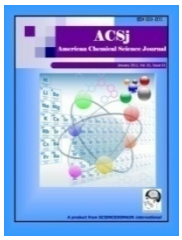

American Chemical Science Journal
2(4): 161-176, 2012

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Determination of Angiotensin-Converting Enzyme Inhibitor, Perindopril Erbumine, in Bulk and Tablet Dosage Form with HPLC

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Authors' contributions

This work was carried out in collaboration between all authors. Authors APR and CBS designed the concept and experiments. The optimization of experimental variables and studies on the validation parameters was carried out by authors CNL and SB. Authors CNL and SB collected the necessary literature for the proposed method. The final version of the manuscript was verified by authors APR and CBS. All authors read and approved the final manuscript.

Research Article

Received 14th August 2012
Accepted 5th November 2012
Published 20th November 2012

ABSTRACT

Aim: To develop and validate a simple HPLC method for the determination of perindopril erbumine (PDE) in bulk and tablets.
Place and Duration of Study: Rainbow Pharma Lab, Hyderabad, India, between April and June 2012.
Methodology: The separation of PDE was carried out on a Zorbax XDB C8 (250 mm × 4.6 mm I.D., 5 µm particle size) analytical column. The mobile phase was phosphate buffer (pH 4.5)-acetonitrile (60:40 v/v). The flow rate and wavelength were set to 1.3 mL/min and 242 nm, respectively. As per ICH guidelines, the proposed method was validated. The developed method was successfully applied for the estimation of PDE in tablets and results were compared statistically with the official method.
Results: The developed method showed a linear response from 8 to 80 µg/mL, with a

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regression coefficient (R^2) of 0.9996. The LOD and LOQ were 2.4 and 8µg/mL, respectively. The selectivity studies showed that the method was selective and free from interfering common excipients. The intra- and inter-day RSD were in the range of 0.043-0.234% and 0.180-0.654%, respectively. The results of recovery studies were good. The stability data of the PDE indicate that the drug was stable for 72 hours.
Conclusion: The developed method was linear, sensitive, selective, precise, accurate and robust, being suitable for routine quality control analyses of PDE.

Keywords: Perindopril erbumine; HPLC; method development; isocratic elution; method validation.

1. INTRODUCTION

Perindopril erbumine (PDE), chemically known as 2-methylpropan-2-amine (2S, 3aS, 7aS)-1-[(2S)-2-[[(1S)-1-(ethoxycarbonyl) butyl] amino] propanoyl] octahydro-1H-indole-2-carboxylic acid (Fig. 1), is a nonsulfhydryl angiotensin-converting enzyme inhibitor. PDE is used in the treatment of hypertensive patients with heart failure, post myocardial infarction, high coronary disease risk, diabetes mellitus, chronic renal failure, and/or cerebrovascular disease (Alfakih and Hall, 2006; Cleland et al., 2006; Hurst and Jarvis, 2001; Jastrzebskal et al., 2004; Leenen et al., 2000).

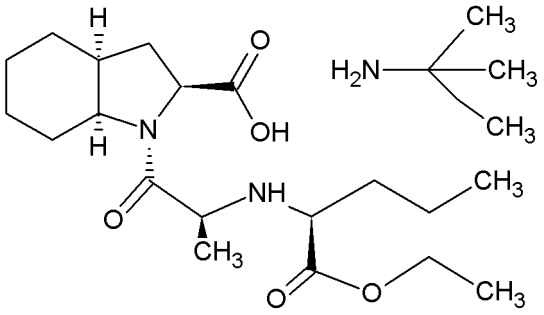

Fig. 1. Perindopril erbumine structure

PDE was official in British Pharmacopoeia (British Pharmacopoeia, 2009) and European Pharmacopoeia (European Pharmacopoeia, 2008), which describes a potentiometric titration method for the assay of PDE. Various analytical methods have been reported in the literature for the assay of PDE in pure, pharmaceuticals preparations and/or biological samples. Procedures using visible spectrophotometry (Abdellatef, 1998; Abdellatef et al., 1999; Nafisur et al., 2006; Nafisur and Habibur, 2011), HPTLC (Dewani et al., 2010), LC–MS/MS (Deepak, et al., 2006a, b; Nirogi, et al., 2006), capillary gas chromatography (Lin et al., 1996), GC-MS (Maurer et al., 1998), radioimmunoassay (Van den Berg et al., 1991), amperometry biosensor (Aboul-Enein et al., 1999; Stefan et al., 1999a; Stefan et al., 1999b; Van Staden et al., 2000) and potentiometry (Stefan et al., 1999c) have been reported by several researchers.

