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A novel RP-HPLC method development and validation of Perindopril Erbumine in bulk drug and tablet dosage form

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ABSTRACT

Some literatures revealed the high performance liquid chromatography (HPLC) method for perindopril erbumine. However, these methods are time consuming, so it is necessary to develop a cost-effective and less time consuming method for the estimation of perindopril erbumine in API as well as pharmaceutical formulation. The separation was performed on a symmetry C_{18} (4.6 x 100 mm, 5µm, Make: Phenomenex) column in an isocratic mode with the mobile phase consisting a mixture of Phosphate Buffer: methanol (30:70 v/v) was used as a mobile phase and the pH was adjusted to 5 by using O-phosphoric acid. The mobile phase was pumped at a flow rate of 0.8 mL min-1 and eluents were monitored at 215 nm. The selected chromatographic conditions were found to analyse perindopril erbumine (retention time = 2.45 min). The proposed HPLC method was validated with respect to linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ). All the parameters were found to be within limits, which indicate that the above method was accurate and precise. Hence, it was concluded that the developed method is suitable for routine analysis of perindopril erbumine due to its less analysis time.

Key words: Perindopril erbumine, Estimation, RP-HPLC, Validation

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INTRODUCTION

Perindopril erbumine is the tert-butyl amine salt of perindopril, the ethyl ester of a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor. It is chemically described as (2S, 3∝S, 7∝S)-1-[(S)-N-[(S)-1-Carboxy-butyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1). Its molecular formula is $C_{19}H_{32}N_2O_5C_4H_{11}N$. Its structural formula is shown in fig.1 [1, 2].

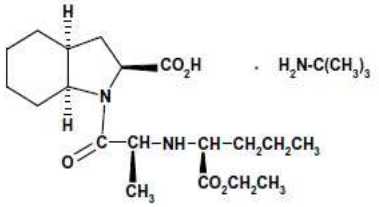

Fig. 1: Structure of perindopril erbumine

It is rapidly metabolized in the liver to perindoprilat, its active metabolite, following oral administration. Perindoprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Perindopril may be used to treat mild to moderate essential hypertension, mild to moderate congestive heart failure, and to reduce the cardiovascular risk of individuals with hypertension or post-myocardial infarction and stable coronary disease [3]. Some literatures revealed the high performance liquid chromatography (HPLC) method for perindopril erbumine [4-6]. However, these methods are time consuming, so it is necessary to develop a cost-effective and less time consuming method for the estimation of perindopril erbumine in API as well as pharmaceutical formulation. In the present study the authors report a rapid, sensitive, accurate and precise RP-HPLC method for the estimation of perindopril erbumine in bulk drug and tablet dosage forms.

MATERIALS AND METHODS

Chemicals and materials: Perindopril erbumine was obtained as a gift sample from Aurobindo pharma ltd. HPLC grade water and methanol were obtained from Merck. Potassium dihydrogen phosphate and ortho phosphoric acid of HPLC grade were obtained from Fisher.

Instrumentation: Quantitative HPLC was performed on waters liquid chromatograph, with a UV detector equipped with automatic injector with injection volume 20 µl, and 515 pump. A symmetry C_{18} column (4.6 x 100 mm, 5 µm, Make: Phenomenex) was used.

Method development and optimization

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of perindopril erbumine in bulk drug and tablet dosage form. Mobile phase tried for this purpose were water: methanol (50: 50), water: methanol (30: 70), buffer pH 3: methanol (40:60), buffer pH 5: methanol (50: 50), buffer pH 5: methanol (30: 70). The condition that gave best resolution and symmetry was selected. HPLC conditions are given in Table-1.

Table-1 HPLC conditions

PARAMETERS	CONDITIONS
Column (stationary phase)	Symmetry C_{18} (4.6 x 100 mm, 5 µm, Make: Phenomenex) or equivalent
Mobile phase	Phosphate buffer (pH 5) : methanol (30%: 70%)
Flow rate	0.8 ml/ min
Run time	5 (min)
Column temperature	Ambient
Volume of injection loop	20 µl
Detection wavelength	215 (nm)
Drug Rt	2.45 min

Preparation of buffer (pH 5):

7gms of potassium dihydrogen phosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. The pH of the solution was then adjusted to 5 with orthophosphoric acid.

Preparation of mobile phase:

300 ml of the phosphate buffer was mixed with 700 ml of methanol. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Diluent preparation:

Mobile phase was used as Diluent.

Preparation of standard solution:

Standard stock solution of 1000 µg/ml was prepared by dissolving 10 mg of perindopril erbumine in 10 ml of diluent. From this stock solution, 0.3 ml was pippetted out and the volume was made upto 10 ml with the diluent to prepare working standard solution of 30 µg/ml.

