INTRODUCTION

Captopril, 1–[(2S)–3–mercapto–2–methyl propionyl]–L–proline, is a sulphahydril containing angiotensin converting enzyme (ACE) inhibitor. It is used in the management of hypertension, heart failure and diabetic nephropathy [1]. The drug is listed in United States Pharmacopoeia [2], which recommends a HPLC method for its assay in bulk and tablet formulations. In order to assure the quantity of captopril in dosage forms several methods have been reported which include gas chromatography [3, 4], high performance liquid chromatography [5, 6], capillary electrophoresis [7 – 9], flow injection analysis [10, 11], titrimetry [12] and atomic absorption spectrophotometry [13 – 15]. The content of captopril in pharmaceutical preparations and biological fluids has also been determined involving electrochemical methods such as potentiometry [16] and amperometry [17].

Several spectrophotometric methods have been reported in the literature which are based on the reaction of drug with Folin-Ciocalteau reagent [18], potassium iodate [19, 20], vanadate [21], bromate-bromide [22], chloramine-T [23], tetrazoline blue [24], palladium(II)chloride [25] and p–chloranilic acid [26]. In the literature no kinetic spectrophotometric method has been reported for the assay of captopril. Therefore, there is a need to develop a kinetic spectrophotometric method for the determination of captopril in commercial dosage forms. This chapter describes a simple and sensitive kinetic spectrophotometric method for the determination of captopril in drug formulations.

EXPERIMENTAL

Apparatus

Spectronic 20 D+ spectrophotometer (Milton Roy, USA) was used for absorbance measurements with matched glass cuvettes.
Materials and reagents

Captopril was kindly provided by Wockhardt India Ltd., and was used as received. Aceten tablets (25 mg each, Wockhardt India Ltd.), the only available commercial dosage forms, were purchased from local market. All chemicals used were of AR grade.

Standard solution of captopril (0.05%) was prepared in doubly distilled water and diluted as necessary. Potassium ferricyanide solution (0.1%) was also prepared in doubly distilled water. Ferric chloride solution (0.1%) was prepared by dissolving in distilled water, adding 70% HNO₃ and finally diluting to the mark with doubly distilled water (final concentration of HNO₃ 0.14%).

General procedure

Aliquots (0.2 – 2.2 mL) of 0.005% captopril test solution were pipetted into a series of 10 mL volumetric flasks. To each flask, 2.0 mL of 0.1% FeCl₃ solution was added followed by 1.0 mL of 0.1% potassium ferricyanide and then diluted with doubly distilled water. The contents of the flask were shaken well and immediately transferred to the spectrophotometric cell. The absorbance was recorded as a function of time at $\lambda_{max}$ 730 nm against the reagent blank prepared simultaneously. The initial rate of the reaction ($R_0$) at different concentrations was evaluated from the slope of the tangent to the absorbance–time curves. The calibration graph was constructed by plotting logarithm of the initial–rate (log $R_0$) against the logarithm of the molar concentration of the drug (log $c$).

Procedure for determination of captopril in pharmaceutical preparations

Ten tablets of Aceten were accurately weighed and powdered. A portion equivalent to 50 mg of captopril was extracted with methanol by shaking and filtered on Whatmann No. 42 filter paper. The filtrate and washings were evaporated to
dryness. The residue was dissolved in doubly distilled water and analysed by the recommended procedure.

**Determination of Stoichiometry**

The stoichiometry of the reaction was studied by the Bent and French method [27]. For this, three sets of experiments were performed. In the first set, the concentration of captopril was varied while keeping excess concentrations of FeCl$_3$ ($2.07 \times 10^{-3}$ M) and potassium ferricyanide ($3.04 \times 10^{-3}$ M). In the second set, excess concentrations of captopril ($2.30 \times 10^{-3}$ M) and potassium ferricyanide ($3.04 \times 10^{-3}$ M) were employed and varying concentration of FeCl$_3$. In the last set, concentration of potassium ferricyanide was varied and keeping excess concentrations of captopril ($2.30 \times 10^{-3}$ M) and FeCl$_3$ ($2.07 \times 10^{-3}$ M).

**Validation**

For evaluation of linearity, the content of captopril was determined at eight concentration levels: 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 11.0 $\mu$g/mL. Each concentration was analyzed for five times.

