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DEVELOPMENT OF DIFFERENCE SPECTROSCOPIC METHOD FOR ESTIMATION OF PERINDOPRIL ERBUMINE IN BULK AND TABLET DOSAGE FORM

Khade Megha Mahadeo*[1], P. D. Lade[2], Dr. P. N. Sable[3]

[1]Assistant Professor in Department of Quality assurance, Siddhi College of Pharmacy, Chikhali, Tal:- Haveli, Dist: - Pune 411062. Maharashtra, India.

[2]Head of Department in Pharmaceutical Chemistry, Shree Sant Krupa College of Pharmacy Ghogaon Karad Tal-Karad Dist; - Satara.415004, Maharashtra, India.

[3]Principal, Siddhi College of Pharmacy, Chikhali, Tal:- Haveli, Dist:- Pune 411062. Maharashtra, India.

***Corresponding Author: Khade Megha Mahadeo**
Assistant Professor in Department of Quality assurance, Siddhi College of Pharmacy, Chikhali, Tal:- Haveli, Dist: - Pune 411062. Maharashtra, India.

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ABSTRACT

A simple, precise and accurate difference spectroscopic method has been developed for the estimation of perindopril erbumine (PDE) in bulk and in pharmaceutical dosage form. The proposed method is based on the principle that perindopril erbumine can exhibit two different chemical forms in basic and acidic medium that differ in the absorption spectra in basic and acidic medium. Perindopril erbumine exhibits a substantial difference in absorbance in the two solvents that is in 0.01 N Hydrochloric acid and 0.01 N sodium hydroxide at 213 nm. The amplitude of difference spectra followed Beers law within the range of 2-12 µg /ml.No change in isobestic point indicates interference due to additives on absorbance is automatically nullified. The results of analysis have been validated statistically and by recovery studies. Recoveries obtained do not differ significantly from 100% showed that there was no interference from the common excipients used in the tablet formulation indicating accuracy and reliability of the method.

KEYWORDS: Perindopril Erbumine, Difference Spectroscopy, 0.01 N Sodium hydroxide,0.01 N Hydrochloric acid, ICH guidelines.

INTRODUCTION

The analytical chemistry has challenge in developing various methods for analysis with the help of number of analytical techniques which are available for estimation of the drug. Analytical monitoring of pharmaceutical product or of specific ingredients within the product is necessary to ensure the safety and efficacy throughout the shelf life, including storage, distribution and use. The selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferences may be markedly improved by the technique of difference spectrophotometry. This is simplest and most commonly employed technique for altering the spectral properties of the analyte is the adjustment of pH by means of aqueous solution of acid, alkali, or buffer. The ultraviolet-visible absorbance spectra of many substances containing ionizable functional group e.g. phenols, aromatic carboxylic acids and amines are dependent on the state of ionization of the functional groups and consequently on the pH of the solution.[1, 2]

Perindopril Erbumine (PDE),chemically known as 2-methylpropan-2-amine (2S,3aS,7aS)-1-[(2S)-2-[(1S)-1-(ehtoxycarbonyl 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 hypertension patients with heart failure, post myocardial infarction, high coronary disease risk, diabetes mellitus, chronic renal failure.[3,4]

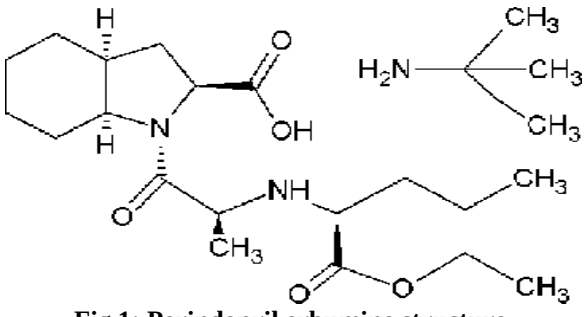

Fig.1: Perindopril erbumine structure.

