DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PERINDOPRIL ERBUMINE IN PHARMACEUTICAL FORMULATIONS USING 2, 4 DINITROFLUOROBENZENE

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(Received: September 27, 2010 - Accepted: January 5, 2012)

ABSTRACT

A simple, rapid and sensitive spectrophotometric method was developed for the determination of perindopril in pharmaceutical formulations. The proposed method is based on the reaction of amine group of drug with 2, 4 dinitrofluorobenzene in dimthylsulfoxide (DMSO) to form yellow colored product, which absorbs maximally at 410 nm. Beer's law was obeyed in the concentration range 2.5 - 25 μg/mL with molar absorptivity 6.71 $\times 10^3$ L/mol/cm. The limits of detection and quantitation of the proposed method were 0.17 and 0.52 μg/mL, respectively. The optimum experimental condition for the proposed procedure was investigated. The results of the proposed method were compared with those of Abdellatef's spectrophotometric method, which indicated excellent agreement with acceptable true bias of all samples within ±2.0%.

Keywords: Perindopril erbumine, 2, 4 dinitrofluorobenzene, pharmaceutical formulations.

INTRODUCTION

Perindopril erbumine is angiotensin-converting enzyme (ACE) inhibitor[1]. It is chemically described as (2S,3αS,7αS)-1-[(S)-N-[(S)-1-carboxy-butyl] alanyl] hexahydro-2- indolinecarboxyilc acid, 1-ethyl ester , compound with tert-butylamine (1:1) [CAS No. 107133-36-8, M.W. 441.6]. It is a pro-drug for perindoprilat, which inhibits ACE in human subjects and animals. The mechanism through which, perindoprilat lowers blood pressure is believed to be primarily inhibition of ACE activity. The drug is officially listed in the monograph of British Pharmacopoeia[2], which describes a potentiometric titration procedure for its assay in formulations. In order to assure the quantity of perindopril in pharmaceutical preparations, several methods have been published for the determination of perindopril. These methods include GC-MS[3], HPLC and RP-HPLC[4-8], capillary electrophoresis[9], atomic absorption spectrophotometry[10], gas chromatography[11], LC- MS/MS[12,13] and radioimmunoassay[14].

Few spectrophotometric methods have also been reported in the literature. Abdellatef et al have developed two extractive spectrophotometric methods for the determination of perindopril, depending upon chloroform extractable complex with eosin and copper and an ion association complex of perindopril with bromothymol blue at pH 5[10,15]. The content of perindopril was determined based on its interaction with $FeCl_3$ in the presence of potassium thiocyanate[15]. UV derivative spectrophotometry using zero-crossing method[16] was utilized for the quantification of perindopril, where sufficient spectra resolutions of drug and its impurity were obtained. The spectrophotometric determination of perindopril was done based on the reaction of the drug[17], 1- chloro 2, 4-dinitrobenzene[18], 3-methylbenzo-thiozolin-2-one hydrazone, horseradish peroxidase[19] and indigo carmine[20]. The reported methods are not much sensitive and few require experimental variables such as heating or extraction steps. Therefore, there is a need to develop a simple and sensitive method for the determination of perindopril without prior extraction and heating.

This manuscript presents a simple and sensitive spectrophotometric method for the determination of perindopril at room temperature (30 ± 1°C) based on its reaction with 2, 4 dinitrofluorobenzene. The assay procedure was validated as per ICH guidelines[21].

EXPERIMENTAL

Apparatus

Spectral runs were made on a Shimadzu 1601 UV-visible spectrophotometer (UV-VIS Shimadzu 1601, Kyoto, Tokyo, Japan). All other spectrophotometric measurements were made on Spectronic 20 D^+ spectrophotometer (Milton Roy Company, USA).

Materials and Reagents

All chemicals and reagents were of analytical or pharmaceutical grade. 2, 4 dinitrofluorobenzene (0.1%, 5.37 $\times 10^{-3}$ M, Fluka Chemie AG) was prepared in dimethyl sulfoxide (Merck).

Standard Solution

Perindopril reference standard (Batch No K32002003) was kindly supplied as a gift sample by Glenmark Pharmaceuticals Ltd. Mumbai, India. Perindopril standard solution (0.25 mg/mL) in DMSO was prepared. The standard drug solution was stable for at least two days in DMSO, at room temperature (30 ± 1°C) when protected from direct sunlight. Quality control tablets of perindopril such as Coversyl 2.0® SERDIA (Serdia Pharmaceuticals India, Ltd.) and Perigard 2.0® (Glenmark Pharmaceuticals LTD (Zoltan) India) were obtained from local drug shops.

