INTRODUCTION

Perindopril erbumine is a non official drug and is chemically, tert-butylamine salt of [[2S-1-(R, R) 2α, 3αβ, 7αβ]-1-[2-(1-ethoxy carbonyl butyl] amino]-oxopropyl, octa-1H indole-2-carboxylic acid. It is an angiotensin converting enzyme inhibitor (ACE) [1] used for management of hypertension and congestive heart failure. Few analytical procedures have been described for its determination in pharmaceutical formulations which include gas chromatography [2], gas chromatography mass spectrometry [3], radioimmunoassay [4] and derivatization-gas chromatography [5]. Spectrophotometry still belongs to the most frequently used analytical technique in pharmaceutical analysis, which provides practical and significant economic advantages over other methods. Abdellatef et. al. have developed two extractive spectrophotometric methods for the determination of perindopril, depending upon chloroform extractable complex with eosin and copper(II) [6]. Another method involves the chloroform extractable ion association complex of perindopril with bromothymol blue at pH 5 [7]. The content of perindopril was determined based on its interaction with FeCl₃ in the presence of potassium thiocyanate. UV derivative spectrophotometry using zero-crossing method [8] was utilised for the quantification of perindopril, where sufficient spectra resolutions of drug and its impurity were obtained.

This chapter describes a kinetic spectrophotometric method for the determination of perindopril in commercial tablets. The method is based on the reaction of perindopril with 1-chloro-2,4-dinitrobenzene (CDNB) in dimethylsulphoxide (DMSO) at 40±1°C. The activation parameters such as E_a, ΔH^*, ΔS^* and ΔG^* were calculated. The proposed kinetic method is validated statistically.
EXPERIMENTAL

Apparatus

All spectrophotometric measurements were made on spectronic 20 D+ spectrophotometer (Milton Roy, USA). A water bath shaker (NSW 133, New Delhi, India) was used to control the heating temperature.

Materials and reagents

Perindopril erbumine was kindly supplied as a gift sample by Glenmark Pharmaceuticals Ltd., Mumbai, India and used without further purification. DMSO and CDNB were purchased from Merck Chemicals, India. Commercial tablets of perindopril erbumine such as Coversyl (Serdia Pharmaceuticals India Ltd.) and Perigard (Glenmark Pharmaceuticals Ltd., India) were purchased from local drug store.

Standard Solutions

- 1-chloro-2,4-dinitrobenzene solution, 0.2%, was prepared in DMSO. It was protected from light and stored in refrigerator.
- Standard perindopril solution, 0.1%, was prepared in DMSO.

Proposed Procedure

Aliquots of standard perindopril solution (0.1%) corresponding to 100 – 700 μg were pipetted into a series of 5 mL volumetric flasks. To each flask, 1 mL of 0.2% CDNB was added and diluted to volume with DMSO. The contents of the mixture were heated on a water bath at 40±1°C. The increase in absorbance at 420 nm was recorded against the reagent blank as a function of heating time. The initial rate (R₀) of the reaction at different concentrations was evaluated from the slope of the initial
tangent to the absorbance-time curves. The calibration graph was constructed by plotting initial rate of reaction versus final concentration of perindopril.

**Method validation**

**Precision**

Precision of the method was determined by analysing the commercial tablets. An amount of the tablet powder equivalent to 100% of the label claim of the perindopril was accurately weighed and assayed. The intraday repeatability was determined by measuring the perindopril content of the sample solution six times within one day at the analytical concentration of 40, 100, and 140 μgmL$^{-1}$. The interday precision was assessed by assaying the sample solution at three concentration levels (40, 80 and 140 μgmL$^{-1}$) on five consecutive days.

**Robustness of the method**

By introducing small changes in the concentration of CDNB and temperature, the effects on the results were examined. The volume of 0.2% CDNB and temperature were varied as optimum value ±0.2 mL and 40±1°C, respectively. Robustness of the method was done at two concentration levels (20 and 100 μgmL$^{-1}$).