Literature survey revealed that few analytical methods have been reported for the estimation of PDE; they include visible spectrophotometry[5], radioimmunoassay[6], Spectophotometric[7], HPTLC[8], HPLC[9], LC-MS/MS[10] and capillary gas chromatographic method.[11] But there is no any method reported method for estimation of PDE by difference spectroscopy. Hence an attempt has been made to

develop difference spectroscopic method for its estimation in bulk and pharmaceutical formulation with good accuracy, simplicity, precision and economy. The purpose of this work was to develop and validate simple, specific, sensitive, accurate, precise, rapid and cost effective difference spectroscopic method for the estimation of perindopril erbumine in bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Apparatus

The instrument used for the present study was PC based Jasco V-530 UV-Visible double beam Spectrophotometer with 1 cm matched pair quartz cell and spectral bandwidth of 2 nm.

Reagents and Materials

PDE were obtained as a gift sample from Cipla, Vapi India. Sodium hydroxide (NaOH) and Hydrochloric acid (HCL) were purchased from Loba fine India. Double distilled water was used throughout the experiment in a tablet dosage form containing PDE were purchased from local commercial sources.

1. Selection of Solvent

0.01 N HCL and 0.01 N NaOH was selected as a common solvent for developing spectral characteristics of drugs. The selection was made after using different acids and bases and their different normalities.

1.1 Preparation of 0.01 N HCL

O.85 ml of HCL dissolved in sufficient quantity of distilled water and final volume make up to 1000 ml distilled water to get 0.01 N HCL.

1.2 Preparation of 0.01 N NaOH

O.4 gm of NaOH dissolved in sufficient quantity of distilled water and final volume make up to 1000 ml distilled water to get 0.01 N NaOH.

2. Preparation of Standard Drug Solution

Standard stock solution containing perindopril erbumine (PDE) was prepared by dissolving 10 mg of PDE separately in 50 ml of 0.01 N HCL and 0.01 N NaOH sonicated for 5 min. and then final volume of both the solutions was made up to 100 ml with same solvents to get stock solution containing 100 µg/ml of PDE in 0.01 N HCL and 0.01 N NaOH in two different 100 ml volumetric flasks.

3. UV Analysis for Detection of Wavelength

By appropriate dilution of two standard drug solutions with 0.01 N HCL and 0.01 N NaOH solutions containing 10 µg/ml of PDE were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for the drug. Difference spectroscopic method developed for analysis of perindopril erbumine, one wavelength was selected for estimation of PDE from the overlain spectra (shown as **Figure 1.2** and **1.3**) .The wavelength for the estimation of drug was found to be 213 nm.

4. Procedure for Plotting Calibration Curve

From standard stock solution of drug, six working standard solutions prepared and scanned in the wavelength range of 200-400 nm. The appropriate aliquots of drug were pipetting out from standard stock solution of the drug in 0.01 N HCL and 0.01 N NaOH into series of 10 ml volumetric flask. The volume was made up to get solution of concentration 2, 4,6,8,10 and 12 of PDE in both 0.01 N HCL and 0.01 N NaOH separately. Overlay spectra of both 0.01 N HCL and 0.01 N NaOH were shown in **Figure 1.4** and **1.5**

Calibration curve was constructed at wavelength 213 nm by recording absorbance difference between two solvents against concentration of drug. PDE obeyed Beer's law in the concentration range of 2-12 µg/ml. By using quantitative modes of instrument slope, intercept and correlation coefficient values for calibration curve was obtained. The concentration of PDE was calculated by using formula: $Y = mx + c$ where m= 0.009, c = 0.004, x = concentration of PDE and correlation coefficient for PDE was 0.999.Calibration curves are shown in **Figure 1.6** and Calibration data shown in **Table 2.1 and 2.2**

5 Analysis of Tablet Formulation

Marketed tablet formulations PDE were analyzed by this method. From the triturate of 20 tablets, an amount equivalent to 4 mg of PDE was weighed and transferred to 100 ml volumetric flask. The contents of the flask were dissolved in the 50 ml of the 0.01 N HCL and 0.01 N NaOH separately with the aid of ultra sonication for 10 min. The solution was filtered through Whatmann filter paper no. 41 and then final volume of the solution was made up to 100 ml with same solvents to get a stock solution containing 40 µg/ml of PDE in 0.01 N HCL and 0.01 N NaOH. After appropriate dilutions, the absorbances were measured and the concentration of each analyte was determined with the equations obtained from calibration curve. The statistical data obtained after replicate determinations (n = 6) is shown in **Table 2.3**

6 Method Validation[12,13]

The proposed method was validated according to International Conference on Harmonization (ICH) Q_2B guidelines for validation of analytical procedures in order to determine accuracy, precision, repeatability, robustness, linearity, range, sensitivity, limit of detection, limit of quantitation, robustness and ruggedness.

