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DEVELOPMENT AND VALIDATION OF NEW HPLC METHOD FOR THE ESTIMATION OF PERINDOPRIL IN TABLET DOSAGE FORMS

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ABSTRACT

An accurate and precise RP-HPLC method was developed for the determination of perindopril in tablet dosage forms. Separation of the drug was achieved on a reverse phase C_{18} column using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 65:35 v/v. The flow rate was 0.6 ml/min and 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 proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of perindopril in tablet dosage forms.

Keywords: Perindopril, Estimation, RP-HPLC, Validation.

INTRODUCTION

Perindopril erbumine[1] is the tert-butylamine salt of perindopril, the ethyl ester of a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor. 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. Perindopril erbumine is chemically described as (2S, 3αS, 7αS)-1-[(S)-N-[(S)-1-carboxy butyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1) (Fig. 1). A few spectrophotometric[2,3], HPLC[4] and LC-MS[5] methods were reported earlier for the determination of perindopril in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise RP-HPLC method for the estimation of perindopril in bulk samples and in tablet dosage forms.

EXPERIMENTAL

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C_{18} column (100 mmx4.6mm; 5 µm), a 2695 binary pump, a 20 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower2 software.

Chemicals and solvents

The reference sample of perindopril was supplied by Glenmark Pharmaceuticals Ltd, Mumbai. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer (pH 3.0)

Seven grams of KH_2PO_4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of Triethyl amine was added and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents

650 ml of the phosphate buffer was mixed with 350 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Procedure

A mixture of buffer and acetonitrile in the ratio of 65:35 v/v was found to be the most suitable mobile phase for ideal separation of perindopril. The solvent mixture was filtered through a 0.45µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.6 ml/min. The column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 209 nm. The run time was set at 6 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 3.122 min. A typical chromatogram showing the separation of the drug is given in Fig. 2.

Calibration plot

About 25 mg of perindopril was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 65:35 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 100 µg/ml solution. From this, a working standard solution of the drug (40µg/ml) was prepared by diluting 2 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 20-100 µg/ml were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of each dilution was injected six times into the column at a flow rate of 0.6 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 20-100 µg/ml of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of perindopril in tablets dosage forms.

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of perindopril. Solution containing 40 µg/ml of perindopril was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2. The accuracy of the HPLC method was assessed by analyzing solutions of perindopril at 50, 100 and 150 % concentrated levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Estimation of perindopril in tablet dosage forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate perindopril in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of perindopril was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 65:35 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

RESULTS AND DISCUSSION

In the proposed method, the retention time of perindopril was found to be 3.122 min. Quantification was linear in the concentration range of 20-100 µg/ml. The regression equation of the linearity plot of concentration of perindopril over its peak area was found to be $Y=32625.5+38432.49X$ ($r^2=0.9997$), where X is the concentration of perindopril (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 3103, which indicates efficient performance of the column. The limit of

detection and limit of quantitation were found to be 0.03 µg/ml and 0.12 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 65:35 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

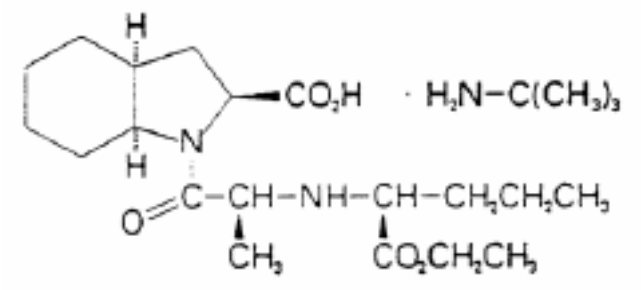

Fig.-1: Chemical structure of perindopril

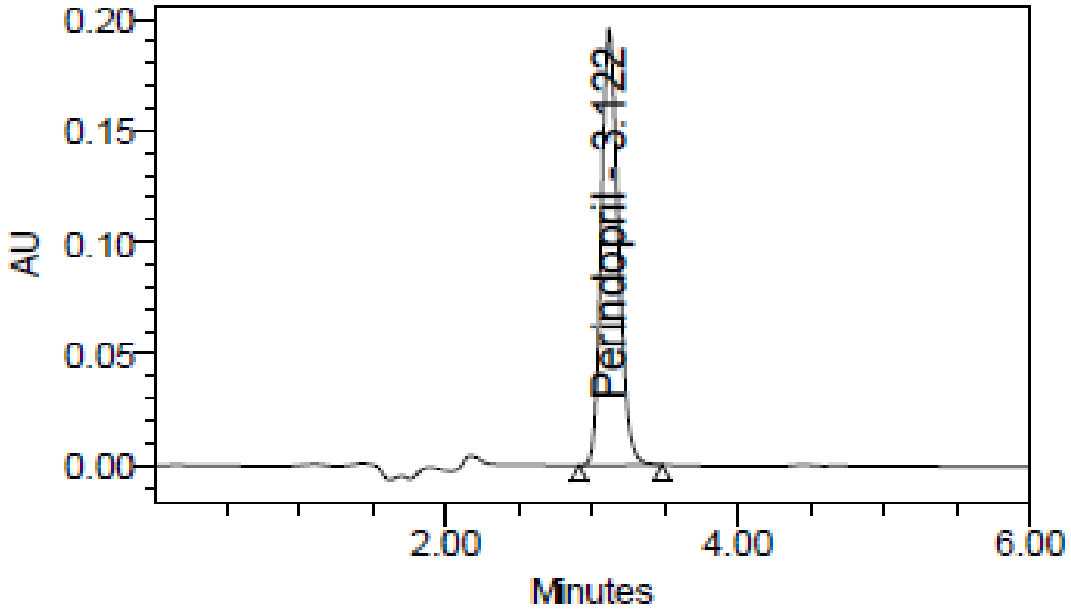

Fig.-2: Typical chromatogram of perindopril

Table-1: Calibration data of the method

Concentration (µg/ml)	Mean peak area (n=5)
20	781547
40	1591955
60	2329492
80	3138212
100	3851667

Table-2: Precision of the proposed HPLC method

Concentration of perindopril (µg/ml)	Measured concentration of perindopril (µg/ml)			
	Intra-day		Inter-day	
	Mean (n=5)	% RSD	Mean (n=5)	% RSD
40	39.93	0.17	39.46	0.10

Table-3: Accuracy studies

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50 %	5.05	4.97	98.4 %	
100 %	9.95	10.0	100.5 %	99.3
150 %	14.85	14.69	98.9 %	

Table-4: System suitability parameters

Parameter	Result
Linearity (µg/ml)	20-100
Correlation coefficient	0.9997
Theoretical plates (N)	3103
Tailing factor	1.15
LOD (µg/ml)	0.03
LOQ (µg/ml)	0.12

Table-5: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Coversyl	2	2.003	100.15
Perigard	2	1.992	99.60

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of perindopril and can be reliably adopted for routine quality control analysis of perindopril in its tablet dosage forms.

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