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Spectrophotometric analysis of Perindopril Erbumine in bulk and tablets using bromophenol blue

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ABSTRACT

A method for spectrophotometric determination of perindopril erbumine (PDE) in bulk and tablet dosage forms has been described. This method is based on the PDE ability with bromophenol blue (BPB), in acidic media, to form an ionic association extractible in chloroform. The absorbance of PDE-BPB ion pair complex is monitored spectrophotometrically at 425 nm and it is related to the concentration of PDE present. The method is optimized for acidity, concentration of BPB and solvent for the extraction required. The range of linearity for ion pair complex was found to be 5-125 μg mL^{-1} of PDE with molar absorptivity 5.210 $x10^3$ L $mol^{-1}cm^{-1}$ and sandell's sensitivity 0.0847 μg cm^{-2}. The detection limit and quantitation limit of the PDE determination are found to be 0.256 μg mL^{-1} and 0.775 μg mL^{-1}, respectively. The proposed method has been successfully applied to the determination of PDE in tablet dosage forms.

Key words: perindopril erbumine, bromophenol blue, ion pair complex, spectrophotometry, analysis.

INTRODUCTION

Perindopril erbumine [1-7], chemically known as 2-Methylpropan-2-amine (2S,3aS,7aS)-1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl)butyl]amino]propanoyl]octahydro-1H-indole-2-carboxylate (Figure 1), is a nonsulfhydryl prodrug and an angiotensin converting enzyme (ACE) inhibitor. PDE is used in the treatment of hypertension and to prevent heart attack in people with coronary artery disease. Perindopril, following oral administration, is metabolized to its active metabolite,

perindoprilat. The perindoprilat inhibits ACE both in vitro and in vivo. Inhibition of ACE results in decreased plasma angiotensin II, leading to decreased vasoconstriction, increased plasma rennin activity and decreased aldosterone secretion. The overall effect is a drop in blood pressure and a decrease in the workload of the heart. Due to the vital significance of the PDE, the development of a simple, sensitive and rapid method for its determination is of urgent need.

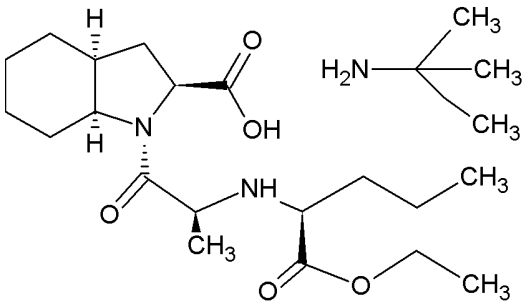

Figure 1: Chemical structure of perindopril erbumine

PDE was official in British pharmacopoeia, which describes a potentiometric titration method for its assay [8]. Different analytical techniques have been adopted for the determination of PDE in bulk, pharmaceutical formulations and/or biological samples. They include radioimmunoassay [9], HPLC [10,11], stability indicated HPTLC [12], amperometry biosensor [13-15], LC–MS/MS [16,17], capillary gas chromatography [18], GC-MS [19] and potentiometry [20]. However, many of the above mentioned techniques are tedious, time consuming and/or require expensive equipment.

Spectrophotometry is by far the instrumental technique of choice in the laboratories of underdeveloped and developing nations for the quantification of drugs, owing mainly to its simplicity, high sensitivity & selectivity and often demanding low-cost equipment. Few visible spectrophotometric methods have been reported for the assay of PDE in tablet dosage forms. Abdellatef HE et al., [21,22] reported six spectrophotometric methods. The first five methods are based on the charge transfer complexation reaction of the PDE with 2,3-dichloro-5,6-dicyano-p-benzoquinone [21], 7,7,8,8- tetracyanoquinodimethane [21], tetracyanoethylene [21], chloranil [21] and p-chloranilic acid [21]. The sixth method involves the formation of a ternary complex, extractable with chloroform, between copper (II), eosin and the PDE [22]. Nafisur Rahman et al.,[23] developed two spectrophotometric methods for the determination of perindopril in pharmaceutical preparations. The first method is based on the formation of ternary complex between zinc(II), eosin and the perindopril, which is extractable with chloroform. The second method is based on the interaction of drug with iodine in dichloromethane resulting in the formation of charge transfer complex. An kinetic spectrophotometric method [24], based on the interaction of PDE with 1-chloro-2,4-dinitrobenzene in dimethylsulfoxide, for the determination of PDE in pharmaceutical preparations was described by Nafisur Rahman et al., The aforementioned spectrophotometric methods present some limitations and drawbacks such as poor sensitivity, use of expensive reagent, unstable colored product, and measurements made at shorter wavelength.