Proposed Procedure for the Determination of Perindopril

Aliquots of (0.05 - 0.50 mL) standard solution of perindopril (0.25 mg/mL) were taken and placed into a series of 5 mL volumetric flasks. Then, 0.50 mL of 5.37 $\times 10^{-3}$ M 2,4 dinitrofluorobenzene was added to each flask and diluted to volume with DMSO. The intensity of the color developed was measured at 410 nm after 10 min of mixing against the reagent blank prepared similarly omitting the drug. The amount of drug in a given sample can be estimated from calibration curve or corresponding regression equation.

Analysis of Perindopril in Pharmaceutical Formulations

Formulations of perindopril were powdered and placed in a conical flask, shaking with 20 mL DMSO for 10 min. The DMSO extract was filtered through Whatmann No. 42 filter paper (Whatmann International Limited, Kent, UK) in 50 mL volumetric flask. The residues were washed well with 3 × 10 mL portions of DMSO for complete recovery of the drug. The resulting solution was further diluted to the working concentration range and analyzed following the proposed procedure.

Limits of detection (LOD) and limits of quantitation (LOQ)

The limits of detection (LOD) and quantitation (LOQ) were calculated using the equation[21]

$$LOD = 3.3 \times S_0 / b \text{ and } LOQ = 10 \times S_0 / b$$

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

RESULTS AND DISCUSSION

In the present study, perindopril reacted with 2, 4 dinitrofluorobenzene in DMSO medium at room temperature (30 ± 1°C) resulting in the formation of yellow colored complex. The absorption spectra of the complex and 2, 4 dinitrofluorobenzene are shown in fig.1. It is evident from the fig.1 that the complex and 2, 4 dinitrofluorobenzene absorbs maximally at 410 nm, and 345 nm, respectively.

The optimum conditions for proposed procedure have been established by studying the reaction as a function of time, concentration of reagent, nature of solvents and stability of the colored species.

The effect of time on the course of the reaction was studied by measuring the absorbance at room temperature (30 ± 1°C). It was found that the reaction got stabilized at 8.0 min and remained stable at room temperature for further 5 h. Therefore, 10 min time was chosen as the optimum time throughout the determination process.

The effect of concentration of 2, 4 dinitrofluorobenzene on the absorbance of the colored product was studied over the concentration range of 2.69 $\times 10^{-5}$ M - 7.52 $\times 10^{-4}$ M; keeping a constant concentration of the drug (25.0 μg/mL). It was observed that increasing the concentration of 2, 4 dinitrofluorobenzene would result in a gradual increase in the absorbance of the colored product up

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to 3.22 × 10^{-4} M and remained constant up to 7.52 × 10^{-4} M. Thus 5.37 × 10^{-4} M of 2, 4 dinitrofluorobenzene was used as an optimum concentration (Fig. 2).

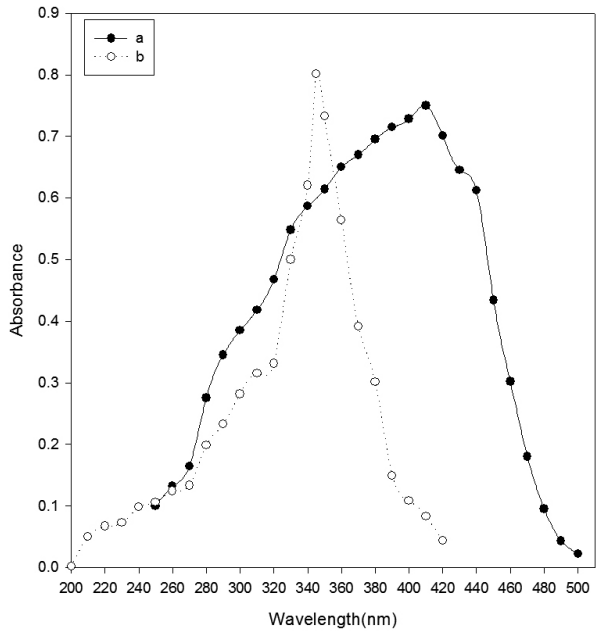


Fig. 1 Absorption spectra of (a) 5.66 ×10^{-5} M perindopril and 5.37 ×10^{-4} M 2, 4 dinitrofluorobenzene in DMSO (b) 3.22 × 10^{-3} M 2, 4 dinitrofluorobenzene in DMSO.

