Optimized and Validated Initial-Rate Method for the Determination of Perindopril Erbumine in Tablets

Nafisur RAHMAN,* Nishat ANWAR, and Mohammad KASHIF

Analytical Research Division, Department of Chemistry, Aligarh Muslim University; Aligarh (U.P.) 202 002 India.
Received June 20, 2005; accepted October 5, 2005

A simple and sensitive kinetic spectrophotometric method for the determination of perindopril in pharmaceutical preparations is described. The method is based on the interaction of drug with 1-chloro-2,4-dinitrobenzene (CDNB) in dimethylsulfoxide (DMSO) at 40±1 °C. The reaction is followed spectrophotometrically by measuring the rate of change of the absorbance at 420 nm. Under the optimized experimental conditions, the calibration curve showed a linear relationship over the concentration range of 20—140 μg/ml. The activation parameter such as E_a, ΔH^*, ΔS^* and ΔG^* for this reaction were calculated and found to be 27.31 kJ/mol, 24.69 kJ/mol, −138.84 J/K/mol and 61.50 kJ/mol, respectively. The method has been successfully applied to the determination of perindopril in commercial dosage forms. Statistical comparison of the results with the Abdellatef's spectrophotometric method[6] shows excellent agreement and indicates no significant difference between the methods compared in terms of accuracy and precision.

Key words perindopril; 1-chloro-2,4-dinitrobenzene; kinetic spectrophotometry; meisenheimer complex

Perindopril erbumine is a non official drug and is chemically, *tert*-butyl-amine salt of [[2*S*-1-(*R*,*R*)2α,3αβ,7αβ]-1-[2-(1-ethoxy carbonyl butyl]amino]-oxopropyl, octa-1*H* 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 the 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 $FeCl_3$ in the presence of potassium thiocyanate. UV derivative spectrophotometry using zero-crossing method[8] was utilized for the quantification of perindopril, where sufficient spectra resolutions of drug and its impurity were obtained.

This paper describes a kinetic spectrophotometric method for the determination of perindopril in commercial tablets. The method is based on the reaction of perindopril with CDNB in DMSO at 40 °C. The activation parameters such as activation energy (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, U.S.A.). 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 (CDNB) 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 flask. 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 (R_0) of the reaction at different concentration 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 analyzing 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 μg/ml. The interday precision was assessed by assaying the sample solution at three concentration levels (40, 80, 140 μg/ml) 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, 100 μg/ml).

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

$$\mathrm{LOD}=3.3\times S_0/b\,;\quad \mathrm{LOQ}=10.0\times S_0/b$$

where S_0 and b are the standard deviation and slope of calibration line.

Selectivity The selectivity of the method was ascertained by analyzing 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 analyzed 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 min. The residue was filtered on Whatman 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 mg/ml. An aliquot of the diluted solution was analyzed for perindopril content following the recommended procedure.

* To whom correspondence should be addressed. e-mail: cht17nr_amu@yahoo.com

Reference Method[6] Aliquots of 0.1—0.6 ml standard solution of perindopril (1 mg/ml) 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 3×3 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 upto 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 h 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 °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 °C, 40 °C and 50 °C in DMSO medium. It was observed that the linear dynamic range for determination increases on increasing temperature and become constant at 40 °C; further increase in temperature caused no change in the linear dynamic range. Therefore, a temperature of 40 °C was chosen as an optimum temperature.

The effect of the concentration of CDNB on the rate of reaction was studied in the range 4.0×10^{-3}—4.4×10^{-2} M. The rate of reaction or absorbance increases with increase in concentration of CDNB and becomes constant at 3.2×10^{-2} M; above this concentration no increase in the rate of reaction was observed. Thus a concentration of 4.0×10^{-2} M 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. 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 participate 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 Chart 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 curve Fig. 2. As can be seen from Fig. 2 that the initial rate increases with increasing perindopril concentration. The kinetic equation for this reaction is written as

$$R_0=dx/dt=K\,[\text{Perindopril}]^n\,[\text{CDNB}]^m \qquad (1)$$

At $[\text{CDNB}]\geq3.2\times10^{-2}$ M, the order of reaction became zero with respect to CDNB concentration. The Eq. 1 is reduced to