Preparation of sample solution:
Weigh accurately tablets, powdered equivalent to about 10 mg of perindopril erbumine and transfer it into a 10 ml volumetric flask. Add about 7 ml of diluent and sonicate to dissolve. The volume was made upto the mark and the solution was filtered through 0.45 µ filter under vacuum. From this stock solution, 0.3 ml was pippetted out and the volume was made upto 10 ml with the diluent to prepare sample solution of 30 µg/ml

Assay:
Inject 20 µl of the standard and sample solution into the chromatographic system and measure the areas for the perindopril erbumine and calculate the % assay by using the formula. The standard and sample chromatograms were shown in fig. 2.

Formula:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.wt}{\text{Label claim}} \times 100$$

Where:
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim of drug mg/ml**.**

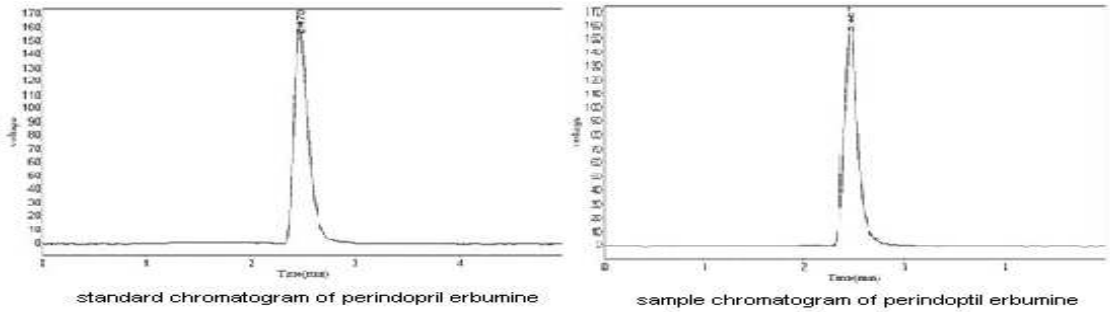

Fig. 2: Standard and sample chromatograms of perindopril erbumine

Method validation:
The method was validated for the following parameters such as linearity, precision, accuracy, limits of detection and quantitation, ruggedness and robustness.

System Suitability:
System suitability was daily performed during entire validation of this method. The results of system suitability were presented in Table 2.

Table 2: System Suitability Parameters

S. No	Parameter	Perindopril erbumine
1	Retention time	2.457
2	Theoretical plates	3078.174
3	Tailing factor	1.287
4	Area	2522658

Accuracy:
The accuracy of an analytical method expresses the closeness of agreement between the value, which is accepted reference value, and the value found. Accuracy studies were done by the standard addition method. Accuracy is expressed as % recovery of the standard spiked to previously analyzed test sample of tablet. The active ingredients were spiked in previously analyzed tablet powder sample at different concentration levels viz. 50%, 100%, and

150% each of the labeled claim and injected in developed chromatographic conditions in triplicate. The percentage recoveries were then calculated. The recovery data for accuracy studies were shown in Table 3. The accuracy chromatograms were shown in fig 3, 4 and 5.

