

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

Chatragadda Nagalakshmi¹, Avula Prameela Rani¹, Sunkara Bhawani¹

Chandra Bala Sekaran^{2*}

¹University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna nagar, India-522 510.

²Department of Biotechnology, Jagarlamudi Kuppaswamy Choudary College, Guntur, India-522 006.

ABSTRACT

A simple HPLC method for the determination of perindopril erbumine (PDE) in bulk and tablets was developed and validated. The separation was carried out on a 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. This method was linear, sensitive, selective, precise and accurate, being suitable for routine quality control analyses of PDE. The developed HPLC method was successfully applied for the estimation of PDE in their tablet dosage forms and results were compared statistically with the official method.

Key words: Perindopril Erbumine, HPLC, Method Development, Isocratic Elution, Method Validation.

* Address for correspondence: balumpil@gmail.com

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 (Figure 1), is a nonsulfhydryl angiotensin-converting enzyme inhibitor (Alfakih, K.K. and Hall, A.S. 2006; Cleland, J. G. et al. 2006; Hurst, M. and Jarvis, B. 2001; Jastrzebskal, M. et al. 2004; Leenen, F.H.H. et al. 2000). 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.

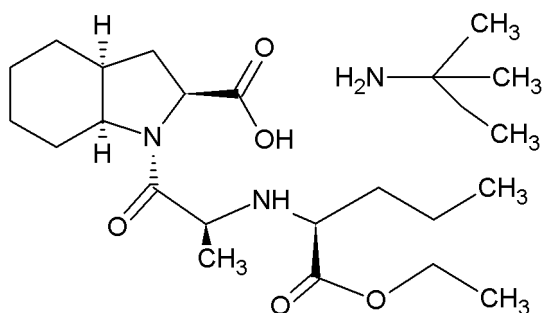


Fig. 1. Perindopril Erbumine structure

PDE was official in British Pharmacopoeia (British Pharmacopoeia, 2009). The British Pharmacopoeia 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, H.E. 1998; Abdellatef, H.E. et al. 1999; Nafisur, R. et al. 2006; Nafisur, R. and Habibur, R. 2011), HPTLC (Dewani, M.G. et al. 2010), LC–MS/MS (Deepak, S. J. et al. a, b2006; Nirogi, R.V. et al. 2006), capillary gas chromatography (Lin, S. J. et al. 1996), GC-MS (Maurer, H. H. et al. 1998), radioimmunoassay (Van den Berg H. et al. 1991), amperometry biosensor (Aboul-Enein, H.Y. et al. 1999; Stefan, R.I. et al. 1999a; Stefan, R.I. et al. 1999b; Van Staden, J. F. et al. 2000) and potentiometry (Stefan, R.I. et al. 1999c) have been reported by several researchers.

Only one HPLC method (Bhaskara Raju, V. and Lakshmana Rao, A. 2011) with ultra violet detection has been described in the literature for determination of PDE in tablet dosage forms.

Separation of the PDE was 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. The reported HPLC method suffers from disadvantages like poor selectivity, sensitivity, accuracy and precision.

In the present work, an attempt is made to develop and validate a simple, sensitive, efficient and reliable method for the determination of PDE in bulk and tablet dosage forms by HPLC with UV detection.

2. MATERIAL AND METHODS

2.1. Apparatus

All HPLC experiments were carried out on a Water Alliance 2695 separation module, having water 2996 photodiode array detector in isocratic mode. The system was associated with the help of Millennium 32 software in a computer system for data collection and processing. The analytical column used for the separation was 250 mm × 4.6 mm I.D., 5 µm particle size, C8 column.

2.2. Chemicals

All chemicals were of HPLC grade quality. Milli-Q-water, potassium dihydrogen phosphate, methanol, orthophosphoric acid 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. Phosphate buffer (pH-4.5)

The buffer was prepared by dissolving 136.09 gm of potassium dihydrogen phosphate in 500 mL of water in a 1000 mL volumetric flask and then made up to the mark with water. The pH of the buffer was adjusted to 4.5 with orthophosphoric acid.