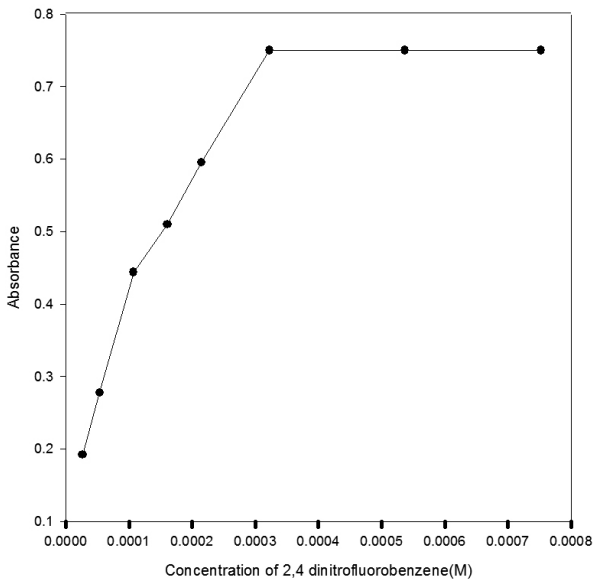


Fig. 2. Effect of the concentration of 2, 4 dinitrofluorobenzene on the absorbance of the colored product (perindopril 5.66 ×10^{-5} M)

Mole ratio method[22] was applied to determine the molar combining ratio between perindopril and 2, 4 dinitrofluorobenzene. The molar combining ratio between drug and 2, 4 dinitrofluorobenzene was found to be 1:1. Therefore it is concluded that one mole of the drug reacted with one mole of 2, 4 dinitrofluorobenzene (Fig. 3). The apparent formation constant (K_f) and standard Gibbs's free energy (ΔG^0) were calculated and found to be 1.19 ×10^7, -40.36 KJ/mol, respectively.

The reaction of amines with polynitro aromatic compounds in DMSO has resulted in the formation of colored Meisenheimer complexes[23]. The formation of such complexes depends on the degree of participation of amino group through its unshared electron pair with the polynitro aromatic compounds. In the present study, perindopril possesses a –NH group in the moiety and hence reacted with 2, 4 dinitrofluorobenzene in DMSO medium resulting in the formation of 1-substituted Meisenheimer complex. On the basis of literature background and experimental observation, a possible mechanism is proposed and given in Scheme 1.

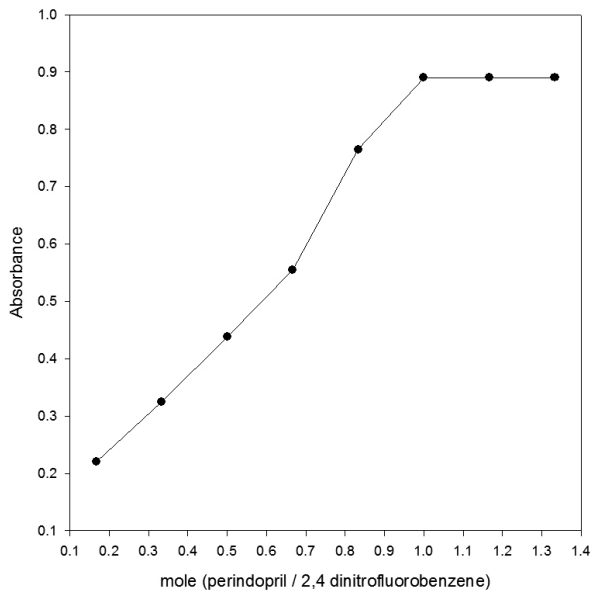
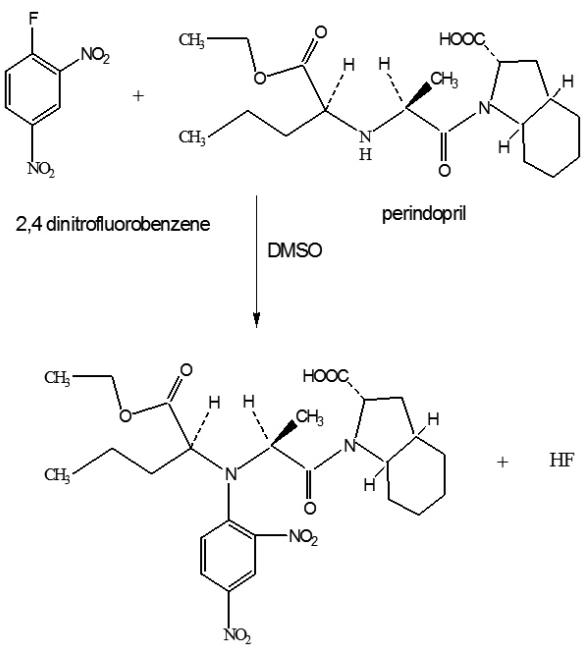


Fig. 3 Mole ratio plot for the reaction between perindopril and 2, 4 dinitrofluorobenzene in DMSO ([Perindopril] = [2, 4 dinitrofluorobenzene] = 2.68 × 10^{-3} M).