The detection limit [28] was evaluated as:

$$\text{LOD} = 3.3 \frac{S_0}{b}$$

where $b$ is the slope and $S_0$ is the standard deviation of the regression line.

Three concentrations of reference captopril solution within the linearity range were selected: 2.0, 4.0, and 7.0 $\mu$g/mL. Six solutions of each concentration were prepared and analysed within one day. This assay was repeated for five consecutive days. Recovery experiments were performed using standard addition method. The total amount was determined by the proposed procedure.
**Ruggedness**

The ruggedness of the proposed method was determined by assaying the captopril content under varying conditions of experimental parameters using different batches of the reagent. The operational parameters challenged were: 2.0 mL of 0.1% FeCl₃ solution (± 0.2 mL); 1.0 mL of 0.1% potassium ferricyanide solution (±0.1 mL). Under this condition a sample containing 5.0 μg/mL⁻¹ captopril was analysed.

**Interval hypothesis**

For practical purposes, the acceptable bias can be calculated statistically [29]. For example, a test method, *i.e.* initial rate method (method 2) is considered acceptable if its true mean value is within ±2.0% of that of the reference method, *i.e.* Sastry’s spectrophotometric method (18) (method 1):

\[-0.02 μ_1 < (μ_2 - μ_1) < 0.02 μ_1\]

this can be written as:

\[0.98 < μ_2 / μ_1 < 1.02\]

which can be generalised to:

\[θ_L < μ_2 / μ_1 < θ_u\]

where  θₐ and θₐ represent the lower and the upper acceptance limits, respectively, when μ₂ is expressed as a proportion of the reference mean μ₁. Statistically, θₐ and θₐ are calculated from the relation:

\[θ^2 \left( \frac{\bar{x}_2 - S^2_p\,t_{tab}}{n_1} \right) - 2θ\bar{x}_2\bar{x}_1 + \left( \frac{\bar{x}_2^2 - S^2_p\,t_{tab}^2}{n_2} \right) = 0\]

where \(\bar{x}_1\) and \(\bar{x}_2\) are estimates of μ₁ and μ₂ based on n₁ and n₂ measurements, respectively. S_p² is the variance of pooled measurements, t_{tab} is the tabulated one-sided *t*-value, with n₁+n₂-2 degrees of freedom at the specified level of significance.
RESULTS AND DISCUSSION

Captopril is reducing agent owing to the presence of thiol group (–SH) in its structure. In aqueous solution, captopril reduces Fe(III) to Fe(II), but it is oxidised to disulphide. Fe(II) immediately reacts with ferricyanide resulting in the formation of blue product which absorbs maximally at 730 nm. The absorbance of the coloured solution increases with time and hence, a kinetically based method was elaborated to assay the captopril in pharmaceutical formulations. However, the following specific advantages of the kinetic method can be expected: (i) some experimental steps such as filtration and extraction are eliminated before the absorbance measurement; (ii) selectivity is improved owing to the measurement of the evolution of absorbance with time instead of the measuring concrete absorbance value; (iii) usually no interference is observed due to coloured background of the samples.

Reaction conditions optimisation

To study the effect of volume of 0.1% FeCl₃ solution, aliquots equivalent to 8.0 μg/mL of drug were pipetted into a series of 10.0 mL volumetric flasks followed by varying volume of 0.1% FeCl₃ solution (0.2-2.0 mL) and 1.0 mL of 0.1% potassium ferricyanide solution. The absorbance was measured at a fixed time of 24 minutes because the equilibrium was attained at this time point. The absorbance increases with increasing volume of FeCl₃ solution and became constant at 1.2 mL (Fig.2.1). The dependence of the volume of 0.1% potassium ferricyanide on the formation of blue product was examined by taking a fixed amount of captopril (8.0 μg/mL), 2.0 mL of 0.1% FeCl₃ solution and varying volume of 0.1% potassium ferricyanide (0.2-1.6 mL). It was observed that maximum absorbance was obtained with 0.9 mL (Fig.2.2). Thus, 2.0 mL of 0.1% FeCl₃ and 1.0 mL of 0.1% potassium
ferricyanide were used as optimum volumes for the maximum concentration of captopril in calibration graph.

**Stoichiometry**

The stoichiometry of the reaction product was established from the Bent and French plots. The plot of log absorbance vs log [Fe(III)], [K₃Fe(CN)₆] and [Captopril] gave values of the slopes of 1.06, 1.0, and 1.0, respectively, (Fig.2.3 A,B&C). Hence it is concluded that the reaction proceeds in the molar ratio of 1:1:1. The reaction sequence is shown in Scheme 2.1.