The objective of the present work is to develop and validate a simple, sensitive and economically viable spectrophotometric method for the determination of PDE in bulk and in their

pharmaceutical formulations. The proposed method was based on the extraction of PDE into chloroform as yellow colored ion pair with bromophenol blue. The yellow colored ion pair was quantitated spectrophotometrically at 425 nm. The proposed method has been validated for linearity, sensitivity, precision, accuracy, selectivity and robustness.

MATERIALS AND METHODS

2.1. Apparatus

All spectrophotometric measurements were carried out using an Elico double beam model SL 159 digital spectrophotometer (Hyderabad, India). The cells used for absorbance measurements were 1-cm matched quartz cells. Samples were weighed by using Essae-Teraoka electronic weighing balance (Goa, India) PG1000 model.

2.2. Reagents

All chemicals used were of analytical reagent grade. Doubly distilled water was used in the preparation of all solutions. All the solutions were prepared afresh daily.

- 0.5% Bromophenol blue (BPB): Prepared by dissolving 500 mg of BPB (Sdfine-Chem limited, Mumbai) in 100 mL of water.
- 0.5 N HCl: 1.825 mL of HCl (Fisher Scientific, Mumbai) was added to 100 mL of distilled water to get 0.5 N HCl.
- Chloroform (Sdfine-Chem limited, Mumbai).

2.3. Standard solutions of perindopril erbumine

Pharmaceutical grade PDE was kindly gifted by Hetero Drugs Ltd, Hyderabad, India and used as received. A stock standard solution containing 1 mg mL^{-1} of PDE was prepared in water. Working standard solution equivalent to 500 µg mL^{-1} of PDE was obtained by appropriate dilution of stock solution with water.

2.4. Tablet dosage forms of perindopril erbumine

Branded PDE products Conversyl and Periguard containing 2 and 4 mg of PDE per tablet, respectively were purchased from a local pharmacy market.

2.5. Recommended procedure

Into a series of 100 mL separating funnels, volumes (0.1-2.5 mL) of PDE standard solution (500 µg mL^{-1}) equivalent to 5–125 µg of the drug were transferred. The volume in each separating funnel was adjusted to 2.5 mL with distilled water. Then, to each separating funnel 1 mL of 0.5 N HCl and 2.0 mL of 0.5% BPB were transferred and mixed well. The funnels were shaken vigorously with 5 mL of chloroform for 2 min and then allowed to stand for clear separation of the two phases. The chloroform phase thus separated was transferred into a 10 mL volumetric flask. Then the extract was made up to the mark with chloroform and mixed well. The absorbance of the chloroform phase was measured at 425 nm against a reagent blank prepared similarly omitting the drug. The absorbances versus final concentration of PDE were plotted and the calibration curve was drawn. The concentration of the drug in unknown samples was read from the calibration curve or computed from the regression equation.

2.6. Analysis of tablet dosage forms

Fifty tablets were accurately weighed and finely powdered. A portion of the powder equivalent to 50 mg of PDE was transferred into a 50 mL volumetric flask and 25 mL of water was added to it. The content of the flask was sonicated for 15 min and diluted to volume with water. This solution was filtered through Whatmann number 1 filter paper. The tablet extract (1 mg mL^{-1}) was diluted with water to give final concentration (500 µg mL^{-1}). A suitable aliquot was then analyzed by the recommended procedure. The amount of PDE in tablet was calculated from the calibration curve or computed from the regression equation.

2.7. Analysis of placebo blank

A placebo blank containing starch (10 mg), acacia (10 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (10 mg), magnesium stearate (10 mg), lactose (10 mg), glucose (10 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under "analysis of tablet dosage forms" and then subjected to analysis.