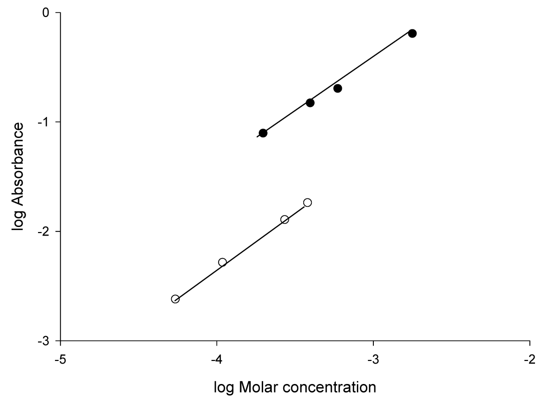

Fig. 1. Limitting Logarithmic Plot for Molar Combining Ratio between Perindopril and CDNB

○, log A *vs.* log [Drug]; ●, log A *vs.* log [CDNB].

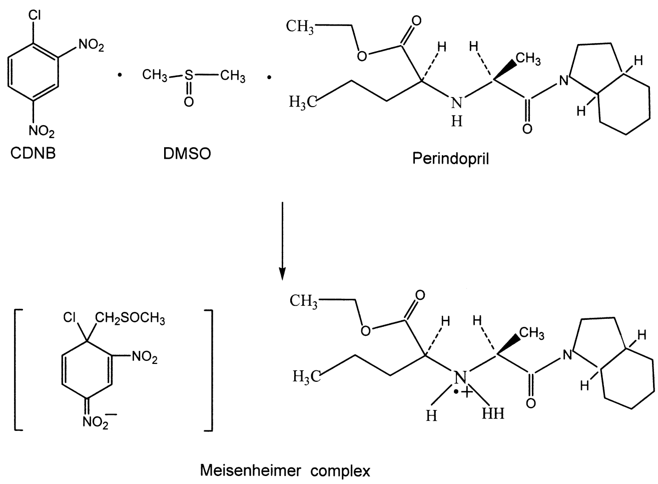

Chart 1

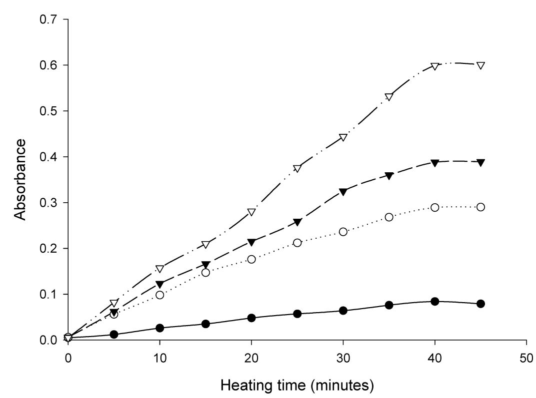

Fig. 2. Absorbance *vs.* Heating Time Graph for the Reaction between Perindopril and CDNB, Showing the Dependence of the Reaction on Perindopril Concentration

●, 5.428×10^{-5} M; ○, 16.283×10^{-5} M; ▼, 21.711×10^{-5} M; ▽, 32.567×10^{-5} M.

$$R_0 = K\,[\text{Perindopril}]^n \quad (2)$$

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 Eq. 2 is transformed into pseudo first order reaction as

$$R_0 = K_\Psi\,[\text{Perindopril}]$$

where K_Ψ 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 μg/ml. The regression analysis using the method of least square was performed to estimate the slope, intercept and correlation coefficient under optimized experimental conditions. The value of LOD, LOQ and variance were also calculated and summarized in Table 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.

$$K = Ae^{-E_a/RT}$$

The plot of ln k *versus* $1/T$ was linear Fig. 3. and E_a calculated from the slope ($-E_a/R$) was 27.31 kJ/mol. The values of ΔH^* and ΔS^* were evaluated using Eyring equation

$$k = (k_b T/h)e^{\Delta S/R}\, e^{-\Delta H/RT}$$

A plot of ln k/T *versus* $1/T$ was linear Fig. 4. The values of ΔH^* and ΔS^* obtained from slope ($-\Delta H^*/R$) and intercept ($\ln K_b/h + \Delta S^*/R$) were 24.69 kJ/mol and −134.84 J/K/mol, respectively. The value of ΔG^* was found to be 61.50 kJ/mol.