**Limits of detection and quantitation**

The limits of detection (LOD) and quantitation (LOQ) were calculated using the following equations [9];

\[
\text{LOD} = 3.3 \times S_0/b; \quad \text{LOQ} = 10.0 \times S_0/b
\]

where $S_0$ and b are the standard deviation and slope of calibration line, respectively.
Selectivity

The selectivity of the method was ascertained by analysing standard perindopril in presence of excipients (lactose, magnesium stearate, cellulose and talc), impurities and its active metabolite perindoprilat.

Accuracy

The samples were spiked with extra 33.33, 100 and 200% of the standard perindopril and the mixtures were analysed by the proposed method. The experiment was repeated 5 times. This was done to check for the recovery of the drug at different levels in the formulations.

Analysis of marketed formulations

To determine the content of perindopril in tablets (label claim: 4 mg/tablet) the contents of 10 tablets were weighed and finely powdered. A portion of the powder equivalent to 40 mg perindopril was stirred with 15 mL DMSO and let stand for 10 minutes. The residue was filtered on Whatmann No. 42 filter paper and washed with DMSO. The filtrate and washings were diluted to 25 mL with DMSO. A suitable volume of this solution was further diluted to give a final concentration of 1 mgmL\(^{-1}\). An aliquot of the diluted solution was analysed for perindopril content following the recommended procedure.

Reference Method [6]

Aliquots of 0.1-0.6 mL standard solution of perindopril (1 mgmL\(^{-1}\)) were transferred into a series of 50 mL separating funnels. The volume of each solution was adjusted to 10 mL with distilled water, and then 3.0 mL of 0.2% copper(II) sulphate solution was added followed by 1.0 mL of 0.1% eosin solution. The complex was extracted with 3 x 3 mL portions of chloroform. The solution was shaken for 1 min. each time and the chloroform layer was passed through a layer of anhydrous sodium sulphate.
into a 10 mL volumetric flask. The volume of chloroform layers was made up to 10 mL and the absorbance was measured at 535 nm against the blank in which drug is omitted. The amount of drug in given sample was computed either from calibration graph or corresponding regression equation.

**RESULTS AND DISCUSSION**

Perindopril was found to react with CDNB in DMSO medium resulting in the formation of coloured product, which absorbed maximally at 420 nm. At room temperature, the reaction was slow and more than 1 hour was required to attain the maximum absorbance. In order to increase the rate of reaction and decrease the time for attaining the equilibrium, the reaction was carried out at 40±1°C. At this temperature the intensity of the coloured product increases with time and so a kinetically based method was elaborated for determination of perindopril.

*Optimization of variables*

The reaction between perindopril and CDNB was studied at 30±1°C, 40±1°C and 50±1°C in DMSO medium. It was observed that the linear dynamic range for determination increases on increasing temperature and become constant at 40±1°C; further increase in temperature caused no change in the linear dynamic range. Therefore, a temperature of 40±1°C was chosen as an optimum temperature.

The effect of the volume of 0.2% CDNB on the rate of reaction was studied in the range 0.1–1.3 mL. The rate of reaction or absorbance increases with increase in volume of CDNB and becomes constant at 0.8 mL; above this volume no increase in the rate of reaction was observed. Thus a volume of 1.0 mL was selected for the determination process.
**Stoichiometry and reaction mechanism**

The molar combining ratio between perindopril and CDNB was evaluated using limiting logarithmic method [10] by performing two sets of experiments Fig. 5.1. In the first, the concentration of the drug was varied and keeping the CDNB concentration constant. In the second, the drug concentration was kept constant and varying the CDNB concentration. The slopes of the plots of logarithm of absorbance *versus* logarithm of respective varied molar concentration were calculated and found to be 1:1 between perindopril and CDNB.

Aromatic nitro compounds react with bases resulting in the formation of brightly coloured solutions. There are various interactions depending upon the degree to which the base participates through its unshared electron pair with the nitro compounds [11]. In the present study, perindopril behaves as a base owing to the presence of –NH group in its structure. Addition of CDNB to perindopril in DMSO yielded the 1-substituted Meisenheimer complex which absorbs maximally at 420 nm. On the basis of our experimental findings and literature background [12], the reaction mechanism is proposed and given in Scheme 5.1.