6.1 Accuracy

The accuracy of the proposed method was examined by determining the recovery of the drug by standard addition technique. Pre-analyzed tablet powder was spiked with pure PDE at three concentration level (80%, 100%, and 120% of that present in tablet powder).The procedure was repeated as per the analysis of formulation. The amplitude was calculated and the amount of PDE recovered was determined. Results are

shown in **Table 2.4**

6.2 Precision

The precision of the method was determined by repeated measurement of standard solution (n=6) without changing the other parameters of the method. The intraday precision of the method was determined by analyzing corresponding responses of same concentration of drug three times on same day. And results were reported in relative standard deviation. Results are shown in **Table 2.5 and 2.6.**The interday precision of the method was determined by analysing corresponding responses of same concentration of drug on three different days. The results were reported in relative standard deviation (%RSD and shown in **Table 2.7**

6.3 Linearity

Six point calibration graphs were constructed covering a concentration range 2-12 µg/ml. six independent determinations were performed at each concentration. The calibration curve was obtained by plotting absorbance verses concentration data.

6.4 Limit of Detection and Limit of Quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The LOD & LOQ were calculated using the formula involving standard deviation of response and further diluted slope of calibration curve. Results are shown in **Table 2.8**

6.5 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Result are shown in **Table 2.9**

6.6 Ruggedness

In ruggedness study, assay of the branded PDE (**COVERSYL**) tablet was performed by two different analysts on two different days. % RSD for tablet was calculated and illustrated in **Table 2.9**.

RESULTS AND DISCUSSION

In this method, the measured value is the difference in absorbance (DA) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics. The difference spectrum of PDE in 0.01 N NaOH was recorded by taking PDE in 0.01 N HCl solutions as blank. The difference spectrum showed that the maxima at 213 nm.In alkaline solutions, drug shows more intense peak than acidic peak. Therefore DA is positive.

1. Selection of Wavelength

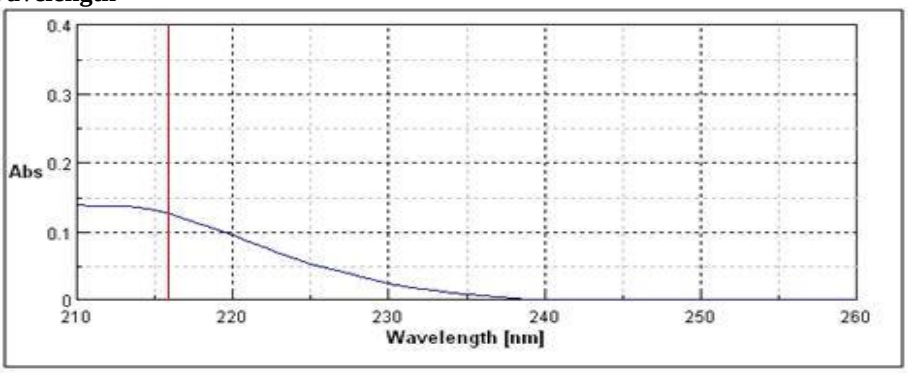

Figure 1.1 Spectra of PDE in 0.01 N HCL.

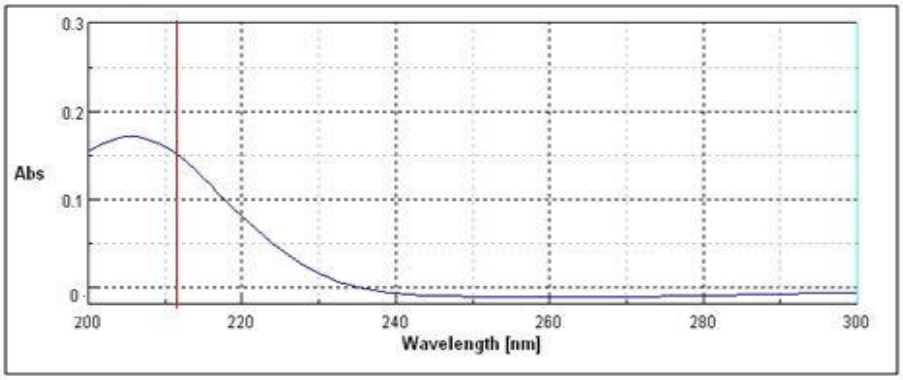

Figure 1.2 Spectra of PDE in 0.01 N NaOH.