2.3.3. Mobile Phase

Mobile phase 'A' consisted of Phosphate buffer. Mobile phase 'B' was acetonitrile. The mobile phase used for the analysis was prepared by mixing mobile phase 'A' and mobile phase 'B' in the ratio, 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 50 mL of diluent in a 100 mL volumetric flask and then made up to the mark with diluent. Working standard solutions equivalent to 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 45°C. The injection volume of samples was 10 µL. The analyte was monitored at a wavelength of 242 nm.

2.5. General Assay Procedure

The mobile phase was pumped for about 30 minutes to saturate the column thereby to get the base line corrected. 10 µL of each working standard solution of PDE (16, 32, 48, 54 ad 80 µg/mL) was injected automatically onto the column in triplicate and the peaks were determined at 242 nm. The peak areas of PPD 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

Ten 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 satisfactory and optimum conditions. HPLC parameters, such as detection wavelength, ideal mobile phase & their proportions and flow rate were carefully studied.

Preliminary experiments indicated that the C8 column (250 mm × 4.6 mm, 5 µm) provides efficient and reproducible separation of PPD at 45°C. Hence C8 column was selected for method development and validation. PDE was determined by injecting the drug solution on to C8 column with ultra violet detector set at 242 nm. After trying different ratios of mixtures of phosphate buffer and acetonitrile, the best results were achieved by using a mixture of phosphate buffer-acetonitrile (60:40 v/v) as mobile phase. At a flow rate of 1.3 mL/min, the retention time for PDE was 3.409 min. The analyte peak area was well defined and free from tailing under the described experimental conditions (Figure 2).

