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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF PERINDOPRIL ERBUMINE IN PHARMACEUTICAL DOSAGE FORM

Dandamudi Santhoshi Priya[1]*, Suchitra D.[3], Sahithi A.[2], Parijatha B.[2], Himaja M.[2] and Krishna Mohan Chinnala[2]

[1]School of Pharmacy, Anurag Group of Institutions.
[2]School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions.
[3]University College of Science, Osmania University, Hyderabad.

***Corresponding Author: Dandamudi Santhoshi Priya**
School of Pharmacy, Anurag Group of Institutions.

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ABSTRACT
A new method was established for estimation of Perindopril erbumine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of perindopril by using Xterra C18 4.6×250 mm 5.0 µm, flow rate was 0.8ml/min, and mobile phase ratio was 65:35%v/v methanol: water, detection wavelength was 218nm. The instrument used was SHIMADZU HPLC, software N-2000.The retention times were found to be 2.482 mins. The analytical method was validated according to ICH guidelines [ICH, Q2 (R1)]. The developed method was found to be simple, rapid, accurate, linear, and précised. The linearity study of perindopril was found in concentration range of 30µg-150µg and correlation coefficient (r2) was found to be 0.997, % recovery was found to be 100.3%. LOD value was 2.97µg/ml and LOQ value was 9.92µg/ml. Hence the suggested RP-HPLC method can be used for routine analysis of perindopril in standard and pharmaceutical dosage form.

KEYWORDS: Perindopril erbumine, RP-HPLC, Xterra C18 and retention time.

INTRODUCTION
Perindopril erbumine is a tert-butylamine salt of perindopril, the ester of a non-sulfhydryl angiotensin converting enzyme (ACE) inhibitor with antihypertensive activity. Upon hydrolysis, perindopril erbumine is converted to its active form perindoprilat, inhibiting ACE and the conversion of angiotensin I to angiotensin II, consequently, angiotensin II- mediated vasoconstriction.[1] Perindopril is an angiotensin converting enzyme (ACE) inhibitor used in the therapy of hypertension and stable coronary artery disease.[2]

Reverse Phase HPLC: In this chromatographic technique, the stationary phase is non-polar and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first.[3]

Steps involved in development of RP-HPLC method
Selection of chromatographic method: The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drug selected is polar and ionic hence reversed phase chromatography was used because of its simplicity and suitability.[4]

Selection of stationary phase: Matching the polarity of sample and stationary phase and using a mobile phase of different polarity achieve a successful separation.[5]

Selection of mobile phase: Reversed phase bonded packing, when used in conjunction with highly polar solvents; approach is ideal and is a universal system for liquid chromatography. Mobile phase may be either single liquid or combination of liquids, which are compatible with sample, column and instrument.[6]

Selection of suitable detector: Detector is the eye of HPLC system that measures the compounds after their separation on the column. There are basically two types of detectors- the bulk property detectors and solute property detectors. Detectors, in order of their popularity are UV, fluorescent, conductivity, polarimeter and refractive index detectors. UV detector is the first choice because of its convenience and applicability in case of most of the samples. The latest versions of equipment's are available with photo diode- array detectors (PAD or DAD).

Method optimization
During the optimization stage, the initial sets of conditions that have evolved from the First stages of development are improved or maximized in terms of

resolution and shape, plate Counts asymmetry, capacity, elution time, detection limits, limit of quantization, and overall ability to quantify the specific analyte of interest.

A literature survey on various analytical methods for perindopril has revealed that a few methods using spectrophotometry and high-performance liquid chromatography (HPLC) has been reported for the assay of perindopril in bulk and pharmaceutical formulations.[7,8] The present work is designed to develop a sensitive, precise, accurate and selective stability-indicating RP-HPLC method for the estimation of perindopril erbumine in tablet dosage form, which were not included in earlier reports. The developed method was validated as per international conference on harmonization (ICH) Q2 (R2) guidelines.[9]

MATERIALS AND METHODS

Drug profile of Perindopril erbumine[10]

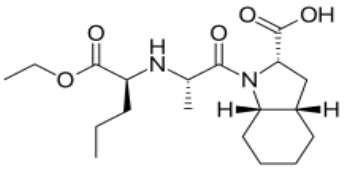

Fig. 1: Structure of Perindopril erbumine.

IUPAC Name : (2S, 3aS, 7aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxopentan-2-yl] amino} propanoyl]-octahydro-1H-indole-2-carboxylic acid.
Chemical formula : $C_{19}H_{32}N_2O_5$
Molecular weight : 368.468 g/mol.
Description : White crystalline powder.
Solubility : Freely soluble in water and alcohol.
Category : ACE inhibitors.
λ_{max} : 218 nm

Drugs used

Table: 1. List of Drugs used.