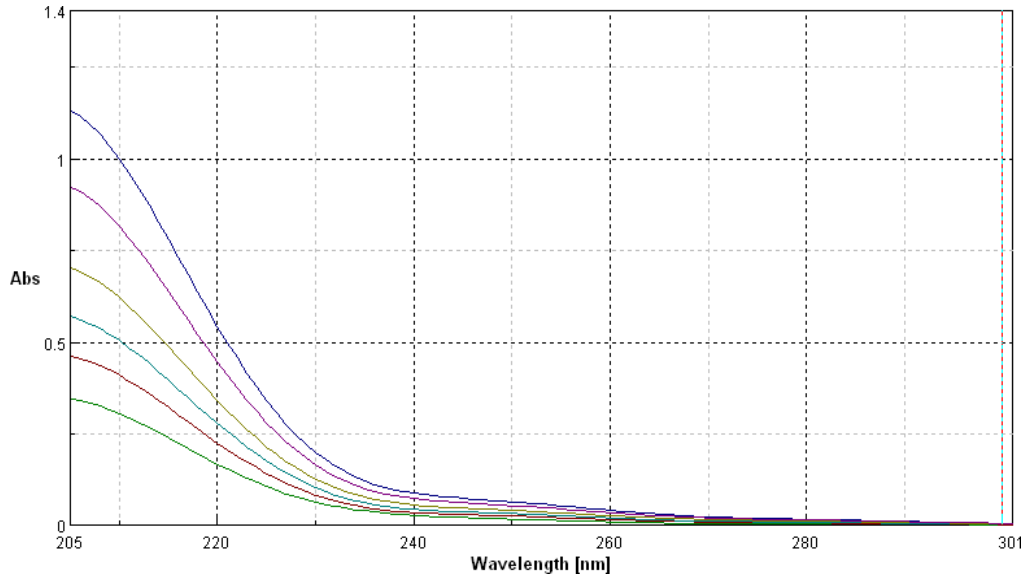

Figure 1.3 Overlay Spectra of PDE in O.O1 N HCL.

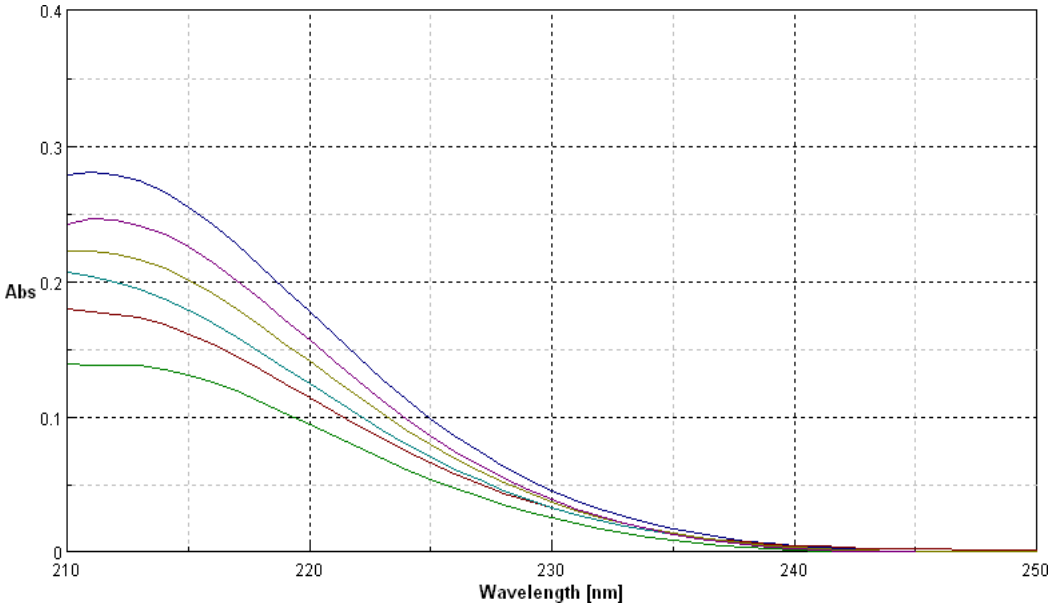

Figure 1.4: Overlay Spectra of PDE in O.O1 N NaOH.