The calibration curve between the absorbance at 410 nm and the perindopril concentration was found to be linear over the concentration range of 2.5 – 25.0 µg/ mL with molar absorptivity of 6.71 × 10^3 L/mol/cm. The linear regression equation of the proposed method has been evaluated by the least square treatment of the calibration data. Table 1 summarizes Beer's law limit, linear regression equation, correlation coefficient, standard deviations for slope and intercept at 95% confidence level, variance, limits of detection (LOD) and limits of quantitation (LOQ) for the proposed method.

Table 1. Optical Characteristics and Statistical Data of the Regression Equation.

Parameters	
λ_{max} (nm)	410
Beer's law limit (μg/mL)	2.5 - 25
Molar absorptivity (L/mol/cm)	6.71×10^3
Linear regression equation	$A = 4.88 \times 10^{-4} + 2.99 \times 10^{-2} C$
S_a	1.22×10^{-3}
tS_a	3.39×10^{-3}
S_b	8.03×10^{-5}
tS_b	2.23×10^{-4}
Correlation coefficient (r)	0.9999
Variance (S_0^2)	2.21×10^{-7}
LOD (μg/mL)	0.17
LOQ (μg/mL)	0.52

Table 2. Test of Precision of the Proposed Method by Intra Day and Inter Day Assays.

Proposed method	Concentration (μg/mL)		Recovery ± RSD*(%)	SAE**	C.L***
	Taken	Found ± SD			
Intra day assay	5. 0	4.995 ±0.060	99.90±1.20	0.027	0.074
	15.0	14.997±0.050	99.98 ±0.33	0.022	0.062
	25.0	25.010±0.055	100.03 ±0.22	0.024	0.068
Inter day assay	5.0	4.999±0.061	99.97±1.23	0.027	0.076
	15.0	15.014±0.062	100.09 ±0.42	0.028	0.078
	25.0	25.001±0.058	100.00±0.23	0.026	0.072

*Mean for five independent determinations.
**SAE, standard analytical error.
***C.L., confidence limit at 95% confidence level and four degrees of freedom (t = 2.776).

The small value of variance (2.21×10^{-7}) has also pointed towards negligible scattering of experimental data points about the line of best fit.

The accuracy and precision of the proposed method was established by determining the content of perindopril in drug formulations at three different concentration levels (5.0, 15.0, and 25.0 μg/mL). The intra day and inter day precisions of the proposed method in pure form was evaluated by carrying out five independent analyses at each concentration level within one day and on five consecutive days (Table 2). The RSD values of intra- day and inter-day studies (< 1.24%) showed that the precision of the method was satisfactory.

The recovery experiments were also performed using the standard addition technique. The assay was carried out by addition of known amounts of the standard drugs to the preanalyzed commercial dosage forms. The results obtained for two concentrations are shown in Table 3. As can be seen from the table that the mean recovery was found to be vary between 99.95% and 100.04%, indicating that the co-formulated substances such as talc, starch, lactose, hydroxyl methyl cellulose and magnesium stearate did not interfere in the assay.

Table 3. Determination of Perindopril in Pharmaceutical Formulations by Standard Addition Technique.

Formulations	Concentration (μg/mL)			Recovery (%)	RSD*(%)	CL**
	Taken	Added	Found ± SD			
Coversyl-2.0® (SERDIA)	5	5	09.99 ±0.06	99.97	0.60	0.074
	10	10	20.01 ±0.06	100.03	0.28	0.069
Perigard-2.0® (Glenmark, (Zoltan)	5	5	10.00 ±0.06	100.04	0.62	0.078
	10	10	19.99 ±0.06	99.95	0.31	0.078

*Mean for five independent analyses.
**CL, confidence limit at 95% confidence level and four degree of freedom (2.776).

The performance of the proposed method was compared with those of existing spectrophotometric methods and other techniques. As can be seen from the Table 4 that the molar absorptivity of the proposed method is the same order of magnitude, but very different values,[8,10-13,16-19] and it can be used to determine the perindopril concentration as low as 2.5 μg/mL. However, sophisticated techniques can be used to detect as low as 0.5 ng/mL, but these instruments are very costly and need pretreatment steps before analysis in some cases.

Table 4. Comparison of the Performance of the Proposed method with other Existing Methods.