The above mentioned visible spectrophotometric methods (Abdellatef, 1998; Abdellatef et al., 1999; Nafisur et al., 2006; Nafisur and Habibur, 2011) undergo one or more drawbacks such as lack of sensitivity, use of expensive reagent and unstable colored product. The

remaining reported methods exhibit some drawbacks such as lack of sensitivity (Dewani et al., 2010; Stefan et al., 1999c), narrow range of linear response (Dewani, et al., 2010), use of expensive detector (Deepak et al., 2006a, b; Nirogi et al., 2006; Maurer et al., 1998), derivatization of sample (Lin et al., 1996), cumbersome procedure (Van den Berg et al., 1991; Aboul-Enein et al., 1999; Stefan et al., 1999a; Stefan et al., 1999b; Van Staden et al., 2000) and lack of accuracy and precision (Stefan et al., 1999c). Hence the above reported methods are not suitable for the routine analysis of PDE.

High performance liquid chromatrography (HPLC) is an analytical technique for the enhancement of sensitivity and specificity in quantitative analysis of various pharmaceutical compounds. Several HPLC methods (Prajapati et al., 2011; Ali et al., 2012; Hitesh et al., 2010; Jain et al., 2012; Joseph et al., 2011; Suraj et al., 2012; Ankit et al., 2010; Patel et al., 2011) have been reported for estimation of PDE in combination with other drugs such as amlodipine besylate (Prajapati, et al., 2011; Ali et al., 2012), indapamide (Hitesh et al., 2010; Jain et al., 2012; Joseph et al., 2011), hydrochlorothiazide (Suraj et al., 2012) and losartan potassium (Ankit et al., 2010; Patel et al., 2011). To the best of our knowledge, there are only four reports (Bhaskara Raju and Lakshmana Rao, 2011; Prameela Rani and Bala Sekaran, 2009; Khomushku, et al., 2011; Gumieniczek and Hopkala, 1998) dealing with the application of HPLC for the individual determination of PDE in bulk and tablet dosage forms.

In a method reported by Bhaskara Raju and Lakshmana Rao (2011) separation and quantification were achieved on a reverse phase C18 column using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 65:35 *v/v*. The detection wavelength was 209 nm. The linearity was observed in the range of 20-100 µg/mL with a correlation coefficient of 0.9997. A HPLC method (Prameela Rani and Bala Sekaran, 2009) with UV detection at 215 nm has been carried out with a C18 column using a mobile phase consisting of methanol and water. The linear range was 4-20 µg/mL. A HPLC method (Khomushku et al., 2011) for PDE in bulk has been reported and was carried out on an ODS-C18 column with acetonitrile, aqueous heptanesulfonic acid and triethylamine as the mobile phase. In a method proposed by Gumieniczek and Hopkala (1998) PDE was determined in tablets by HPLC at 211 nm on LiChrosorb RP-18 column with buffer:acetonitrile (6:4, v/v) as the mobile phase. This method uses quinapril as internal standard and exhibit linearity over the concentration range of 0.1-0.5 mg/ml. The reported HPLC methods (Bhaskara Raju and Lakshmana Rao, 2011; Prameela Rani and Bala Sekaran, 2009; Khomushku et al., 2011; Gumieniczek and Hopkala, 1998) suffers from disadvantages like poor selectivity, sensitivity, long retention time, use of internal standard, lack of accuracy and precision.

In the present work, an attempt is made to develop and validate a simple, sensitive, efficient and reliable method (without the use of internal standard) for the determination of PDE in bulk and tablet dosage forms by HPLC with UV detection.