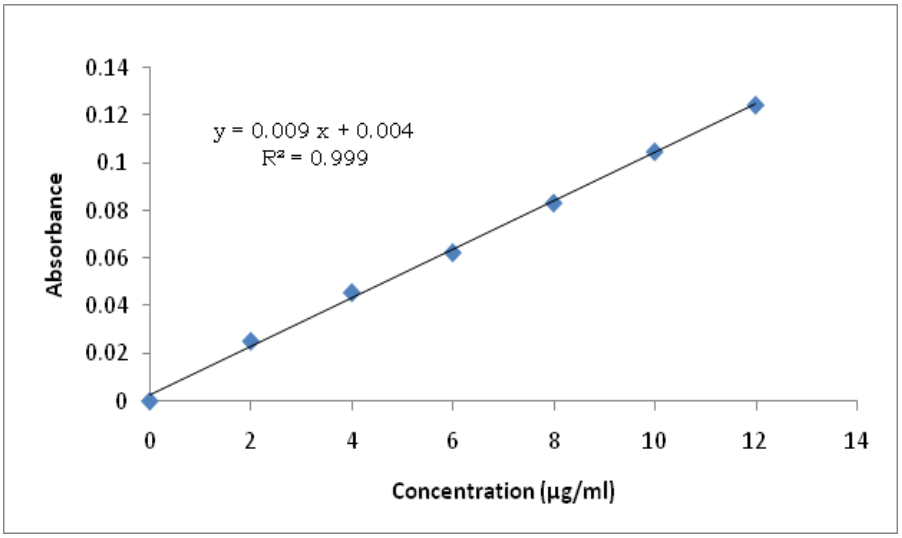

Figure 1.5: Calibration Plot of PDE.

Table 2.1 Absorbance Values for Calibration Curve of PDE at 213 nm.

Sr. No.	Concentration (µg/ml)	Absorbance
1.	2	0.09550
2.	4	0.1822
3.	6	0.28185
4.	8	0.38955
5.	10	0.48608
6.	12	0.59308

Table 2.2: Optical Parameter's for the Performance of the Proposed Method.

Parameters	Values
l_{max} (nm)	213
Beer's law limit (µg/ml)	2-12
Correlation coefficient	0.999
Regression equation (Y)= mx + C	
Slope (m)	0.009
Intercept (c)	0.004

Y= mx+c where x is the concentration in µg/ml and Y is absorbance unit.

7. Assay Result

Perindopril erbumine tablet was analysed by proposed method, the percentage of drug in tablet was determined and presented in the below table. Assay results obtained are within limit.

Table 2.3 Result of Tablet Formulation.

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S. D.)	% R.S.D.
PDE	4	100.11 ± 0.5564	0.5557

*****Average of six determinations; R.S.D., Relative Standard Deviation.S.D., standard deviation

8. Validation of Analytical Parameter [12, 13]

The method was validated according to International Conference on Harmonization (ICH) Q_2B guidelines for validation of analytical procedures in order to determine the Precision, linearity, sensitivity, precision and accuracy, ruggedness, robustness, Limit of detection and limit of quantitation for the analyte.

8.1 Accuracy

All the formulations may contain excipients, lubricating agents and binders which are added along with the active drug constituents. These substances may cause some interference during the estimation of the active drug constituents. This was determined by accuracy. The data for accuracy were expressed in terms of percentage recoveries of PDE in the real samples.

These results are summarized in below table. The mean recovery data of PDE in real sample were within the range of 99.90 % and 100.12%, mean % RSD was 0.1625, and Recoveries obtained do not differ significantly from 100% showed that there was no interference from the common excipients used in the tablet formulation indicating accuracy and reliability of the method, satisfying the acceptance criteria for the study.

Table 2.4 Result of Accuracy Study.