Techniques/ Reagents	λ_{max} (nm)	Linear Range	RSD (%)	Molar Absorptivity	References
Spectrophotometry					
Cu (II)-eosin	535	10 - 60 µg/mL	1.99	6.55×10^3	10
2,3- dichloro-5,6-dicyano-p-benzoquinone	588	20 - 200 µg/mL	0.98	6.201×10^3	17
7,7,8,8- tetracyanoquinodimethane	843	20 - 200 µg/mL	1.20	4.431×10^3	17
Tetracyanoethylene	419	20 - 200 µg/mL	1.03	2.86×10^3	17
Chloranil	550	20 - 200 µg/mL	0.95	1.137×10^3	17
p-chloranilic acid	520	20 - 200 µg/mL	1.33	1.883×10^3	17
1- chloro 2, 4-dinitrobenzene	420	20 - 140 µg/mL	1.07	-	18
3-methylbenzo-thiozolin-2-one hydrazone	425	10 - 50 µg/ mL	-	-	19
2, 4 dinitrofluorobenzene	410	2.5 - 25 µg/mL	1.23	6.71×10^3	This work
HPLC	215	4 - 20 µg/mL	0.86	-	8
	215	5 - 70 µg/mL	0.21	-	16
GC	-	20 - 300 nmol	-	-	11
LC-MS/MS	-	0.5 - 350 ng/mL	-	-	12
	-	0.1 - 100 ng/mL	< 6.1	-	13

The applicability of the proposed method has been tested for the determination of perindopril in pharmaceutical preparations. The results of the proposed method were compared with those obtained by the Abdellatef's method[17]. The student's t and F-values at 95% confidence level did not exceed the tabulated t- and F-values, confirming no significant differences between the performance of the proposed method and the reference method (Table 5). Interval hypothesis[24] has also been tested and the true bias based on recovery experiments were calculated using the equation:

$$\theta^2\left(\overline{x_1^2} - S_p^2 t^2 / n_1\right) - 2\theta\overline{x_1}\,\overline{x_2} + \theta^2\left(\overline{x_2^2} - S_p^2 t^2 / n_2\right) = 0$$

The lower limit (θ_L) and upper limit (θ_U) of the confidence interval are obtained as:

$$\theta_L = \frac{-b - \sqrt{b^2 - 4ac}}{2a}$$

$$\theta_U = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

where

$$a = \overline{x_1}^2 - S_p{}^2 t^2 / n_1$$

$$b = -2\overline{x_1}\,\overline{x_2}$$

$$c = \overline{x_2}^2 - S_p{}^2 t^2 / n_2$$

Table 5. Analysis of perindopril of the proposed method using point and interval hypothesis tests at 95% confidence level.

Formulations			Reference method[17]	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Coversyl 2.0®	99.95	0.31	99.94	0.38
(SERDIA)	θ_L=0.986	θ_U=1.015		
	t =0.012	F =1.436		
Perigard 2.0®	100.03	0.28	99.97	0.38
(Glenmark, Zoltan)	θ_L=0.991	θ_U=1.008		
	t =0.157	F=1.925		

where $\overline{x_1}$ and $\overline{x_2}$ are the means obtained by proposed and reference methods, respectively. n_1 and n_2 are the number of respective measurements. S_p is the pooled standard deviation and t- is the tabulated one sided t- value, with $n_1 + n_2$ degree of freedom at 95% confidence level. q_L and q_U values were 0.986 and 1.015; 0.991 and 1.008 for the assay of Coversyl-2.0® (SERDIA) and Perigard 2.0® (Glenmark, Zoltan), respectively. In the pharmaceutical analysis, a bias of < ± 2.0 % is acceptable.[25]

It is evident from Table 5 that the true bias of all samples is < ± 2 %. The interval hypothesis tests have confirmed that the accuracy and precision are within the acceptable limits and indicated no significant differences between the performances of the proposed method with the reference method at 95% confidence level.

CONCLUSIONS

The proposed method is compared with other methods using different techniques (Table 4) shows that this method is simple, sensitive and accurate and performed at room temperature related to other existing methods. The proposed method also not requires any pretreatment of drug sample. Hence the present spectrophotometric method is extended for routine analysis of perindopril in pharmaceutical industries, hospitals and research laboratories.

ACKNOWLEDGEMENTS

Authors are thankful to Aligarh Muslim University, Aligarh for providing necessary research facilities. Thanks are extended to Dr. Mubeen Ahmad Khan, Vice President, Glenmark India Limited, Mumbai, for providing a gift sample of pure perindopril.

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