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A VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF PERINDOPRIL ERBUMINE IN PHARMACEUTICAL FORMULATIONS

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Abstract: A new high performance liquid chromatographic (HPLC) method was developed and validated for the determination of Perindopril Erbumine (PE) in pharmaceutical formulations. Optimum separation was achieved in 10 min using C_{18} column (250 mm × 4.6 mm, i.d., particle size 5 mm), and elution was accomplished using a mobile phase (1 ml/min). Detection was carried out using a UV detector set at 215 nm. A linear relationship between mean peak area and concentration of PE was observed in the range 4-20 µg/ml, with a detection limit of 2 µg /ml and a quantization limit of 7.0 µg/ml. Intra-day and Inter-day precision, and accuracy of the methods have been established according to the current ICH guidelines. The developed method was successfully applied to the determination of PE in pharmaceutical formulations. The results were statistically compared with those of the reference method (UV method) by applying Student's t-test and F-test. Accuracy, evaluated by means of the recovery method, was in the range 99.00 - 100.5 %, with precision (RSD) 0.865%. No interference was observed from the coformulated substances. The proposed method was successfully employed for the determination of PE in various pharmaceutical preparations.

Key words: Perindopril erbumine, LOD, LOQ, pharmaceutical formulations.

Introduction

Perindopril erbumine[1],(2S,3(infinity)S,7(infinity)S)-1-[(S)-N-[(S)-1-Carboxy- butyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1), belongs to a group called angiotensin converting enzyme (ACE) inhibitors. Inhibition of ACE results in decreased plasma angiotensin II, leading to decreased vasoconstriction, increased plasma rennin activity and decreased aldosterone secretion. The overall effect of this is a drop in blood pressure and a decrease in the workload of the heart. Literature survey revealed that few analytical methods have been reported for the estimation of PDE; they include immunoassay[2], spectrophotometric[3,4], HPLC[5], biosensor method[6-8], LC–MS/MS[9,10], capillary gas chromatographic method[11].

The purpose of the present study was to develop a simple, sensitive, accurate and precise HPLC method for the determination of PE in pharmaceutical formulations. The developed method has been validated by evaluation of the system suitability, linearity, limits of detection and quantification, precision and accuracy. The validated method was applied to the commercially available pharmaceutical formulations containing PE.

Experimental

Apparatus

A High pressure liquid chromatograph (Shimadzu HPLC class VP series) with two LC-10 AT VP pumps, variable wavelength programmable UV-Visible detector SPD-10 A VP, SCL-10A VP system controller (Shimadzu) and C-18 column was used. The HPLC system was equipped with the soft ware Class VP series version 5.03 (Shimadzu).

Reagents and standards

All chemicals used were of analytical reagent grade and HPLC grade methanol was used. Distilled water filtered through 0.45 µm filter (Millipore) was used to prepare solutions. Mobile phase A consisted of methanol. Mobile phase B was water. The mobile phase used was prepared

by mixing mobile phase A and mobile phase B in the ratio, 4:1. The same mobile phase was also used as a diluent for the sample preparations.

Pharmaceutical grade PE, certified to be 99.8% pure was procured from local pharmaceutical industry and was used as received. A stock standard containing 1mg/ml PE solution was prepared by dissolving accurately weighed 100 mg of pure drug in the diluent and diluting to 100 ml with the diluent in a calibrated flask.

Procedures

Chromatographic conditions

The separation was achieved at a temperature of 35 °C on the column using the mobile phase at a flow rate of 1 ml/min. The detector wavelength was set at 215 nm.

Calibration graph

Working standard solutions equivalent to 4 to 20µg/ml PE were prepared by appropriate dilution of stock standard solution with the diluent solution. 20 µl aliquot of each solution was injected automatically on to the column for five times and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of PE. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the mean peak area-concentration data.

Assay in dosage forms

Twenty tablets were weighed accurately and ground into a fine powder using pestle and mortar. A quantity of tablet powder equivalent 50 mg of PE was accurately weighed into a 100 ml calibrated flask, 60 ml of diluent solution added and contents are shaken for 20 min; then, the volume was diluted to the mark with the diluent and mixed well. A small portion of the extract (say 10 ml) was withdrawn and filtered through 0.2 µm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent solution for analysis as described already.