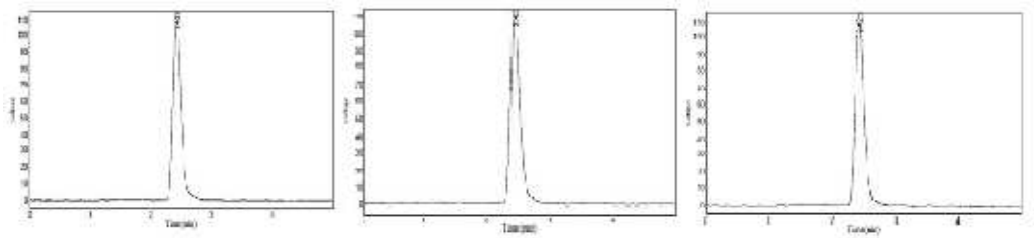

Fig 3: Accuracy chromatograms for 50% solution

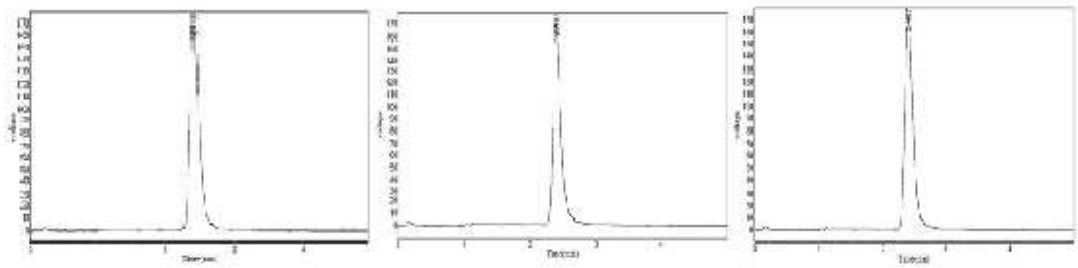

Fig. 4: Accuracy chromatograms for 100% solution

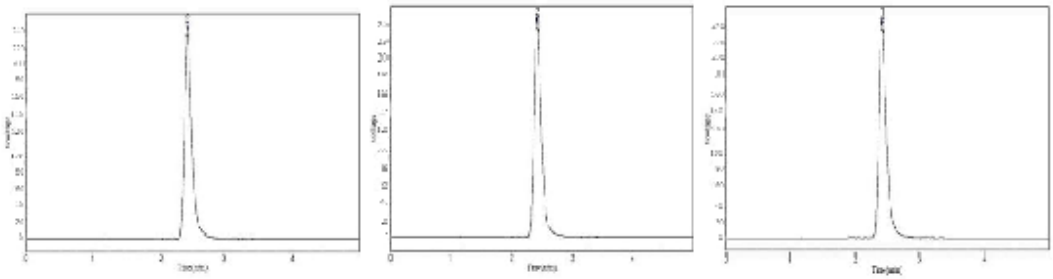

Fig. 5: Accuracy chromatograms for 150% solution

Table 3: Accuracy Result of Perindopril erbumine

% Concentration* (at specification Level)	Area	Amount Added (mcg)	Amount Found (mcg)	% Recovery	Mean Recovery
50 %	1214992	15	14.8	98.66667	100.7926
100 %	2478531	30	30.7	102.6667	
150 %	4936984	45	45.5	101.0444	

Precision:

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

$$\%CV = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

The standard solution was injected for five times and the area was measured for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. Results were reported in Table 4. Chromatograms were reported in fig 6.

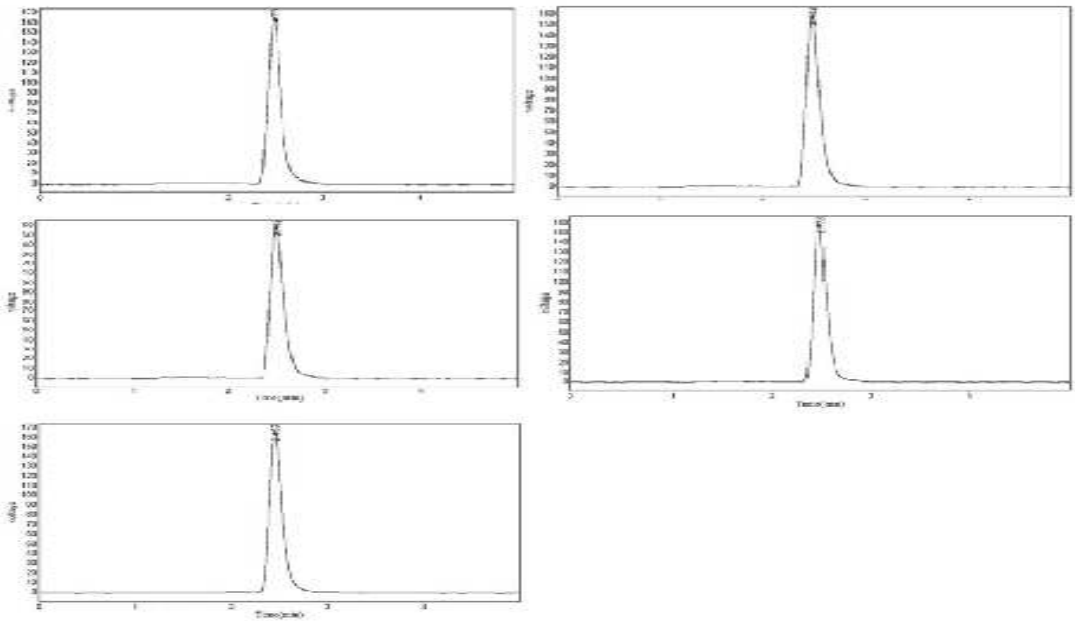

Fig. 6: Precision chromatograms

Table 4: Result of System Precision

Injection	Peak area
Injection 1	2537347
Injection 2	2526781
Injection 3	2509433
Injection 4	2501556
Injection 5	2563978
Average	2527819
Standard Deviation	24632.92347
% RSD	0.9745

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. Results were reported in Table 5.