Table 1. Optical and Regression Characteristics of Initial Rate Method

Parameters	Data
Linear dynamic range (μg/ml)	20—140
Regression equation	$R_0 = 1.90\times10^{-6} + 0.7797$ [Perindopril]
S_a	4.41×10^{-6}
$\pm tS_a$	8.57×10^{-6}
S_b	1.54×10^{-2}
$\pm tS_b$	2.98×10^{-2}
Correlation coefficient (r)	0.9990
LOD (μg/ml)	6.33
LOQ (μg/ml)	19.20
Variance (S_0^2)	1.65×0^{-11}

$\pm tS_a$=confidence limit for intercept. $\pm tS_b$=confidence limit for slope.

The intra and interday precision were evaluated at three concentration levels and the results are shown in Table 2. It is evident from the Table 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 analyzed 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 determining the perindopril content in the presence of ex-

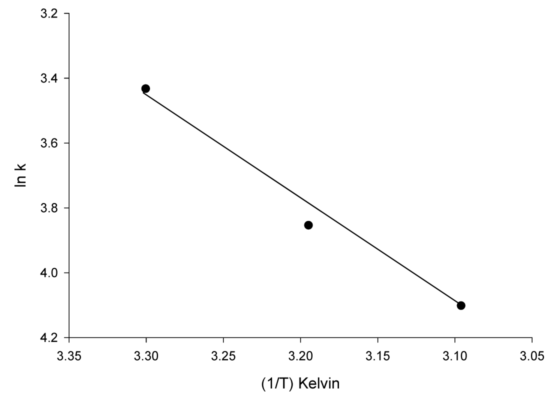

Fig. 3. Arrhenius Plot for the Reaction between Perindopril (2.714×10^{-4} M) and CDNB (4.937×10^{-4} M)

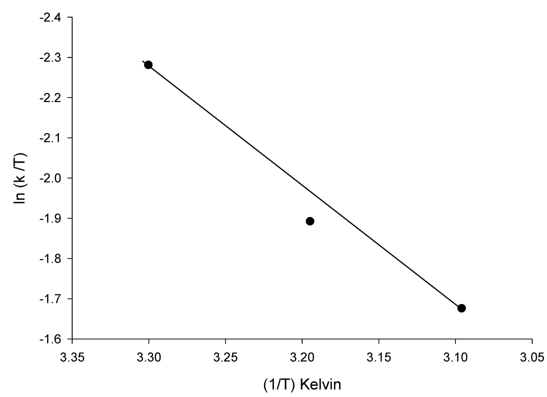

Fig. 4. Eyring Plot for the Reaction between Perindopril (2.714×10^{-4} M) and CDNB (4.937×10^{-4} M)

Table 2. Evaluation of Accuracy and Precision of the Proposed Method

Intraday assay amount (μg/ml)		Recovery[a]±RSD (%)	SAE[b]	CL[c]
Taken	Found±S.D.			
40	39.60±0.34	98.99±0.86	0.139	0.280
100	100.91±0.98	100.91±0.97	0.400	0.808
140	139.97±0.96	99.98±0.69	0.393	0.792
Interday assay amount (μg/ml)				
40	39.74±0.34	99.36±0.85	0.408	0.822
80	80.12±0.74	100.14±0.92	0.301	0.606
140	140.01±0.92	100.01±0.66	0.374	0.754

a) Average of six independent analysis. *b*) Standard analytical error. *c*) Confidence limit at 95% confidence level.