**Initial-rate method**

The absorbance vs time curves are shown in Fig. 2.4. The order with respect to the Fe(III) was determined by studying the reaction at different initial concentration of Fe(III) with fixed captopril concentration. The plot of initial rate (ΔA / Δt) against initial absorbance was linear passing through the origin suggested that the order of the reaction with respect to Fe(III) at the start is one. The order with respect to captopril was determined from the measurement of initial rates at several concentrations of captopril, keeping a constant concentration of Fe(III), which was also found to be one. The simple rate expression can be written as:

\[
\text{rate} = k [\text{Fe(III)}]^m [c]^n
\]

Under the optimised experimental parameters, pseudo-first order condition was worked out by taking excess concentrations of FeCl₃ and potassium ferricyanide with respect to the initial concentration of captopril. Therefore the above equation is reduced to:

\[
\text{rate} = K_\psi [c]^n
\]

where \( K_\psi \) is the pseudo-first order rate constant, \( c \) is the molar concentration of captopril. The above equation is written in logarithmic form as:
\[ \log \text{rate} = \log K + n \log c = 4.248 + 1.173 \log c \]

The calibration graph (Fig. 2.5) was obtained by plotting the log initial rate \( \psi \) versus log captopril concentration which showed a linear relationship over the concentration range \( 4.60 \times 10^{-6} \text{-} 5.06 \times 10^{-5} \text{ M (1-11\mu g mL}^{-1}) \). The value of \( n \) also confirmed that the order of reaction with respect to captopril concentration at the initial stage is one. The regression equation, confidence interval of slope and intercept of line of regression at 95% confidence level, correlation coefficient, variance of calibration line and detection limit were calculated and summarised in Table 2.1.

The small value of variance of calibration line \( (2.38 \times 10^{-4}) \) also confirmed the negligible scattering of the calibration data points around the line of regression. The low value of detection limit \( (0.04 \mu g mL^{-1}) \) pointed towards good sensitivity of the proposed procedure.

The intraday and interday precisions were found to be in the range 0.7 – 1.6% and 0.6 – 1.0%, respectively, (Table 2.2). The percent error was found to vary from 0.1 – 1.0%. The results of recovery studies are summarised in Table 2.3. The mean percentage recovery ranged from 99.8 – 101.4% with relative standard deviation values <2.0%. This clearly suggested that there is no interference from common excipients present in dosage forms. For evaluation of the method ruggedness, two parameters were changed; volume of \( \text{FeCl}_3 \) and potassium ferricyanide solutions. The mean assayed concentration was \( 4.98 \mu g mL^{-1} \) with 1.02% RSD as a measure of ruggedness.

The proposed procedure has been successfully applied to the determination of captopril in pharmaceutical preparations. The results are summarised in Table 2.4. The results obtained by the proposed procedure were compared to those of Sastry’s spectrophotometric method [18] using point and interval hypotheses. The results (Table 2.4) show that the Student’s \( t- \) and \( F- \) values at 95% confidence level are less than the theoretical values which confirmed that there is no significant
difference between the performances of the methods compared. The Canadian Health Protection Branch [29] has recommended that a bias, based on recovery experiments, of ±2.0% is acceptable for pharmaceutical analysis. The data obtained for the analysis of commercial dosage forms show that the true bias is less than ±2.0%. Thus, the interval hypothesis test has also indicated the acceptable performance of the proposed method.
References


Table 2.1: Regression data for captopril using initial-rate method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial-rate method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear dynamic range (µg mL⁻¹)</td>
<td>1-11</td>
</tr>
<tr>
<td>Regression equation</td>
<td>log ( R_0 = 4.248 + 1.173 \log c )</td>
</tr>
<tr>
<td>Coefficient of correlation (r)</td>
<td>0.9992</td>
</tr>
<tr>
<td>( \pm t s_a )</td>
<td>2.768 \times 10^{-2}</td>
</tr>
<tr>
<td>( \pm t s_b )</td>
<td>2.705 \times 10^{-2}</td>
</tr>
<tr>
<td>Detection limit (µg mL⁻¹)</td>
<td>0.04</td>
</tr>
<tr>
<td>Variance of regression line (( S_0^2 ))</td>
<td>2.38 \times 10^{-4}</td>
</tr>
</tbody>
</table>

