151.60</td>
<td>4.4</td>
<td>2.9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>162.40</td>
<td>3.2</td>
<td>2.0</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>1.09</td>
<td>0.0</td>
<td>4.4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.50</td>
<td>0.0</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>78.20</td>
<td>2.4</td>
<td>3.1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>152.20</td>
<td>5.8</td>
<td>3.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>164.40</td>
<td>4.6</td>
<td>2.8</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1. Mean Regression Parameters for Five Calibration Curves During Validation

Table 2. Within Assay Imprecision and Inaccuracy in Plasma
Table 3. Between Assay Precision and Inaccuracy in Plasma

<table>
<thead>
<tr>
<th>Nominal Conc. (ng/mL)</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
<th>Mean Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>1.06</td>
<td>0.1</td>
<td>5.0</td>
<td>6</td>
</tr>
<tr>
<td>1.5</td>
<td>1.49</td>
<td>0.1</td>
<td>4.7</td>
<td>-1</td>
</tr>
<tr>
<td>75</td>
<td>75.73</td>
<td>5.0</td>
<td>6.6</td>
<td>1</td>
</tr>
<tr>
<td>150</td>
<td>148.93</td>
<td>7.2</td>
<td>4.8</td>
<td>-1</td>
</tr>
<tr>
<td>160</td>
<td>162.40</td>
<td>4.0</td>
<td>2.5</td>
<td>2</td>
</tr>
</tbody>
</table>

single oral dose, two way randomised crossover design with a one week washout period between the doses. In total, 66 healthy subjects received the generic products and Capoten® as reference formulation. Blood samples were collected into heparinised tubes at 0, 20, 40 min, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 hours post dose. Fig. (4) shows the plot for mean captopril concentration in plasma from 0 to 10 hours post dose obtained from three bioequivalence studies conducted on three generic products.

DISCUSSION AND CONCLUSION

The chromatographic and derivatisation procedure used in this study was similar to the method published by Franklin et al. (1998), however, we have simplified the extraction procedure by using solid phase extraction. The solid phase extraction procedure developed in this study was relatively rapid when compared to the published methods utilizing liquid-liquid extraction. Hence, this method is suitable and reliable for the analysis of a large number of samples such as those obtained from bioequivalence studies. In conclusion, a simple solid phase extraction and analysis method for captopril in plasma using GCMS has been developed and validated. This method has been utilized in the bioequivalence studies of various captopril pharmaceutical products (unpublished data).

REFERENCES