2. MATERIALS AND METHODS

2.1 Apparatus

All HPLC experiments were carried out on a Waters Alliance 2695 separation module, having waters 2996 photodiode array detector in isocratic mode. Data collection and processing was done using Millennium 32 software. The analytical column used for the separation was 250 mm × 4.6 mm I.D., 5 µm particle size, Zorbax XDB C8 column.

2.2 Chemicals

All chemicals were of HPLC grade quality. Milli-Q-water, potassium dihydrogen phosphate (≥99.9% purity), methanol (99.9% purity), orthophosphoric acid (85 % purity) used in the process was obtained from Thermo Fisher Scientific India Pvt. Ltd, Mumbai, India.

2.3 Preparation of Solutions

2.3.1 Diluent

Diluent was prepared by mixing methanol and water in the ratio of 50:50 *v/v*

2.3.2 1 M Phosphate buffer (pH-4.5)

The 1M phosphate buffer was prepared by dissolving 136.09 gm of potassium dihydrogen phosphate in 1000 mL deionized water. The pH of the buffer was adjusted to 4.5 with orthophosphoric acid.

2.3.3 Mobile phase

The mobile phase consisted of phosphate buffer, pH 4.5 and acetonitrile (60:40, *v/v)*.

2.3.4 Standard solutions of PDE

Pharmaceutical grade PDE was kindly obtained as gift sample from Hetero Drugs Ltd, Hyderabad. Stock solution of PDE (1 mg/mL) was prepared by dissolving 100 mg of PDE in methanol:water (50:50, *v/v*). Working standard solutions equivalent to 8, 16, 32, 48, 54 ad 80 µg/mL PDE were prepared by appropriate dilution of the stock standard solution (1 mg/mL) with the diluent.

2.3.5 Tablet dosage form of PDE

The tablet dosage form, Conversyl (Serdia Pharmaceuticals Pvt. Ltd. Mumbai), containing 4 and 8 mg of PDE was purchased from the local pharmacy and used in the present investigation.

2.3.6 Tablet extract

Twenty five tablets containing PDE were exactly weighed and ground into a fine powder. From this powder, an amount of the tablet powder equivalent to 25 mg PDE was transferred to a 25 mL standard flask containing 10 mL of diluent and shaken for 10 minutes. The volume was made up to the mark with diluent and mixed well. The solution was filtered through a 0.45 µm membrane filter. The filtered solution was appropriately diluted with diluent to obtain a concentration of 50 µg/mL.

2.3.7 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 “Tablet extract” and then subjected to analysis.

2.4 Chromatographic Conditions

The mobile phase was a mixture of phosphate buffer and acetonitrile (60:40 *v/v*). The contents of the mobile phase were filtered, before it was used, through 0.45 µm membrane filter, degassed with a helium sparge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1.3 mL/min. The column temperature was maintained at 45ºC. The injection volume of samples was 10 µL. The analyte was monitored at a wavelength of 242 nm.

2.5 General Assay Procedure

To saturate the column, the mobile phase was pumped for about 30 minutes thereby to get the base line corrected. 10 µL of each working standard solution of PDE (8, 16, 32, 48, 54 ad 80 µg/mL) was injected automatically into the column in triplicate and the peaks were recorded at 242 nm. The peak areas of PDE were plotted against the corresponding nominal concentration to obtain calibration graph. The concentration of the drug was calculated from the calibration graph or the regression equation.

2.6 Procedure for Tablet Dosage Form

Aliquot (10 µL) of tablet extract was injected into the column in triplicate. The area under the peak was noted and the drug content in the tablets was quantified using the calibration graph or regression equation.

3. RESULTS AND DISCUSSION

3.1 Optimization of the Chromatographic Conditions

In order to develop an efficient and simple HPLC method for the analysis of the PDE in bulk and in its tablet dosage forms, preliminary tests were conducted to select the satisfactory and optimum conditions. HPLC parameters, such as detection wavelength, ideal mobile phase and their proportions, flow rate and column temperature were carefully studied. The HPLC parameters were finally chosen based on the criteria of peak properties (retention time and peak asymmetry) and sensitivity (height and area).