S. No	Drugs	Manufacturer
1.	Perindopril erbumine	Aurobindo pharma Ltd
2.	Perindopril erbumine Commercial Tablets (4 mg)	Aurobindo pharma Ltd

Reagents Used

Table: 2. List of Reagents used

S. No.	Chemicals	Manufacturer name	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Ortho Phosphoric acid	Merck	G.R
5	Potassium dihydrogen Phosphate	Merck	G.R

Equipment and Apparatus Used

Table: 3. Equipment and Apparatus Used

S. No.	Istrument name	Model number	software	Manufactures name
1	HPLC	L C 10 AT UV-Visible detector-SPD-10A	N2000	SHIMADZU
2	U.V Double beam spectrophotometer	SL 210	-	ELICO
3	Digital weighing balance (Sensitivity 5 mg)	BL-200H	-	SHIMADZU
4	PH-meter	LI-120	-	ELICO
5	Sonicator	3305013	-	SISCO

Preparation of mobile phase

A combination of water (35%) and methanol (65%) was mixed and degassed in ultrasonic water for 5 minutes finally filtered through 0.45µ membrane filter. This prepared solution was used as mobile phase.

Diluents

HPLC grade water was used as diluent.

Preparation of Standard stock solution (1000 ppm)
Accurately weighted 10mg of perindopril standard and transferred to a 10ml of clean dry volumetric flask and add about 7ml of diluent and sonicate to dissolve it completely and make up to the mark with the same solvent. This solution is used for the preparation of working solutions.

Preparation of standard solution (100 ppm)
Accurately weighted 10mg of perindopril and transferred into a volumetric flask and add about 7ml of diluents and sonicate to dissolve it completely and make up volume up to the mark with the same solvent (stock solution). Further pipette out 1ml from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluents.

Preparation of sample solution[11]
20 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 to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent.

Wavelength selection
About 200 ppm of perindopril solution was accurately prepared by dissolving the active in water. The perindopril solution scanned in the 200-400 nm UV regions. The wavelength maximum (λmax) was observed at 218 nm and this wavelength was adopted for absorbance measurement.

Optimizedchromatographic conditions

Column	: Xterra C_{18} 4.6×250mm, 5µm
Column temperature	: Ambient
Wave length	: 218nm
Mobile phase ratio	: Methanol: water 65:35%v/v
Flow rate	: 0.8 min/ml
Injection volume	: 20µl
Run time	: 6 minutes

Validation of developed RP-HPLC method
As per the International conference on harmonization (ICH) guidelines the method validation parameters such as linearity, precision, accuracy, system suitability, limit of detection and limit of quantitation were optimized.

Assay
Sample and standard was injected into the chromatographic system and measured the area for perindopril and calculated the % assay by using the formulae.

CALCULATION

$$Assay\,\% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where
Avg.wt = average weight of tablets
P = percentage purity of working standard
LC = label claim of perindopril mg/ml

RESULTS AND DISCUSSION
Optimized method
It was performed on Xterra C_{18} 4.6×250mm 5µm with a mobile phase composition of methanol: water in the ratio of (65:35% v/v) at a flow rate of 0.8 min/ml. 20µl of sample was injected and the run time was 7 minutes.

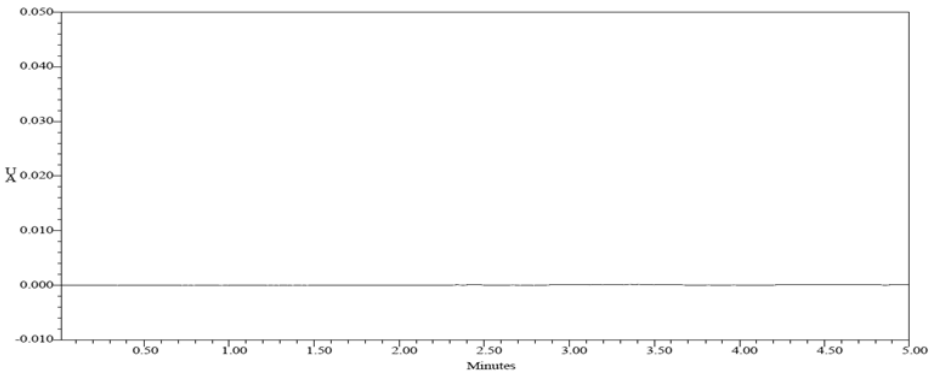

Fig. 2: Chromatogram showing blank preparation (mobile phase).