\( a t_s_a \) – confidence interval of the intercept at 95% confidence level.
\( b t_s_b \) – confidence interval of the slope at 95% confidence level.
Table 2.2: Intraday and Interday assays

<table>
<thead>
<tr>
<th>Concentration (µg/mL⁻¹)</th>
<th>Taken</th>
<th>Found</th>
<th>Error (%)</th>
<th>RSD (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SAE&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>2.01</td>
<td>0.50</td>
<td>1.1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>4.01</td>
<td>0.25</td>
<td>0.7</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>6.93</td>
<td>1.00</td>
<td>1.6</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Interday assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>2.01</td>
<td>0.50</td>
<td>1.0</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>4.01</td>
<td>0.25</td>
<td>0.6</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>6.98</td>
<td>0.28</td>
<td>0.7</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Six independent analyses of reference captopril solution.  
<sup>b</sup> Standard analytical error.
Table 2.3: Determination of captopril in pharmaceutical formulations using the standard addition technique

<table>
<thead>
<tr>
<th>Concentration ((μg/mL⁻¹))</th>
<th>Formulation</th>
<th>Taken</th>
<th>Added</th>
<th>Found</th>
<th>Recovery (%)</th>
<th>RSD (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SAE&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aceten</td>
<td>2.0</td>
<td>2.0</td>
<td>4.05</td>
<td>101.4</td>
<td>1.8</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>6.0</td>
<td>7.98</td>
<td>99.8</td>
<td>1.1</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.0</td>
<td>3.0</td>
<td>10.06</td>
<td>100.6</td>
<td>0.6</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> Six independent analyses.
<sup>b</sup> Standard analytical error.
Table 2.4: Point and interval hypotheses: comparison of the proposed method with the reference method.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Proposed Method</th>
<th>Reference Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Aceten</td>
<td>99.3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

$^a$Average of six independent analyses.
$^b$Theoretical $t$- and $F$-value at 95% confidence level are 1.812 and 5.05, respectively.
$^c$In pharmaceutical analysis, a bias on recovery experiments, of $\pm 2\%$ ($\theta_L = 0.98$ and $\theta_U = 1.02$) is acceptable.
**Step I**

\[
2 \text{HS}-\text{N}-(\text{CH}_3)\text{COOH} + 2 \text{Fe}^{3+} \rightarrow \text{Captopril} \rightarrow \text{COOH}-\text{N}-(\text{CH}_3)\text{COOH} + 2 \text{Fe}^{2+} + 2 \text{H}^+ + \text{Fe}^{2+}[\text{Fe}^{2+}(\text{CN})_6]_3^- + \text{K}^+ + \text{H}_2\text{O} \]

**Step II**

\[
\text{Fe}^{2+} + [\text{Fe}(\text{CN})_6]^{3-} + \text{K}^+ + \text{H}_2\text{O} \rightarrow \text{Fe}^{3+}\text{K}[\text{Fe}^{2+}(\text{CN})_6] \times \text{H}_2\text{O} \]

Blue salt

**Scheme 2.1**
Fig. 2.5: Calibration curve for the determination of captopril by initial rate method
Fig. 2.1: Effect of the volume of 0.1% FeCl$_3$ solution on the intensity of colour produced during the reaction.
Fig 2.2   Effect of the volume of 0.1% potassium ferricyanide solution on the intensity of colour produced during the reaction.
Fig 2.4: Absorbance vs. time graph showing the dependence of the reaction on captopril concentration (●) $9.204 \times 10^{-6}$M (○) $2.761 \times 10^{-5}$M (▼) $4.602 \times 10^{-5}$M
Fig 2.3A: Bent and French plot for the molar ratio: log A vs log [captopril] with FeCl$_3$=2.07×10$^{-3}$ M and [K$_3$Fe(CN)$_6$]=3.04×10$^{-3}$ M.
Fig 2.3B: Bent and French plot for the molar ratio: log A vs log [FeCl₃] with [captopril]=2.03×10⁻³ M and [K₃Fe(CN)₆]=3.04×10⁻³ M.
Fig 2.3C: Bent and French plot for the molar ratio: log A vs log [K₃Fe(CN)₆] with [captopril]=2.03x10⁻³ M and FeCl₃=2.07x10⁻³ M