Two different columns (Phenomenex ODS C18 column, 250 mm × 4.6 mm I.D. particle size 5 µm and Zorbax XDB, C8 column, 250 mm × 4.6 mm I.D., particle size 5 µm) were tested initially. The peak shape and retention time was taken into consideration. The best results were obtained for Zorbax XDB, C8 column, which was choosen for the determination process.

In order to probe the appropriate wavelength for the determination of PDE, the solution of PDE (20 µg/mL) was scanned by UV spectrophotometer in the range 200-400 nm. Alternatively, the solution of PDE (20 µg/mL) was also injected to HPLC directly at different wavelengths. The PDE exhibited two peaks, one at 212 nm and another at 242 nm. The results of linearity and selectivity studies were not satisfactory at 212 nm. Therefore, it was concluded that 242 nm is the most suitable wavelength for the analysis of PDE.

In the investigation of an apt mobile phase, initially four different buffers (sodium acetate, potassium dihydrogen orthophosphate, sodium dihydrogen orthophosphate and ammonium acetate) were employed in the study to achieve the better sensitivity and short analysis time. In this attempt, potassium dihydrogen orthophosphate buffer was chosen for further studies as it produced sharp peak with less retention time than other three buffers. The phosphate buffer with different pH (3-7) was tried. It was found that a sharp and symmetrical peak shape with less retention time was observed at pH 4.5. Hence, phosphate buffer with pH 4.5 was chosen.

Mobile phases consisting of phosphate buffer system (pH 4.5) with acetonitrile, methanol or isopropanol as organic solvents in different volumetric ratios were investigated to meet the required system parameters. The experiments indicated that better sensitivity was not achieved while using methanol or isopropanol with phosphate buffer. The mixture of phosphate buffer (pH 4.5) and acetonitrile was capable of providing good results. After trying different ratios of mixtures of phosphate buffer and acetonitrile (50:50, 60:40, 70:30 *v/v*), the best results were achieved by using a mixture of phosphate buffer and acetonitrile (60:40 *v/v*) as mobile phase.

The mobile phase with different flow rates (0.8-1.6 mL/min) was investigated. At the flow rate 1.3 mL/min, symmetric and well retained peak was obtained. Therefore, for the present study the flow rate 1.3 ml/min was selected.

The effect of temperature on the column efficiency was studied. Column temperature was maintained at 30, 35, 40, 45 and 50ºC and the chromatograms were recorded. The best peak shape along with the uppermost column efficiency was achieved at 45ºC. Under the stated chromatographic conditions, the retention time was 3.409 min. A model chromatogram is shown in Fig. 2.

3.2 Validation

As per ICH guidelines, the proposed method was validated (Validation of Analytical Procedures ICH, 2005).

3.2.1 System suitability

System suitability test was carried out on freshly prepared solution of PDE (48 µg/mL) to ensure the validity of the proposed method. Data from five injections were used to confirm system suitability parameters like retention time, peak area, peak asymmetry, theoretical plates, plates per meter and height equivalent to theoretical plate. The results are presented in Table 1. The values obtained established the suitability of the system for the analysis of the PDE.