2.8. Analysis of synthetic mixture:

A synthetic mixture was separately prepared by adding pure PDE (50 mg) to the above mentioned placebo blank and the mixture was homogenized. The entire mixture containing 50 mg of PDE was taken and its extract was prepared as described above. Aliquots of extracts containing three different concentrations of PDE (within Beer's limit) were subjected to assay by following the recommended procedure.

RESULTS AND DISCUSSION

The ion-pair complex is a special form of molecular complex resulting from two oppositely charged ions extractable into organic solvents from aqueous phase at suitable pH. In the recent years ion pair extraction spectrophometry has received substantial significance for the quantification of many pharmaceutical compounds [25-28].

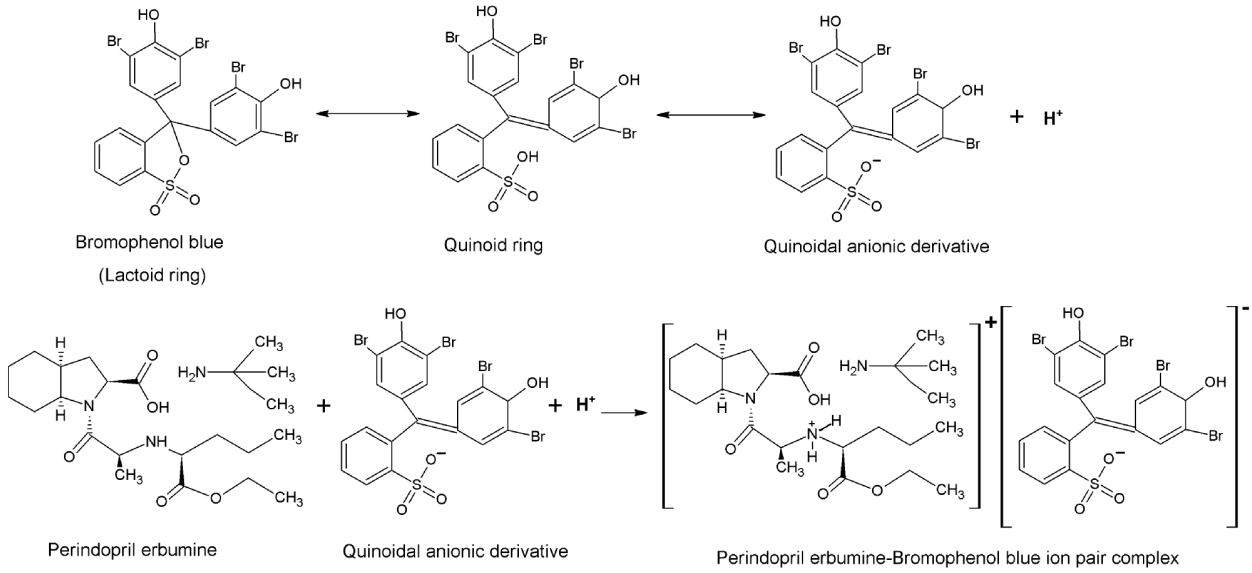

Figure 2: Suggested mechanism of PDE–BPB ion pair complex formation

Considering basic drugs capacity to generate ion-pairs with anionic dyes, a new spectrophotometric method for PDE assay in bulk and in tablet dosage forms was developed. PDE exhibits basic character owing to the presence of secondary amino group. In acidic media, the secondary amino group of PDE is protonated, while sulphonic group present in BPB undergoes dissociation to form an open quinoidal anionic derivative. The results obtained in the proposed method are based on ion pair formation of PDE with BPB under acidic conditions. The resulted yellow colored ion pair complex was extracted with chloroform and the absorbance was measured at 425 nm. The proposed reaction mechanism of PDE with BPB has been given in the Figure 2.

3.1. Selection of the optimum wavelength

The absorbance of the ion-pair complex, formed between PDE and BPB, was measured against the reagent blank in the range of 390-470 nm (Figure 3). The yellow colored PDE-BPB ion pair complex show maximum absorbance at 425 nm. The reagent blank showed negligible absorbance at 425 nm.