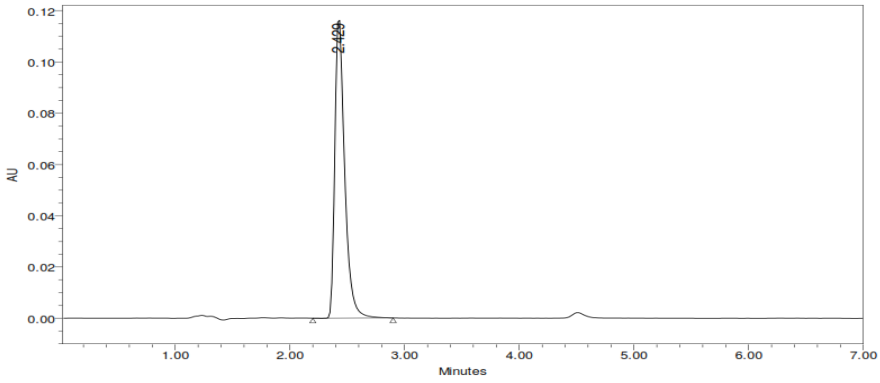

Fig. 3: Chromatogram of Perindopril standard peak.

Linearity

30, 60, 90, 120, 150 ppm was injected into the chromatographic system and peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and Y-axis peak area) and the correlation coefficient was calculated.

Acceptance criteria

Correlation coefficient should be not less than 0.999.

Table 4: Showing the results for the linearity.

Conc.(ppm)	Rt	Area
30	2.428	1608152
60	2.422	2592905
90	2.430	3778327
120	2.426	5170038
150	2.433	6249400
Co efficient of correlation(R^2)		0.997

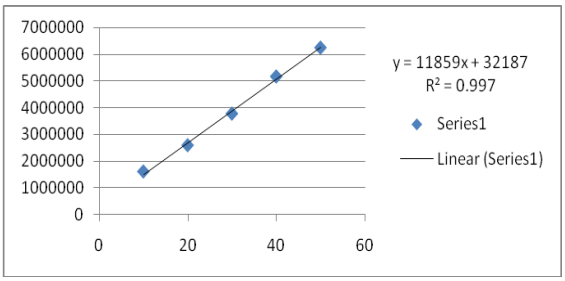

Fig. 4: Calibration graph of perindopril.

Precision

The standard solution (100 ppm) was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria

The %RSD for the area of five standard injections results should not be more than 2.

Table 5: showing the results for precision.

S. No	Conc.(ppm)	Rt	Area
1	100	2.423	693078
2	100	2.424	693338
3	100	2.424	695080
4	100	2.424	694843
5	100	2.423	695336
Mean			694335
Std. Dev			1047.5
% Rsd			0.15

Accuracy

The standard solution of concentration 50,100 and 150 ppm were injected into chromatographic system. Calculate the amount found and amount added for perindopril calculated the individual % recovery and mean % recovery values.

Acceptance criteria

The % recovery for each level should be between 98.0 to 102.0%.

Table 6: Showing accuracy results for perindopril.

S. No	Conc(µg/ml)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean% recovery
1	50	1048287	5	5.04	100.8%	100.3%
2	100	1378200	10	10.01	100.1%	
3	150	1715480	15	15.02	100.1%	

System suitability

The standard I solution was injected one time and standard II solution was injected 5 times.

Table 7: Showing system suitability results for perindopril.

S. No.	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	4352	1.1
2	1	4024	1.2
3	1.2	3730	1.2

Limit of quantitation (LOQ)

From the above preparation 0.5ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluent.

Assay

The developed and validated method was applied to the determination of perindopril erbumine in marketed tablets containing 4 mg of drug per tablet. Three injections of sample were injected into chromatographic system. Assay % was calculated by using the formula mentioned above and it was found to be 99%.

Table 10: Showing the results of assay.

S. No.	Name	Rt	Area
1	Perindopril	2.425	695226
2	Perindopril	2.429	694341
3	Perindopril	2.426	694434

CONCLUSION

A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of perindopril erbumine in pure form and in tablets. The analytical conditions and solvent system developed provided a good separation for perindopril erbumine 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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Limit of detection (LOD)

From the above preparation 1ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluents.

Table 8: Showing results for Limit of Detection.

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg)
Perindopril	371827.90	563365963	2.97

Table 9: Showing results for Limit of Quantitation.

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg)
Perindopril	371827.90	563365963	9.92

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