Results and Discussion

Method development

Drug quality control, stability, metabolism, pharmacokinetics, and toxicity studies all necessitate the determination of drugs in pharmaceutical formulations and biological samples. Consequently, efficient and validated analytical methods are very critical requirements for all these investigations. Chromatographic parameters were preliminarily optimized to develop the present method for the determination of PE with short analysis time (10 min). A solution of PE was injected five times on to the column and was monitored by UV detection at 215 nm. A mobile phase consisting of methanol and water in the ratio 4:1 was selected after several preliminary experiments. At a flow rate of 1 ml/min the retention time was 2.793 min (Fig.1). Under the described experimental conditions, the peak was well-defined and free from tailing.

Method Validation

In order to determine the adequate resolution and reproducibility of the proposed method, suitability parameters including retention time, plate number and tailing factor were investigated,
and were found to be 2.793 min (Fig. 1), 5547.27 and 1.02, respectively, which indicate the method suitability.

Linearity and range

Calibration curve was constructed by plotting the mean peak area versus concentration which was linear over the concentration range 4-20 µg/ml. Using the regression analysis, the linear equation, $Y = 113.9 + 8.062 \times 10^2 X$, was obtained, where Y is the mean peak area and X concentration in µg/ml. The Linearity co-efficient of mean response of replicate determination plotted against respective concentration was found to be 0.9998. The % RSD for peak area response of five replicates was 0.865.

Detection and quantification limits

Limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise ratio method[12]. LOD is taken as the concentration of analyte where signal to- noise ratio was 3, and it was found to be 2 µg /ml. LOQ is taken as the concentration of analyte where signal-to-noise ratio was 10, and it was found to be 7 µg /ml.

Precision and Accuracy

The precision of the method was evaluated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of PE were analysed in five replicates during the same day (intra-day precision) and three consecutive days (inter-day precision). Within each series, every solution was injected in triplicate. The RSD values of intra-day and intra-day studies (<1%) showed that the precision of the method was satisfactory. The results of this study are given in Table-1.

The accuracy of an analytical method expresses the closeness between the reference value and found value[12]. Accuracy was evaluated as percentage relative error between the measured mean concentrations and taken concentrations. The results obtained for three concentrations are shown in Table-1 from which it is clear that the accuracy is excellent. The accuracy was also assessed by analyzing the pharmaceutical formulation containing the PE and calculated the percent recovery of the active ingredient which was found to be in between 99.95 ± 0.25 to 100.26 ± 0.37, indicating that the co-formulated substances such as talc, starch, gum acacia, lactose, dextrose, hydroxyl methyl cellulose, sodium alginate and magnesium stearate did not interfere in the assay.

Application of the method for the analysis of commercial formulation

The developed and validated method was applied to the determination of PE in two brands of tablets containing PE in two doses (2 and 4 mg PE per tablet) which are available in the local market using the procedure

described earlier. Evaluation was performed using the calibration curve method since no significance difference between the slopes of the calibration curves for standards and tablet extracts was observed. The results obtained by the proposed method were statistically compared with those of the reference method by applying the Student's t-test for accuracy and *F*-test for precision. As shown by the results compiled in Table-2, the calculated *t*- and *F*-values did not exceed the tabulated values, $t = 2.77$ and $F = 6.39$ at the 95% confidence level for four degrees of freedom suggesting that the proposed method and the reference method (UV method) do not differ significantly with respect to accuracy and precision. The accuracy and validity of the proposed method were further ascertained by performing recovery experiments. The recovery studies were carried out by mixing a known quantity of drug with pre analyzed sample and the contents were reanalyzed by the proposed method. The recovery of pure drug from the pharmaceutical formulation revealed that co-formulated substances did not interfere in the determination (Table - 3).