Table 5: Result of intermediate system precision

Injection	Peak area
Injection 1	2479986
Injection 2	2497204
Injection 3	2518597
Injection 4	2558034
Injection 5	2583434
Average	2527451
Standard Deviation	42765.69
% RSD	1.692

Linearity:

Aliquots of standard perindopril erbumine stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of perindopril erbumine were in the range of 10-50 µg/ml. Each of these drug solutions (20 µL) was injected into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 215 nm and a Calibration graph was obtained by plotting peak area versus concentration of perindopril erbumine (Fig 7). The linearity Chromatograms were presented in Fig 8. Results were reported in table 6.

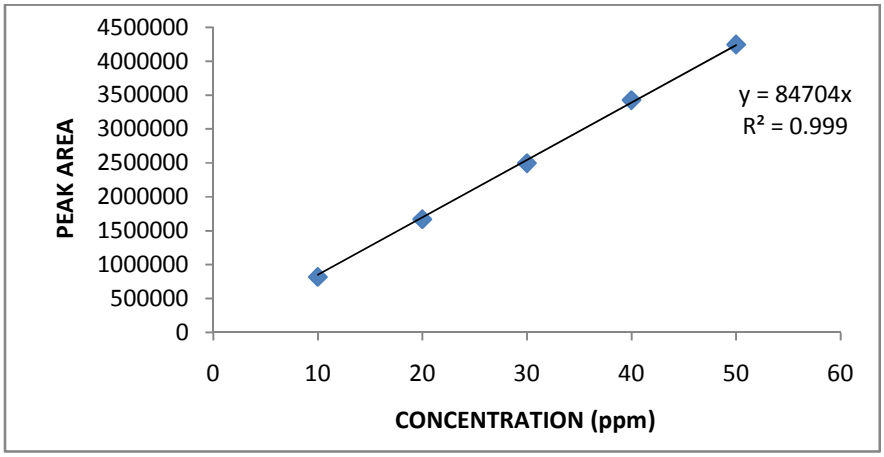

Fig. 7: Calibration Curve for Perindopril erbumine

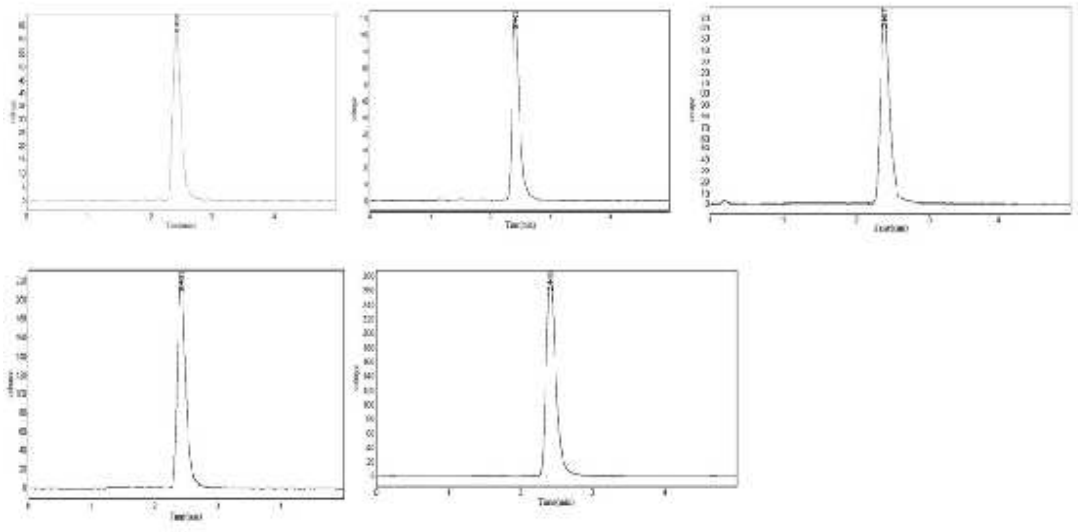

Fig 8: Linearity chromatograms of perindopril erbumine

Table 6: Linearity result of Perindopril erbumine

S.No	Linearity Level	Concentration	Area
1	I	10 µg/ml	819987
2	II	20 µg/ml	1669678
3	III	30 µg/ml	2496322
4	IV	40 µg/ml	3437463
5	V	50 µg/ml	4245812
Correlation Coefficient			0.999546

Limit of Detection [LOD] and Limit of Quantification [LOQ]:

The LOD and LOQ were determined for perindopril erbumine, based on the standard deviation (SD) of the response and slope (S) of the regression line as per ICH guideline according to the formulae given below.

$$LOD = \frac{3.3 \times SD}{S}$$

$$LOQ = \frac{10 \times SD}{S}$$

Method robustness:

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 7 and 8. The chromatograms for flow rate variation and mobile phase variation were shown in fig 9 and 10 respectively.