**Analytical data**

The rate of reaction was found to be dependent on perindopril concentration. The initial rate of reaction was obtained from the slope of the initial tangent to the absorbance-time curves (Fig.5.2). As can be seen from Fig.5.2 that the initial rate increases with increasing perindopril concentration. The kinetic equation for this reaction is written as

$$R_0 = \frac{dx}{dt} = k[\text{Perindopril}]^n[\text{CDNB}]^m$$  \hspace{1cm} (1)

At [CDNB] ≥ 3.2×10^{-2} M, the order of reaction became zero with respect to CDNB concentration. The equation (1) is reduced to
R₀ = k [Perindopril]ⁿ  

(2)

The plot of logarithm of initial rate of reaction versus logarithm of molar concentration of perindopril indicated the first order reaction with respect to perindopril concentration. The equation (2) is transformed into pseudo first order reaction as

R₀ = KΨ [Perindopril]

where KΨ is the pseudo first order rate constant.

The calibration curve obtained by plotting initial rate of reaction versus final concentration of perindopril showed a linear relationship over the range 20–140 μgmL⁻¹ (Fig.5.3). The regression analysis using the method of least squares was performed to estimate the slope, intercept and correlation coefficient under optimised experimental conditions. The value of LOD, LOQ and variance of the calibration line were also calculated and summarised in Table 5.1.

In order to evaluate the activation parameters, the reaction was studied at 30°C, 40°C and 50°C keeping [perindopril] = 2.71×10⁻⁴ M and [CDNB] = 4.94×10⁻⁴ M. The rate data obtained from pseudo first order kinetics were subjected to fitting in Arrhenius Equation.

K = A e⁻ Ea/RT


The plot of ln k versus 1/T was linear Fig.5.4. and Eₐ calculated from the slope (−Eₐ/R) was 27.31 KJmol⁻¹. The values of ΔH* and ΔS* were evaluated using Eyring equation

k = (kₜ T/h) e^{ΔS/R} e^{-ΔH/RT}

A plot of ln k/T versus 1/T was linear Fig.5.5. The values of ΔH* and ΔS* obtained from slope (−ΔH*/R) and intercept (ln Kᵢ/h + ΔS*/R) were 24.69 KJmol⁻¹ and -134.84 JK⁻¹mol⁻¹, respectively. The value of ΔG* was found to be 61.50 KJmol⁻¹.
The intra and interday precisions were evaluated at three concentration levels and the results are shown in Table 5.2. It is evident from the Table 5.2 that %RSD was found to be less than 1.0% which is considered to be very satisfactory.

**Robustness**

The robustness of the proposed method was evaluated by challenging the operational parameters. The operative parameters tested are given as

- volume of 0.2% CDNB (±0.2 mL)
- Temperature (±1°C)

Under these conditions, sample solution containing 100 μg/mL of perindopril was analysed by the proposed method. The low values of RSD (<1.1%) indicated robustness of the method.