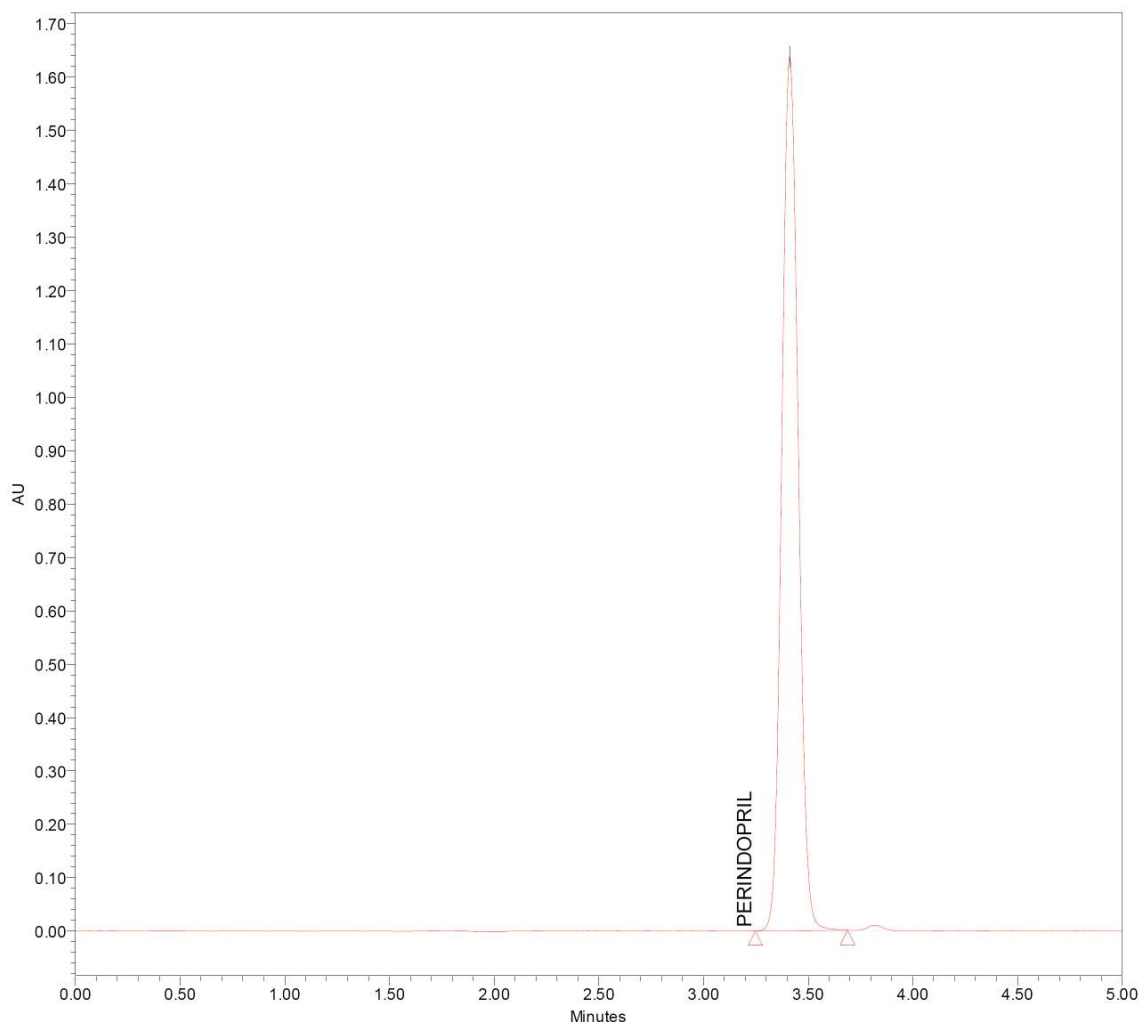


Fig. 2. Chromatogram of PDE (48 µg/mL)

3.2. Validation

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.

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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