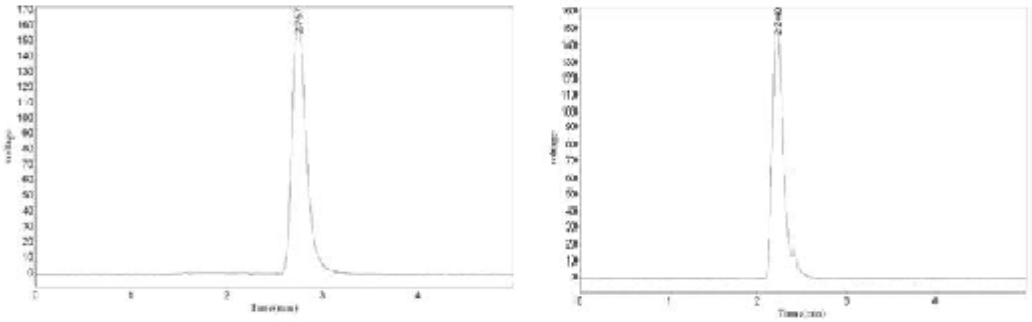

Fig.9: Flow rate variation chromatograms of perindopril erbumine

Table 7: Flow rate variation result of Perindopril erbumine

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.7	2790.621	1.277
2	*0.8	2627.451	1.287
3	0.9	2563.609	1.294

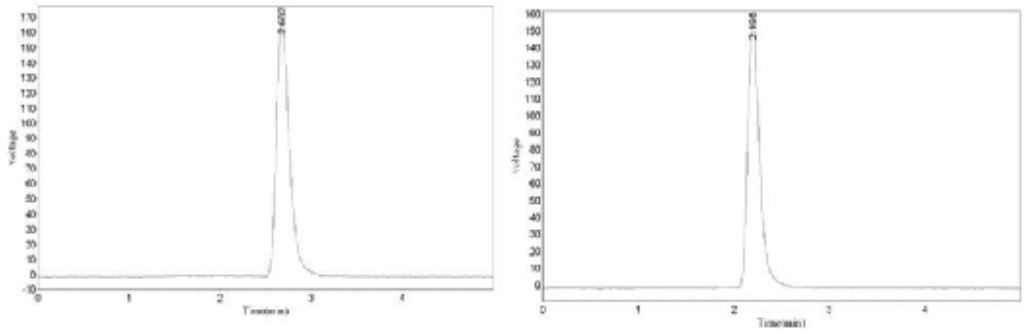

Fig 10: Mobile phase variation results of perindopril erbumine

Table 8: Mobile phase variation result of Perindopril erbumine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2744.850	1.104
2	*Actual	2627.451	1.287
3	10% more	2538.547	1.288

RESULTS AND DISCUSSION

To optimize the mobile phase, various proportions of buffers with methanol were tested. Mobile phase composition was changed and the method development was started by symmetry C_{18} (4.6 x 100 mm, 5 µm, Make: Phenomenex) column and with a flow rate of 0.8 ml/min, and detection wavelength of 215 nm. Injection volume was 20 µL, and run time was for 5 min. The mobile phase consists of phosphate buffer (pH 5) and methanol. The retention time of perindopril erbumine was found to be 2.45 minutes. The assay result was found to be 100.08%. Quantitative

linearity was observed over the concentration range of 10-50 µg/ml. The regression equation of the linearity plot of concentration of perindopril erbumine over its peak area was found to be y = 84704x (R^2=0.999). The numbers of theoretical plates obtained were 3078.17, which indicates the efficiency of the column. The limit of detection and limit of quantitation were found to be 1.09 and 3.63 µg/ml, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate.

CONCLUSION

A simple and rapid RP-HPLC method was developed for the estimation of Perindopril erbumine in API and pharmaceutical dosage forms.

Method was developed on Symmetry C_{18} (4.6 x 100 mm, 5µm, Make: Phenomenex). The mobile phase was phosphate buffer (pH 5): Methanol 30:70 % ratio with a flow rate of 0.8 ml/min. The chromatograms were recorded at 215 nm wave length. The retention time for Perindopril erbumine was found to be 2.457.

The developed method was validated in terms of accuracy, precision, linearity and robustness and results were validated according to ICH guidelines.

Therefore it was concluded that the proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical and can be used for the estimation of Perindopril erbumine in API as well as in pharmaceutical dosage forms.

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