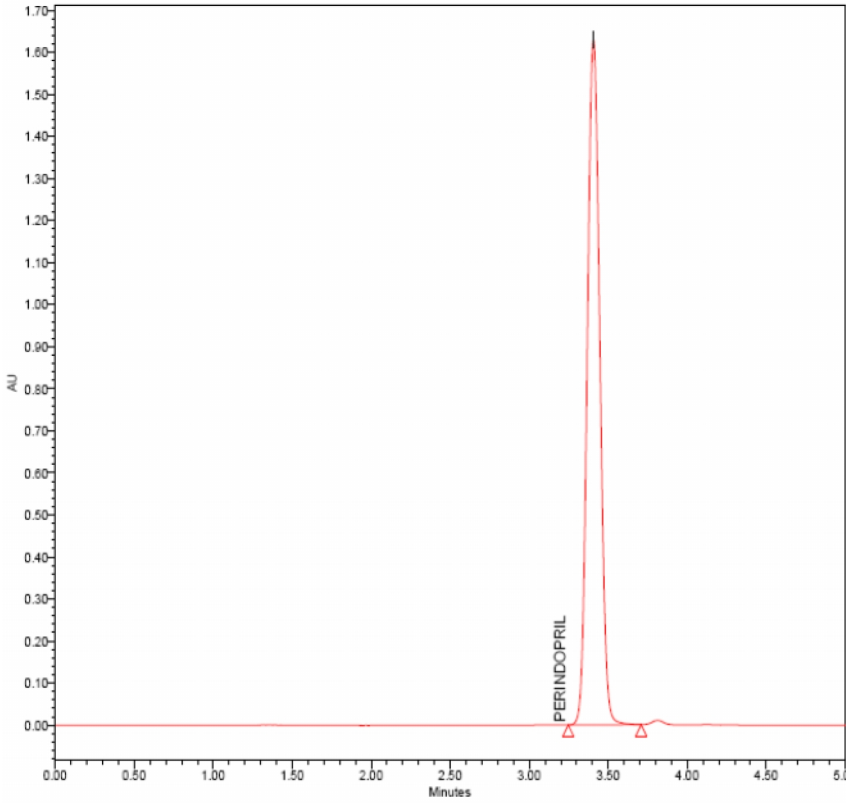

Fig. 2. Chromatogram of PDE (48 µg/mL)

Table 1. System suitability test results

Parameter	Value	RSD%
Retention time (t) (Min)	3.409	0.065
Peak area	8947390	0.100
Theoretical Plates (n)	8923	0.489
Plates per Meter (N)	35692	0.608
Height equivalent to theoretical plate(HETP) (mm)	2.79×10^{-5}	0.759
Peak asymmetry	1.080	0.257

3.2.2 Selectivity

Selectivity is the ability of an analytical method to distinguish between the analyte of interest and other components present in the sample. To identify the interference by the excipients, the tablet extract and placebo blank solution were prepared according to procedure described under “tablet extract” and “placebo blank”, respectively. 10 µL of tablet extract and placebo blank was injected into the column. The resulting chromatograms (Fig. 3 and 4) did not show any peak at the retention time of 3.409 minute, which confirmed the selectivity of the method. The selectivity of the method was also confirmed by interference check by injecting the diluent blank to determine whether any peaks in the diluent are co-eluting with PDE peak. No interference of peaks eluted in the diluent blank with PDE peak was observed (Fig. 5).

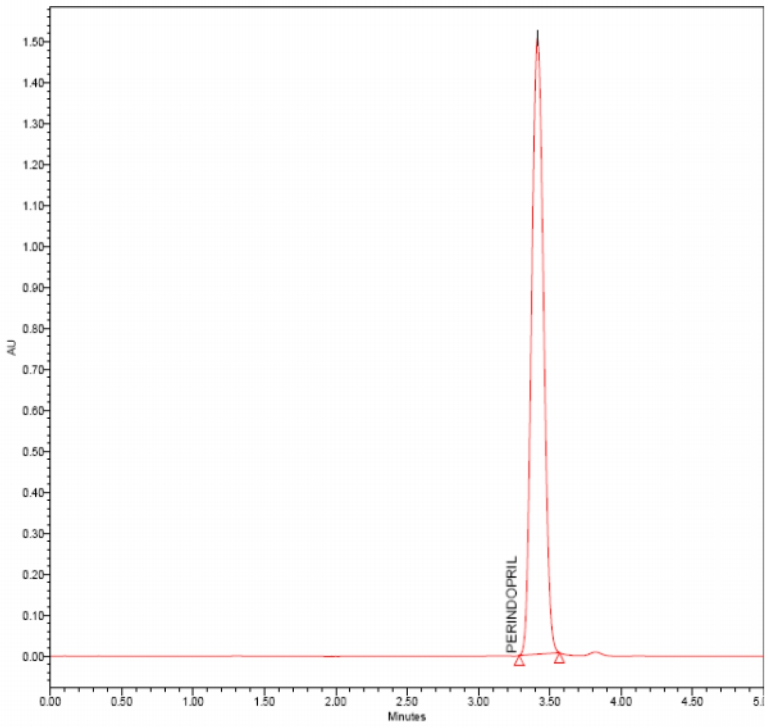

Fig. 3. Chromatogram of tablet extract (50 µg/mL)