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147 **3.2.2. Selectivity**

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

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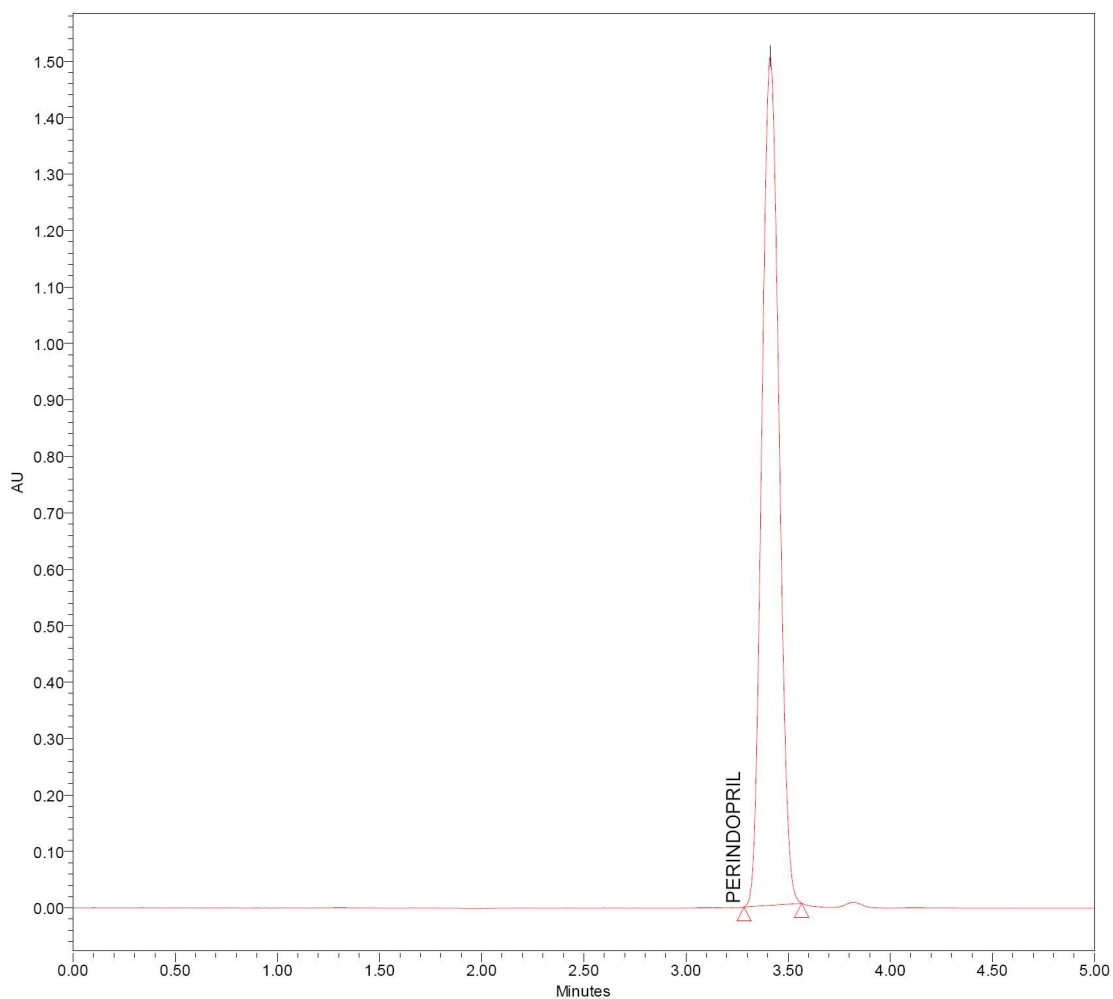