**Selectivity**

The selectivity of the method was assessed by analysing standard perindopril in presence of excipients (lactose, magnesium stearate, cellulose and talc), impurities and its active metabolite perindoprilat. It was observed that impurities such as (2S)–2-[(3S, 5aS, 9aS, 10aS)–3–methyl-1, 4-dioxodecahydro-2(1H)-yl] pentanoic acid, ethyl (2S)–2-[(3S, 5aS, 9aS, 10aS)–3–methyl-1, 4-dioxodecahydroazirinol[1, 2-a]-indole-2(1H)-yl]pentanoate, (2S)–2-[(2S, 5aS, 9aS, 10aR)–3–methyl-1, 4-dioxodecahydroazirinol[1, 2-a]-indole-2(1H)-yl]pentanoic acid, (2S, 3aS, 7aS)-1-[(2S)-2-[(5RS)-3-cyclohexyl-2, 4-dioxo-5-propylimidazolidine-1-yl] propanoyl]octahydro-1H-indol-2-carboxylic acid, and (2S,3aS,7aS)-1-[(2S)-2-[(5RS)-3-cyclohexyl-2-(cyclohexylimino)-4-oxo-5-propylimidazolidine-1-yl]propanoyl] octahydro-1H-indol-2-carboxylic acid do not interfere with the determination. However other impurities such as (2S,3aS,7aS)-1-[(2S)-2-[(1S)-1-[(1-carboxybutyl)amino]propanoyl]octahydro-1H-indol-2-carboxylic acid, (2S,3aS,7aS)-1-[(2S)-2-[(1S)-1-[(1-methylethoxy) carbonyl]...
butyl][amino]propanoyl]octahydro-1H-indol-2-carboxylic acid, and (2S,3aS,7aS)-1-[(2S)-2-][(1R)-1-[(1-ethoxycarbonyl) butyl][amino] propanoyl] octahydro-1H-indol-2-carboxylic acid interfere with assay procedure. The perindoprilat, the principal metabolite of perindopril, also interferes with the determination. However, the perindopril can be determined after separation from perindoprilat on Dowex AG1×2 column using 0.2 M formic acid as eluent.

**Accuracy**

The proposed method when used for estimation of perindopril from tablets after spiking with 33.33, 100 and 200% of additional drug afforded recovery of 99.82 – 99.93% as listed in Table 5.3.

The developed method was successfully applied to the assay of perindopril in tablets; the results are given in Table 5.4. The same batch tablets were also analysed by Abdellatef’s spectrophotometric method [6]. This reference method is chosen due to its simplicity and sensitivity with good precision and accuracy. The results of the proposed method were compared with those of reference method using point and interval hypotheses. It is apparent that the calculated \( t \)– and \( F \)– values are less than the theoretical ones at 95% confidence level which showed no significant difference between methods compared with regard to accuracy and precision. The interval hypothesis test has also confirmed that the true bias of all samples is \(<\pm2.0\%\) at 95% confidence level. For pharmaceutical analysis, a bias of \(\pm2.0\%\) is acceptable and thus the limit of acceptance interval is within \(\theta_L = 0.98\) and \(\theta_U = 1.02\).
References


Table 5.1: Optical and regression characteristics of initial rate method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear dynamic range (μg/mL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>20 – 140</td>
</tr>
<tr>
<td>Regression equation</td>
<td>( R_0 = 1.90 \times 10^{-6} + 0.7797 ) [Perindopril]</td>
</tr>
<tr>
<td>( S_a )</td>
<td>4.41x10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>( \pm tS_a )</td>
<td>8.57x10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>( S_b )</td>
<td>1.54x10&lt;sup&gt;-2&lt;/sup&gt;</td>
</tr>
<tr>
<td>( \pm tS_b )</td>
<td>2.98x10&lt;sup&gt;-2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9990</td>
</tr>
<tr>
<td>LOD (μg/mL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>6.33</td>
</tr>
<tr>
<td>LOQ (μg/mL&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>19.20</td>
</tr>
<tr>
<td>Variance ( (S_o^2) )</td>
<td>1.65x10&lt;sup&gt;-11&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

\( \pm tS_a = \) Confidence limit for intercept.
\( \pm tS_b = \) Confidence limit for slope.
Table 5.2: Evaluation of accuracy and precision of the proposed method.

<table>
<thead>
<tr>
<th>Amount (µg/mL)</th>
<th>Intraday Assay</th>
<th>Recovery(^a) ± RSD</th>
<th>SAE(^b)</th>
<th>CL(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken</td>
<td>Found ± SD</td>
<td>(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>39.60 ± 0.34</td>
<td>98.99 ± 0.86</td>
<td>0.139</td>
<td>0.280</td>
</tr>
<tr>
<td>100</td>
<td>100.91 ± 0.98</td>
<td>100.91 ± 0.97</td>
<td>0.400</td>
<td>0.808</td>
</tr>
<tr>
<td>140</td>
<td>139.97 ± 0.96</td>
<td>99.98 ± 0.69</td>
<td>0.393</td>
<td>0.792</td>
</tr>
</tbody>
</table>