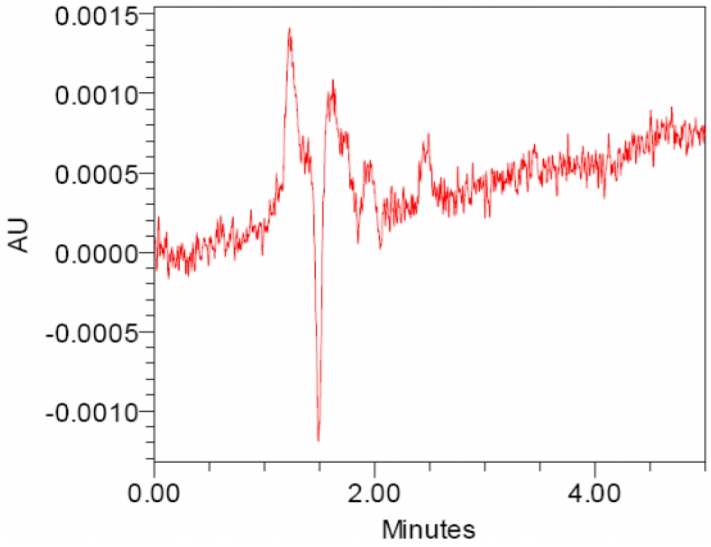

Fig. 4. Chromatogram of placebo blank

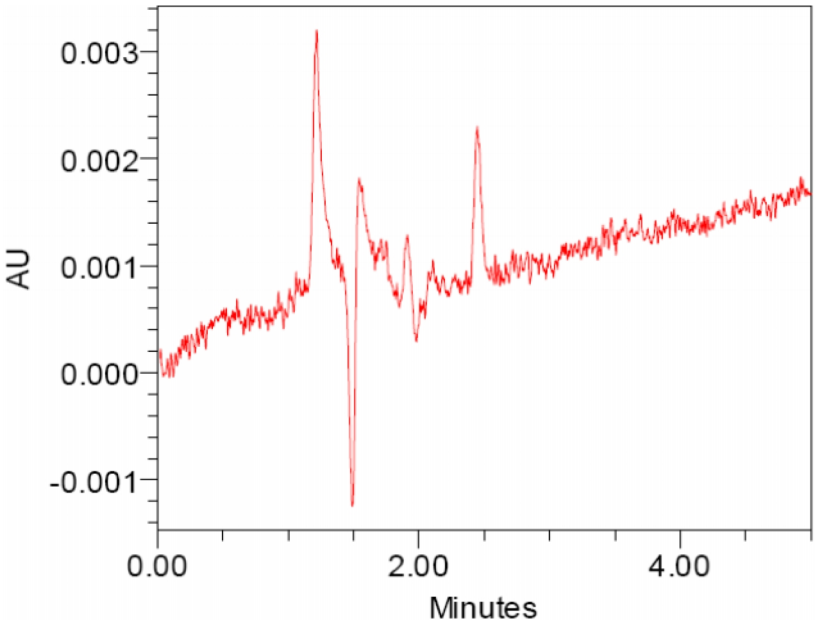

Fig. 5. Chromatogram of diluent Blank

3.2.3 Linearity

The linearity was determined by constructing calibration curve. A calibration curve was constructed using least squares method by plotting the peak area *vs* concentration of PDE. The calibration curve for PDE showed good linearity in the concentration range of 8-80 µg/mL. The corresponding linear regression equation was y=1672x+1298 (where y is the peak area and x is the concentration of PDE in µg/mL with excellent regression coefficient (0.9996). The percentage RSD (n=3) of slope and intercept are 0.986 and 1.021, respectively.

3.2.4 The limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the proposed method were determined as S/N ratio 3 for LOD and 10 fold for LOQ. The low values of LOD and LOQ, that is, 2.4 and 8.0 µg/mL for PDE, respectively suggest the high detection capability of the proposed method.