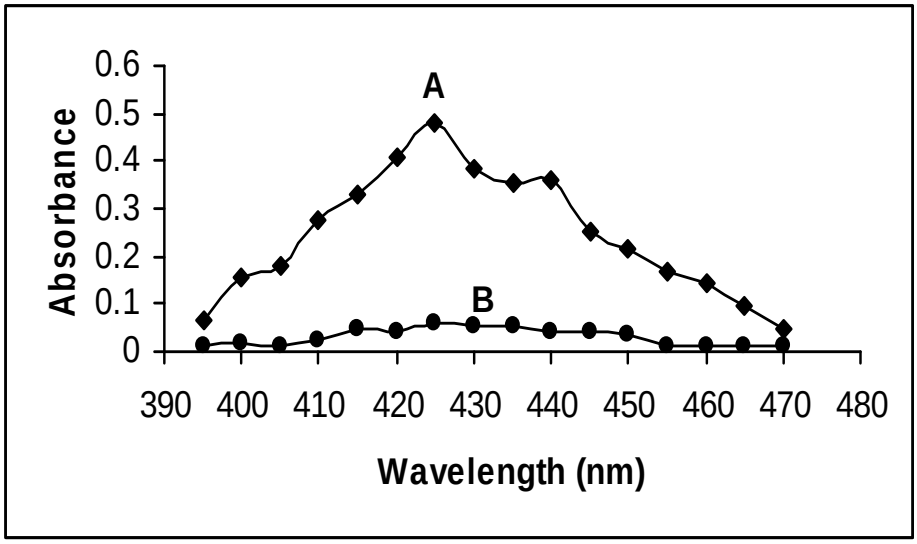

Figure 3: A. Absorption curve of PDE-BPB ion pair complex
B. Absorption curve of reagent blank

3.2. Optimization of experimental variables

The different experimental parameters (Concentration of the BPB, acidity and extracting solvent) affecting the formation of the PDE-BPB ion pair complex were extensively studied to determine the optimal reaction conditions for the determination of PDE. The optimization of experimental variables is commonly accomplished by sequentially optimizing one variable at a time while keeping all other variables constant. The optimum values of the reaction conditions were maintained throughout the experiment.

3.2.1. Effect of bromophenol blue concentration

The effect of the concentration of BPB was studied by treating 50 µg mL^{-1} PDE with 1 mL of 0.5 N HCl and varying volumes (0.5–4.0 mL) of 0.5% BPB. The rate of formation of the PDE-BPB ion pair complex was increased with increasing volume of 0.5% BPB and became constant at 2.0

mL; above this volume, the rate of PDE-BPB ion pair complex formation remained unchanged (Figure 4). Thus a volume of 2 mL of 0.5% BPB was chosen for the quantification process.