Level	Label Claim (mg/tab	% Label claim estimated (mean ± S. D.)	% R.S.D.
80%	4	99.90 ± 0.2122	0.2124
100 %	4	100.12 ± 0.1365	0.1363
120%	4	100.06 ± 0.1389	0.1388

***** Mean of three observations; R.S.D., relative Standard Deviation.

8.2 Precision

The repeatability study (n = 6) was carried out and the amount of PDE was found to be 100.22±0.5252 with % RSD value of 0.5238. It showed that the method was precise and the equipment used for the study worked correctly for the developed analytical method and being highly repetitive.

For the intermediate precision, a study carried out by the same analyst working on three times in the same day and three consecutive days indicated the % RSD of 0.8222 and 0.5238 for inter day and intraday analysis, respectively. Both the percentage RSD values were of below 2%, indicated that the intermediate precision was confirmed.

Table 2.5 Result of Repeatability.

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean ± S.D.)	% R.S.D.
PDE	4	100.64 ± 0.7433	0.7385

* Average of six determinations; R.S.D., relative Standard Deviation.

Table 2.6 Result of Intraday Precision.

Time	% Label claim estimated* (Mean ± S.D.)	% R.S.D.
Morning	100.06 ± 0.4520	0.4517
Afternoon	100.2 ± 0.4355	0.4346
Evening	100.41 ± 0.6882	0.6853

*Mean obtained from six determinations (6 determinations per day).R.S.D., Relative Standard deviation, S.D., Standard deviation.

Table 2.7 Result of Interday Precision.

Day	% Label claim estimated* (Mean ± S.D.)	% R.S.D.
Day -1	100.50± 0.8376	0.8334
Day -2	100.33 ± 0.8319	0.8291
Day -3	100.51 ±0.8084	0.8042

***** Mean obtained from six determinations; R.S.D., Relative standard deviation; S.D., Standard deviation.

8.3 Linearity

Six point calibration graphs were constructed covering a concentration range 2-12 µg/ml. six independent determinations were performed at each concentration. Linear relationships between amplitude of maxima and minima of difference spectra versus the corresponding drug concentrations were observed (**Figure 1.5**). The standard deviation of the slope and intercept were low. The correlation coefficient (R^2) exceeded 0.999.

8.4 Limit of Detection and Limit of Quantitation

The LOD and LOQ were found to 0.48 and 0.67 µg /ml.

Table 2.8 Result of Limit of Detection and Limit of Quantitation.

DRUG	LOD (µg /ml) *	LOQ (µg ml) *
PDE	0.48	0.67

***** Average of six determinations

8.5 Robustness and Ruggedness

Robustness was studied by changing the normality's (±0.1N) of both hydrochloric acid and sodium hydroxide. The calculated %RSD was 0.4367. PDE which proves the ability of the method to remain unaffected by small but deliberate changes in the conditions of analysis. It indicates that the robustness for proposed method was acceptable.

Ruggedness was studied by inter-analyst and instrument. %RSD for Ruggedness was found to be < 0.7540, it indicating the acceptance of the ruggedness.

Table 2.9 Result of Robustness and Ruggedness Studies of PDE.

Validation Parameter	Conditions varied	% RSD
Robustness (n=6)	Small changes in the normality (± 0.1 N)	0.4367
	Analyst-1	0.7547
Ruggedness (n =6)	Analyst-2	0.5988
	Instrument-1	0.6853
	Instrument-2	0.8334

R.S.D., relative Standard Deviation.

The developed difference spectroscopic method was found to be rapid, simple, precise, accurate and economic for routine estimation of PDE in bulk and its commercial dosage form.

CONCLUSION

Based on the data obtained in our study, the proposed difference spectroscopic techniques are simple, rapid and precise. The accuracy of the method was determined by estimating the recovery of Perindopril erbumine. Accuracy of analysis was determined by performing recovery studies by spiking different concentration of pure drug in the pre-analyzed tablet samples. The proposed method is simple, accurate, precise and selective for the estimation of PDE in bulk and in tablet dosage forms. The method is economical, rapid and do not require any sophisticated instruments contrast to chromatographic method. Hence it can be effectively applied for the routine quality control analysis of PDE in bulk and in tablet dosage forms.

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