3.2.5 Accuracy and precision

The intra-day and inter-day precision and accuracy of the proposed method was assessed by performing analysis of three different standard solutions containing 16, 48 and 80 µg/mL PDE under the optimized experimental conditions. In intra-day studies, the PDE was analyzed on the same day whereas inter-day studies were determined by analyzing the PDE for three consecutive days. The precision and accuracy are expressed as relative standard deviation and relative error, respectively. The results are presented in Table 2. The results were found to be satisfactory. Hence the proposed method is precise and accurate.

Table 2. Precision and accuracy results

	Concentration of PDE (µg/mL)		%	%	%
	Taken	Found ±SD	RSD	Recovery	Error
Intra-day[a]	16	16.030 ± 0.037	0.234	100.187	0.187
	48	48.002 ± 0.020	0.043	100.004	0.004
	80	79.820 ± 0.145	0.181	99.775	0.225
Inter-day[b]	16	16.050 ± 0.094	0.585	100.312	0.312
	48	47.960 ± 0.314	0.654	99.916	0.084
	80	80.120 ± 0.145	0.180	100.150	0.150

a. Found value is the average of five determinations
b. Found value is the average of three determinations

3.2.6 Recovery studies

The accuracy of the proposed method was also further assessed by performing recovery experiments with the standard addition method. Known amount of the pure PDE (at three different concentration levels) was added to pre-analyzed formulation and the total concentration was once again determined by the proposed method. The results are summarized in Table 3. The results revealed that any small change in the drug concentration in the solutions could be accurately determined by the proposed method. The closeness of the recoveries suggests lack of interference from tablet excipients and thus establishes some degree of selectivity.

Table 3. Standard addition method results

Labelled claim (mg/tablet)	Added amount (mg)	Found (mg) ± S.D (n=5)	% RSD	% Recovery
8	4	11.980 ± 0.021	0.180	99.833
8	8	16.025 ± 0.086	0.539	100.156
8	12	19.983 ± 0.048	0.241	99.915

3.2.7 Robustness

To determine the robustness of the proposed method, small deliberate changes in the chromatographic conditions like mobile phase composition, flow rate, detection wavelength and column temperature were made. The results are summarized in Table 4. The low % RSD values indicate that the proposed method was adequately robust for generally expected variations in chromatographic conditions.

3.2.8 Stability of PDE standard solution

Standard solutions of PDE (10 and 70 µg/mL) were prepared and stored in refrigerator below 10ºC (stability samples) for 72 hours. At the time of analysis, freshly prepared standard solutions of PDE were used as comparison samples. The stability of standard solutions of PDE was successfully assessed by comparing mean responses of six replicates of stability samples versus six replicates of comparison samples over a period of 72 hours. The results are presented in Tables 5 and 6. These experiments reveal that standard solutions of PDE are stable for at least 72 hours.

Table 4. Robustness results

Parameter	Concentration of PDE (µg/mL)		% RSD	% Recovery
	Taken	Found ±SD (n=3)		
Mobile phase[a]	10	9.95 ± 0.024	0.241	99.500
	70	70.12 ± 0.216	0.308	100.171
	10	10.08 ± 0.061	0.605	100.800
Flow rate[b]	70	69.94 ± 0.564	0.806	99.914
Wavelength[c]	10	9.96 ± 0.082	0.823	99.600
	70	69.89 ± 0.413	0.590	99.842
Column temperature[d]	10	10.15 ± 0.054	0.532	100.150
	70	70.07 ± 0.601	0.857	100.100

a. Phosphate buffer-acetonitrile ratios (v/v) – 60:40, 58:42 and 62:38; b. flow rate (mL/min) – 1.2, 1.3 and 1.4; c. Wavelength (nm) – 241,242 and 243; d. column temperature (ºC) – 43, 45 and 47