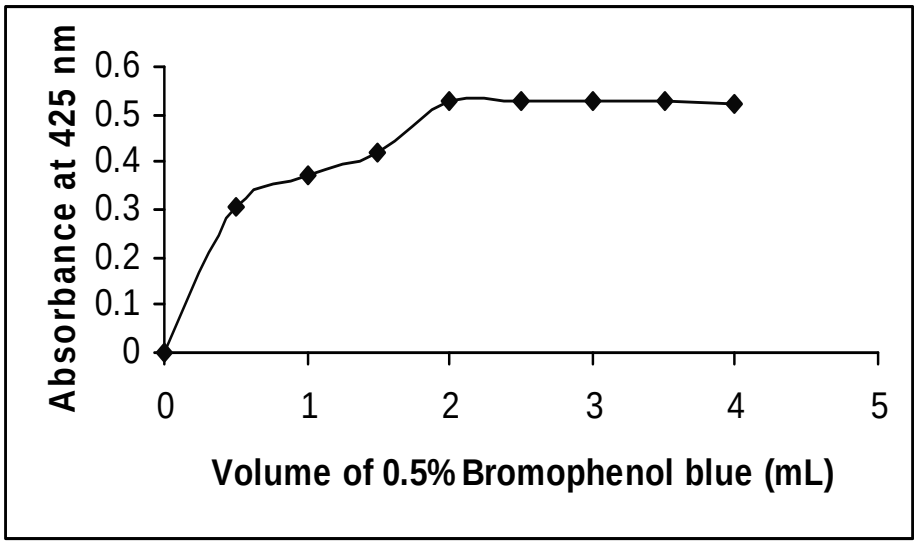

Figure 4: Effect of concentration of bromophenol blue

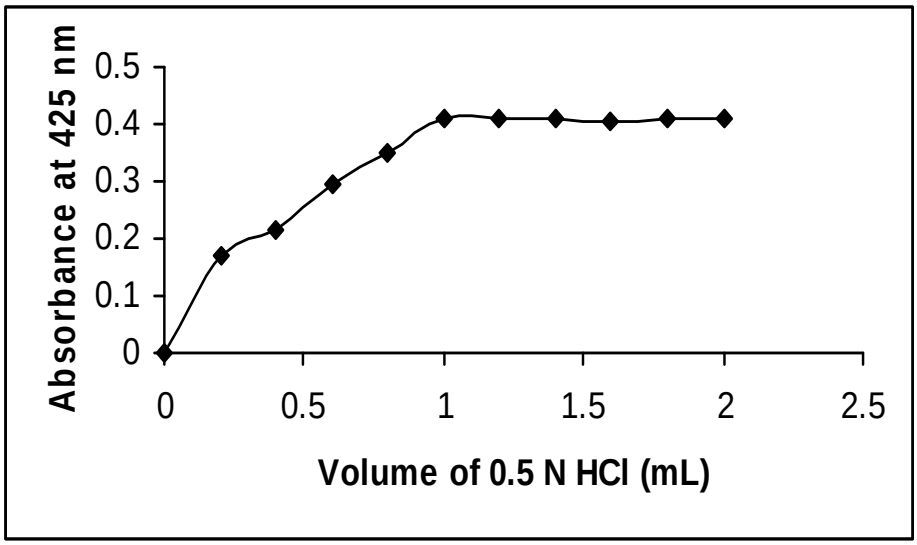

Figure 5: Effect of concentration of HCl

3.2.2. Effect of HCl concentration

The effect of HCl concentration on the formation of the PDE-BPB ion pair complex was studied by adding varying volumes (0.2–2.0 mL) of 0.5 N HCl and 2 mL of 0.5% BPB to 1.0 mL of PDE (50 µg mL^{-1}). As shown in Figure 5, the rate of formation of the PDE-BPB ion pair complex was found to increase with increasing HCl concentration and became constant at 1 mL. Beyond this volume, the formation of the PDE-BPB ion pair complex remained constant. Therefore all subsequent measurements were made using 1 mL of 0.5 N HCl.

3.2.3. Effect of extracting solvent

Different organic solvents (chloroform, benzene, toluene, cyclohexane, carbon tetrachloride and *i*-amylalcohol) have been tried in order to investigate their ability to extract the PDE-BPB ion pair complex. Among the various organic solvents tried, chloroform was found to be the most appropriate solvent. Hence it was chosen for extraction of the PDE-BPB ion pair complex from the aqueous phase.

3.3. Method validation

3.3.1. Linearity

Under the optimized experimental conditions, the absorbance at 425 nm was found to be directly proportional to the concentration of PDE. The standard curve obtained by plotting absorbance at 425 nm *versus* final concentration of PDE showed a linear relationship over the range 5-125 µg mL^{-1} (Figure 6). The regression analysis using the method of least square was performed to calculate the slope, intercept and regression coefficient. The results are summarized in Table 1. The high value of the regression coefficient and the small value of the intercept of the regression equation proved the linearity of the proposed method.