Fig. 3. Chromatogram of tablet extract (50 µg/mL)

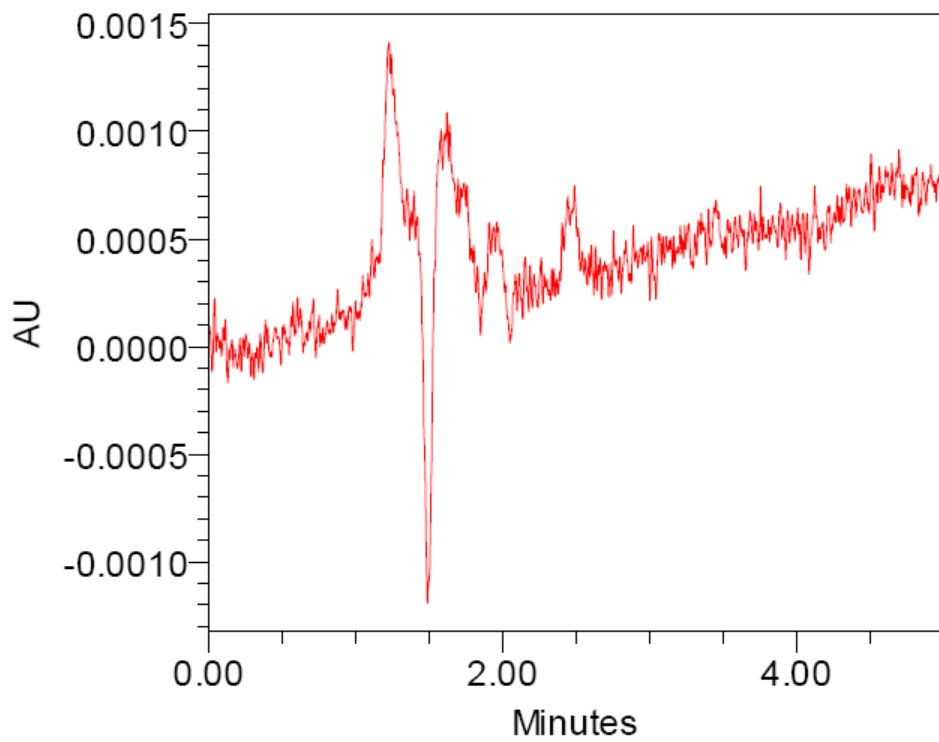


Fig. 4. Chromatogram of placebo blank

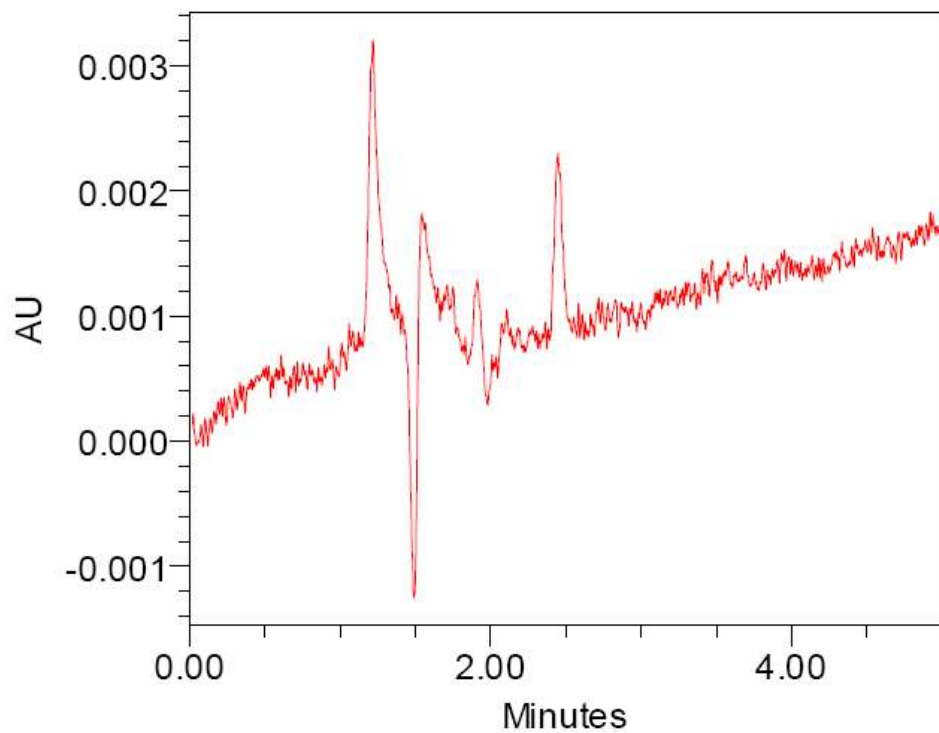


Fig. 5. Chromatogram of diluent Blank

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 16-80 µg/mL. The corresponding linear regression equation was $y = 164234x + 217948$ (where y is the peak area and x is the concentration of PDE in µg/mL) with excellent regression coefficient (0.9976).

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 8.2 ng/mL for PDE, respectively suggest the high detection capability of the proposed method.

3.2.5. Accuracy and Precision

The precision and accuracy of the proposed method was assessed by performing five repeated analysis of three different standard solutions containing 16, 48 and 80 µg/mL PDE under the optimized experimental conditions. 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 (n=5)	RSD	Recovery	Error
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

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 2. 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 2. Standard Addition Method Results

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

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 3) it was found that the proposed method does not differ significantly in precision and accuracy from the official method.

Table 3. Assay of tablets results

Method	Labelled claim (mg)	Found (mg) $\pm$ S.D (n=5)	% RSD	% Recovery
HPLC	8	7.986 $\pm$ 0.065	0.822	99.825
	4	4.026 $\pm$ 0.038	0.943	99.825
Potentiometric titration	8	8.035 $\pm$ 0.058	0.721	100.437
	4	3.968 $\pm$ 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. 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.

AUTHORS' CONTRIBUTIONS

A.P. Rani and C.B. Sekaran designed the concept and experiments. The optimization of experimental variables and studies on the validation parameters was carried out by C.N. Lakshmi, and S. Bhawani. C.N. Lakshmi, and S. Bhawani collected the necessary literature for the proposed method. The final version of the manuscript was verified by A.P.Rani and C.B.Sekaran.

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