cipients such as lactose, magnesium stearate, cellulose and talc, varying amount of impurities and perindoprilat. It was observed that impurities such as (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazino[1,2-a-]indole-2(1*H*)-yl]pentanoic acid, ethyl (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*S*)-3-methyl-1,4-dioxodecahydropyrazinol[1,2-a-]indole-2(1*H*)-yl]-pentanoate, (2*S*)-2-[(3*S*,5a*S*,9a*S*,10a*R*)-3-methyl-1,4-dioxodecahydropyrazinol[1,2-a-]indole-2(1*H*)-yl]pentanoic acid, (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[(5*RS*)-3-cyclohexyl-2,4-dioxo-5-propylimidazolidine-1-yl]propanoyl]octahydro-1*H*-indol-2-carboxylic acid, and (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[(5*RS*)-3-cyclohexyl-2-(cyclohexylimino)-4-oxo-5-propylimidazolidine-1-yl]propanoyl]octahydro-1*H*-indol-2-carboxylic acid do not interfere with the determination. However other impurities such as (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-[(1-carboxybutyl]-amino]propanoyl]octahydro-1*H*-indol-2-carboxylic acid, (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-[(1-methylethoxy)carbonyl]-butyl]amino]propanoyl]octahydro-1*H*-indol-2-carboxylic acid, and (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(1*R*)-1-[(1-ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1*H*-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 3.

Table 3. Accuracy and Recovery

Excess of drug added to the analyte (%)	Recovery[a] ± RSD (%)	SAE[b]	CL[c]
0	99.93±0.86	0.422	0.851
33.33	99.09±1.01	0.163	0.329
100	99.84±1.01	0.330	0.665
200	99.82±0.72	0.352	0.710

a) Average of six independent analysis. *b*) Standard analytical error. *c*) Confidence limit at 95% confidence level.

The developed method was successfully applied to the assay of perindopril in tablets; the results are given in Table 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 hypothesis. 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 <±2% at 95% confidence level. For pharmaceutical analysis, a bias of ±2% is acceptable and thus the limit of acceptance interval is within $\theta_L = 0.98$ and $\theta_U = 1.02$.

Acknowledgements Authors are thankful to Chairman, Department of Chemistry, AMU, Aligarh for providing necessary research facilities. Thanks are extended to Dr. Mubeen Ahmad Khan, Vice President, Analytical Research, Glenmark India Limited, Mumbai, for providing a gift sample of pure perindopril.

References

1) Laubie M., Vincent M., Schmitt H., *J. Cardio-Vasc. Pharmacol.*, **6**, 1076—1082 (1984).
2) Sereda K. M., Hardman T. C., Dilloway M. R., Lant A. F., *Anal. Proc.* (London), **30**, 371—372 (1993).
3) Tasconas C., Devissaguet M., Padieu P., *J. Chromatogr.*, **488**, 249—265 (1989).
4) Doucet I., Verac B. De., Delaage M., Cailla C., Berheim C., Devissaguet M., *J. Pharm. Sci.*, **79**, 741—745 (1990).
5) Lin S. J., Wu H. L., Chem S. H., Wen Y. H., *Anal. Lett.*, **29**, 1751—1762 (1996).
6) Abdellatef E. H., Ayad M. M., Taha A. E., *J. Pharm. Biomed. Anal.*, **18**, 1021—1027 (1999).
7) Abdellatef E. H., Ragab H. G., Baraka M. M., *J. Pharm. Sci.*, **7**, 59—65 (1998).
8) Erk N., *J. Pharm. Biomed. Anal.*, **26**, 43—52 (2001).
9) Morelli B., *Analyst* (London), **108**, 870—879 (1983).
10) Roso J., "Advanced Physicochemical Experiments," Sir Issac Pitman and Sons, London, 1964, p. 67.
11) Buncell E., Norris A. R., Russell K. E., *Q. Rev. Chem. Soc.*, **22**, 123—125 (1968).
12) Crampton M. R., *Adv. Phys. Org. Chem.*, **7**, 211—217 (1969).

Table 4. Comparison of Proposed Method with Abdellatef's Spectrophotometric Method

Formulation	Labelled amount (mg)	Proposed method	Abdellatef's method[6]	*t*-value[b]	*F*-value[b]	θ_L[c]	θ_U[c]
		Recovery[a] ± RSD (%)	Recovery[a] ± RSD (%)				
Coversyl	4	99.89±1.07	99.64±0.51	0.514	4.385	0.994	1.011
Perigard	4	100.15±0.92	99.69±0.57	1.035	2.665	0.997	1.012

a) Average of six independent analysis. *b*) Theoretical *t*-value and *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.