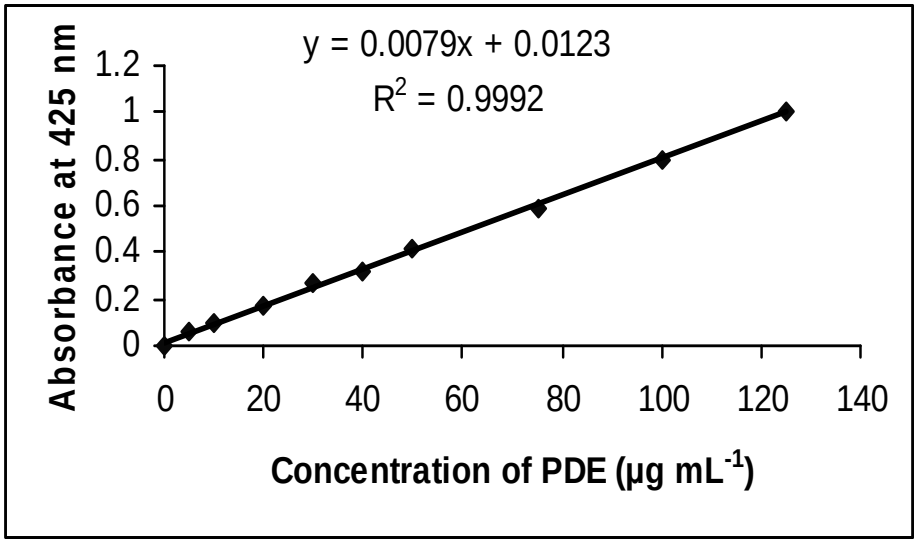

Figure 6: Linearity curve

3.3.2. Sensitivity

The sensitivity of the proposed method was assessed by calculating molar absorptivity, sandell's sensitivity, limit of detection and limit of quantification according to ICH guidelines [29]. The results are compiled in Table 1 which revealed the high sensitivity of the method.

3.3.3. Stability of PDE-BPB ion-pair complex

The stability of the PDE-BPB ion pair complex was monitored by keeping the solution at room temperature (25±1°C) and then measuring the absorbance of the solution at 425 nm at regular intervals of time. There was no change in the absorbance for at least 10 hours. The colored ion pair complex was stable for at least 10 hours at 25 ± 1°C. The stability of this ion pair complex helped in proceeding with large batches of samples and their comfortable measurements easily.

Table 1: Optical and regression characteristics of proposed method

Parameters	Values
λ_{max} (nm)	425
Beer's Limit (µg mL^{-1})	5-125
Molar Absorbitivity (L $mole^{-1}$ cm^{-1})	5.210 x10^3
Sandell's sensitivity (µg cm^{-2}/0.001 Absorbance unit)	0.0847
Regression equation (Y= mx + c)$^{\$}$	
Slope (m)	0.0079
Intercept (c)	0.0123
Regression coefficient (r^2)	0.9992
LOD (µg mL^{-1})	0.256
LOQ (µg mL^{-1})	0.775

$^{\$}$*Y = mx + c, where Y is the absorbance and x is the concentration of drug in µg* mL^{-1}

3.3.4. Selectivity

The selectivity of the proposed method was evaluated by analysis of synthetic mixture and placebo blank. The synthetic mixture solution was assayed by the proposed method at three different concentration levels (within linearity limit) in the presence of excipients such as starch, acacia, hydroxyl cellulose, sodium citrate, talc, magnesium stearate, lactose, glucose and sodium alginate, which are normally added to tablet. The percentage recoveries and RSD values are in the range of 99.93-100.50% and 0.716–0.942%, respectively. The results are presented in Table 2 which demonstrated the accuracy as well as the precision of the proposed method. This confirms the selectivity of the proposed method. The absorbance values of the placebo blank solution were almost equal to the absorbance of the reagent blank which revealed no significant interference from the excipients.

Table 2: Results of selectivity of proposed method

Concentration of PDE (µg mL^{-1})		RSD (%)	Recovery (%)
Taken	Found ± SD (n=5)		
10	10.05 ± 0.072	0.716	100.50
65	64.96 ± 0.612	0.942	99.93
120	120.08 ± 0.937	0.780	100.06

Table 3: Results of inter-day and intra-day precision and accuracy

	Concentration of PDE (µg mL^{-1})		RSD (%)	Recovery (%)	Error (%)
	Taken	Found ± SD (n=5)			
Intra-day	10	9.96 ± 0.089	0.893	99.60	0.400
	65	65.06 ± 0.584	0.897	100.09	0.092
	120	120.04 ± 0.986	0.821	100.03	0.033
Inter-day	10	10.05 ± 0.096	0.955	100.50	0.500
	65	64.96 ± 0.782	1.203	99.93	0.061
	120	119.95 ± 1.041	0.867	99.95	0.041

3.3.5. Precision

The precision of the proposed method was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was carried out by performing five repeated

analysis of PDE at three different concentrations levels on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis for five consecutive days (inter-day). The RSD values of repeatability (intra-day) and intermediate precision (inter-day) showed that the precision of the proposed method was acceptable. The results of this study are given in Table 3.