Interday Assay

<table>
<thead>
<tr>
<th>Taken</th>
<th>Found ± SD</th>
<th>Recovery(^a) ± RSD</th>
<th>SAE(^b)</th>
<th>CL(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>39.74 ± 0.34</td>
<td>99.36 ± 0.85</td>
<td>0.408</td>
<td>0.822</td>
</tr>
<tr>
<td>80</td>
<td>80.12 ± 0.74</td>
<td>100.14 ± 0.92</td>
<td>0.301</td>
<td>0.606</td>
</tr>
<tr>
<td>140</td>
<td>140.01 ± 0.92</td>
<td>100.01 ± 0.66</td>
<td>0.374</td>
<td>0.754</td>
</tr>
</tbody>
</table>

\(^a\) Average of six independent analysis.
\(^b\) Standard analytical error.
\(^c\) Confidence limit at 95% confidence level.
Table 5.3: Accuracy and Recovery

<table>
<thead>
<tr>
<th>Excess of Drug added to the analyte (%)</th>
<th>Recovery&lt;sup&gt;a&lt;/sup&gt; ± RSD (%)</th>
<th>SAE&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CL&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>99.93 ± 0.86</td>
<td>0.422</td>
<td>0.851</td>
</tr>
<tr>
<td>33.33</td>
<td>99.09 ± 1.01</td>
<td>0.163</td>
<td>0.329</td>
</tr>
<tr>
<td>100</td>
<td>99.84 ± 1.01</td>
<td>0.330</td>
<td>0.665</td>
</tr>
<tr>
<td>200</td>
<td>99.82 ± 0.72</td>
<td>0.352</td>
<td>0.710</td>
</tr>
</tbody>
</table>

<sup>a</sup>Average of six independent analysis.
<sup>b</sup>Standard analytical error.
<sup>c</sup>Confidence limit at 95% confidence level.
Table 5.4: Comparison of proposed method with reference method.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labelled amount (mg)</th>
<th>Proposed method</th>
<th>Abdellatef's method</th>
<th>t-value</th>
<th>F-value</th>
<th>$\theta_L$</th>
<th>$\theta_U$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery$^a$ ± RSD (%)</td>
<td>Recovery$^a$ ± RSD (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coversyl</td>
<td>4</td>
<td>99.89 ± 1.07</td>
<td>99.64 ± 0.51</td>
<td>0.514</td>
<td>4.385</td>
<td>0.994</td>
<td>1.011</td>
</tr>
<tr>
<td>Perigard</td>
<td>4</td>
<td>100.15 ± 0.92</td>
<td>99.69 ± 0.57</td>
<td>1.035</td>
<td>2.665</td>
<td>0.997</td>
<td>1.012</td>
</tr>
</tbody>
</table>

$^a$Average of six independent analysis.

$^b$Theoretical $t$-value anf $F$-value at 95% confidence level are 1.812 and 5.05, respectively.

$^c$In pharmaceutical analysis, a bias, based on recovery experiments, of ±2% ($\theta_L=0.98$ and $\theta_U=1.02$) is acceptable.
Scheme 5.1

Meisenheimer complex

Scheme 5.1
Fig 5.1: Limiting logarithmic plot for molar combining ratio between perindopril and CDNB: (o) log A vs log [Drug]; (●) log A vs log [CDNB].
Fig 5.2: Absorbance vs heating time graph for the reaction between perindopril and CDNB, showing the dependence of the reaction on perindopril concentration (●)5.428×10^{-5} M; (○) 16.283×10^{-5} M; (▲) 21.711×10^{-5} M; (△) 32.567×10^{-5} M
Fig 5.4: Arrhenius plot for the reaction between perindopril ($2.714 \times 10^{-4}$ M) and CDNB ($4.937 \times 10^{-4}$ M).
Fig 5.5: Eyring plot for the reaction between perindopril \((2.714 \times 10^{-4} \text{ M})\) and CDNB \((4.937 \times 10^{-4} \text{ M})\).
Fig. 5.3: Calibration curve for the determination of perindopril by initial rate method