Table 5. Results of stability study of PDE (10 µg/mL) over 72 hours

Parameters	After 24 hours		After 48 hours		After 72 hours	
	Stability sample	Comparison sample	Stability sample	Comparison sample	Stability sample	Comparison Sample
Mean area[a]	1915056	1908102	1903757	1914585	1902917	1914776
% RSD	0.648	0.349	0.294	0.317	0.482	0.439
% Stability	100.36		99.43		99.38	

a. Average of six responses

Table 6. Results of stability study of PDE (70 µg/mL) over 72 hours

Parameters	After 24 hours		After 48 hours		After 72 hours	
	Stability sample	Comparison sample	Stability sample	Comparison sample	Stability sample	Comparison Sample
Mean area[a]	13379102	13356717	13038827	13319887	13078136	13379102
% RSD	0.961	0.293	0.618	0.384	0.267	0.293
% Stability	100.16		99.88		99.75	

a. Average of six responses

3.3 Application to Tablet Dosage Form

To find out the appropriateness of the proposed method for the assay of tablet dosage forms containing PDE was analyzed by the proposed method. The results obtained from the proposed method were compared statistically with official potentiometric titration method (British Pharmacopoeia, 2009) by applying Student's t-test for accuracy and F-test for precision. From the results (Table 7) it was found that the proposed method does not differ significantly in precision and accuracy from the official method.

Table 7. Assay of tablets results

Method	Labelled claim (mg)	Found (mg) ± S.D (n=5)	% RSD	% Recovery
HPLC	8	7.986 ± 0.065	0.822	99.825
	4	4.026 ± 0.038	0.943	99.825
Potentiometric titration	8	8.035 ± 0.058	0.721	100.437
	4	3.968 ± 0.026	0.655	99.200
	t- value*: 0.935			
	F- value**: 2.629			

**Tabulated t-value at 95% confidence level is 2.306*
***Tabulated F- value at 95 % confidence level is 6.390*

4. COMPARISION WITH REPORTED HPLC METHODS

In comparison with the reported HPLC methods for the determination of perindpril in tablets (Table 8), the proposed method is more sensitive (Gumieniczek and Hopkala, 1998), selective, (Bhaskara Raju and Lakshmana Rao, 2011; Prameela Rani and Bala Sekaran, 2009; Gumieniczek and Hopkala, 1998), precise and accurate (Bhaskara Raju and Lakshmana Rao, 2011; Prameela Rani and Bala Sekaran, 2009; Gumieniczek and Hopkala, 1998). Unlike the Gumieniczek and Hopkala (1998) method, the proposed method does not require internal standard which increases the simplicity of the method and decreases the cost of the analysis. The parameters like selectivity, robustness and stability of the drug were not reported in the earlier HPLC methods (Bhaskara Raju and Lakshmana Rao, 2011; Prameela Rani and Bala Sekaran, 2009; Khomushku et al., 2011; Gumieniczek and Hopkala, 1998). The proposed method is suitable for the processing of several samples in a limited time due to the short retention time (Khomushku et al., 2011; Gumieniczek and Hopkala, 1998).

Table 8. Comparison between proposed and reported methods

Parameters	References				Proposed method
	Bhaskara Raju, V., Lakshmana Rao, A. 2011	Prameela Rani, A., Bala Sekaran, C. 2009	Gumieniczek, A., Hopkala, H. 1998	Khomushku, G.M. et al., 2011	
Retention time (min)	3.122	2.793	4.16	11.5	3.409
LOD (µg/mL)	0.03	2	50	Not reported	2.4
LOQ (µg/mL)	0.12	7		Not reported	8
Linearity (µg/mL)	20-100	2-20	100-500	Not reported	8-80
Tailing factor	1.15	1.002	Not reported	< 2	1.08
% Recovery in tablets	99.60-100.15	99.00-100.50	95-102.75	Not reported	99.825
% RSD	Not reported	0.64-1.12	1.54-2.84	Not reported	0.822-0.943

5. CONCLUSION

A simple, sensitive, selective, accurate and precise HPLC method was developed for the determination of PDE in bulk and in tablet dosage forms. The short chromatographic time makes this method suitable for the processing of numerous samples in a limited time. The method has good accuracy and precision. The method shows no interference from tablet excipients. Hence, the proposed method could be useful and fit for the quantification of PDE in bulk and tablet dosage forms.

ACKNOWLEDGEMENTS

The authors, Chatragadda Nagalakshmi and Sunkara Bhavani, express their gratitude to the principal, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna nagar, Andhra Pradesh for providing research facilities.

COMPETING INTERESTS

The authors did not have any competing interests.

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