3.3.6. Accuracy

The accuracy of the proposed method was determined by analyzing a known amount of PDE at three different concentrations levels by the proposed method. Accuracy was evaluated as percentage relative error between the measured mean concentrations and taken concentrations. The results are shown in Table 3, from which it is clear that the accuracy is excellent.

The accuracy of the proposed method was further ascertained by performing recovery studies using standard addition method. For this purpose, preanalysed tablet powder was spiked with pure PDE at two different concentration levels and the total amount was once again estimated by the proposed method. Each determination was repeated five times. The results obtained through the standard addition method showed that the mean recoveries and relative standard deviations were in the range of 99.66% - 100.37% and 0.702% - 0.821%, respectively. The results (Table 4) revealed that tablet excipients did not interfere in the assay procedure.

Table 4: Results of recovery studies

Concentration of PDE (mg)		Found ± SD (n=5)	RSD (%)	Recovery (%)
Tablet	Spiked			
4	2	5.98 ± 0.042	0.702	99.66
4	4	8.03 ± 0.066	0.821	100.37

Table 5: Results of robustness of proposed method

Parameter	Concentration of PDE (µg mL^{-1})		RSD (%)	Recovery (%)
	Taken	Found ± SD (n=5)		
0.5 % BPB ± 0.2 mL	10	9.95 ± 0.094	0.944	99.50
	120	120.04 ± 0.876	0.729	100.03
0.5 N HCl ± 0.1 mL	10	10.08 ± 0.099	0.982	100.80
	120	120.05 ± 0.993	0.827	100.04

3.3.7. Robustness

The robustness of the proposed method was assessed by introducing slight deliberate changes in the concentration of BPB & HCl and the effects on the results were examined. The volume of 0.5% bromophenol blue and 0.5 N HCl was varied as optimum value ±0.2 and ±0.1 mL, respectively. Robustness was carried out at two different concentration levels (10 and 120 µg mL^{-1}). Under these conditions, perindopril was analyzed by the proposed method. The results are compiled in Table 5, indicated robustness of the method.

3.4. Application of the proposed method

The developed and validated method was applied to the quantification of PDE in two brands of tablet dosage forms. The amount of PDE in tablet was computed from the calibration curve or from the regression equation. The results of the proposed method were statistically compared

with those obtained by the official method by applying Student's *t*-test for accuracy and *F*-test for precision. The results are presented in Table 6. The calculated *t*- and *F*-values did not exceed the theoretical values of 2.77 and 6.39 for t- and F- tests, respectively at the 95% confidence level for four degrees of freedom. The results confirmed that the proposed method and the official method did not differ significantly with respect to accuracy and precision.

Table 6: Results of tablet dosage forms containing PDE analyzed by proposed and official methods

Method	Brand name of tablet	Concentration of PDE (mg)		RSD (%)	Recovery (%)	t value	F value
		Tablet	Found ± SD (n=5)				
Proposed	Conversyl[1]	2	2.10 ± 0.017	0.944	105.00	0.893	2.637
	Periguard[2]	4	3.96 ± 0.026	0.729	99.00	1.068	2.951
Official	Conversyl	2	2.06 ± 0.019	0.982	103.00	-	-
	Periguard	4	4.02 ± 0..32	0.827	100.50	-	-

1. Serdia pharmaceuticals Pvt. Ltd., Mumbai, India
2. Glenkmark pharmaceuticals Ltd., Mumbai, India.

CONCLUSION

A simple, selective, robust, precise and accurate spectrophotometric method was developed and validated for the assay of PDE in bulk and tablet dosage forms. The proposed method was found to be the best among the reported spectrophotometric methods with respect to sensitivity, cost and stability of the colored product formed. The method is economical, rapid and do not require any sophisticated instruments in contrast to radioimmunoassay, amperometry biosensor and chromatographic methods. The common excipients, normally added to the tablets, did not interfere in the assay of PDE by the proposed method. These advantages encourage the application of the method for the routine analysis of PDE in quality control laboratories.

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