Conclusions

A simple, rapid, accurate and precise HPLC method was developed for the determination of PE in pure form and in tablets. The analytical conditions and solvent system developed provided a good separation for PE within a short analysis time. The method was validated and demonstrated a wide linear dynamic range, a good precision and accuracy. Thus, the method can be proposed for routine analysis laboratories and for quality control.

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Table: 1 Intra-day and Inter-day precision

Concentration of Perindopril erbumine (µg/ml)	Observed concentration of Perindopril erbumine (µg/ml)					
	Intra- day			Inter-day		
	*Mean**	*% RE*	*%RSD*	*Mean**	*% RE*	*%RSD*
5	4.99	0.20	0.59	5.05	1.00	1.03
10	9.93	0.77	0.39	9.98	0.20	0.82
15	15.07	0.46	0.46	14.86	0.93	0.63

RE- Relative error, RSD- relative standard deviation
*Mean value for five determinants

Table: 2: Determination of Perindopril erbumine in tablets and statistical comparison with reference method

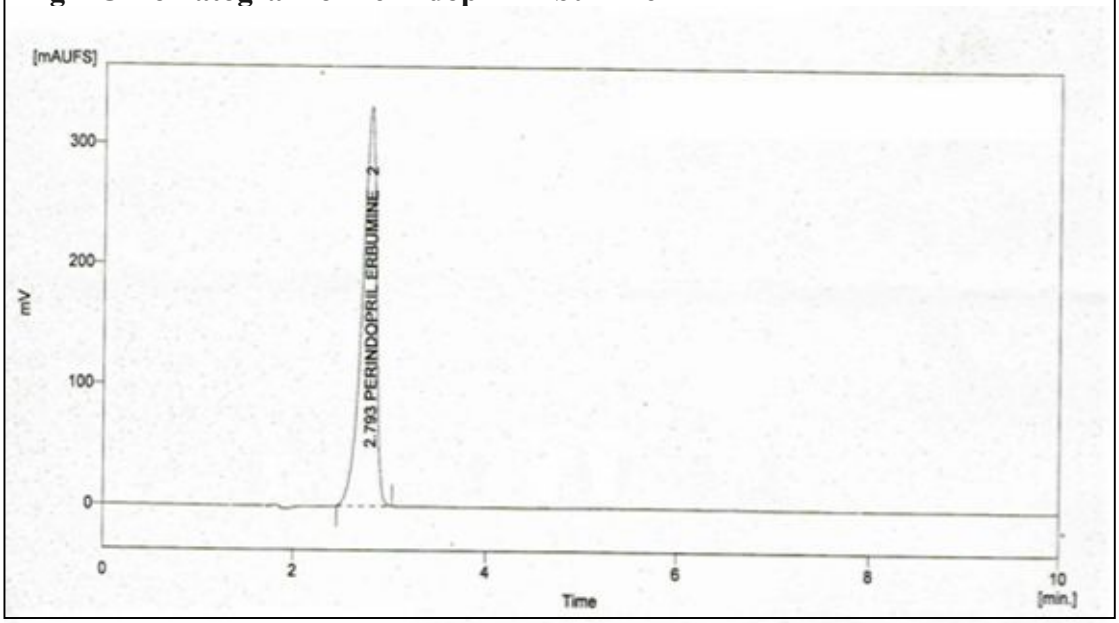
Brand name	Labeled amount(mg)	% Found** (± s.d)			
		Reference method*	Proposed method	t-value	F-value
Tablet I	2	100.25 ± 0.73	99.95 ± 0.25	2.19	3.44
Tablet II	4	99.53 ± 0.65	100.26 ± 0.37	2.36	5.28

* Reference method was UV method developed in the laboratory.
** Recovery amount was the average of five determinants
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39

Table-3: Recovery of Perindopril erbumine

Amount of Drug added (mg)	Mean (±s.d) amount(mg) found(n=5)	Mean (±s.d)% of recovery (n=5)
2	1.98 ± 0.29	99.00 ± 1.12
4	4.02 ± 0.13	100.5 ± 0.64

Fig 1 Chromatogram of Perindopril Erbumine



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