EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 11
Year: 2024

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF PERINDOPRIL ARGININE IN BULK DRUG AND TABLET DOSAGE FORM

Vetrichelvan T.[1]*, Bhuvana M.[2], Deepika R.[2], Girija M.[2], Gowshika J.[2] and Hari Priya V.[2]

[1]HOD Cum Dean Research, Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319, Tamilnadu.
[2]Students, Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319, Tamilnadu.

***Corresponding Author: Vetrichelvan T.**
HOD Cum Dean Research, Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319, Tamilnadu.

Article Received on 06/09/2024 **Article Revised on 26/09/2024** **Article Accepted on 16/10/2024**

ABSTRACT
In the present work, a new validated HPLC method for quantitative determination of Perindopril arginine in tablet formulation was developed. The column was Phenomenex Luna C_{18} column (150 mm × 4.6 mm id; 5µm particle size) and the mobile phase was composed of Methanol: Acetonitrile: Water in 0.1% Triethylamine (40:30:30, v/v/v) with a flow rate 1 ml/min. Eluents were monitored by UV detector at 229 nm. Calibration curve was linear in the concentration range 10 – 50 µg/ml (R^2 value is 0.9999). The proposed method was successfully applied for the assay of perindopril arginine in tablet formulation and validated as per ICH guidelines.

KEYWORDS: Perindopril arginine, HPLC Method, Validation and ICH guidelines.

INTRODUCTION
Perindopril arginine (Figure1), chemically (2s,3as,7as)-1-[(2s)-2[[(1s)-1-(ethoxy carbonyl) butyl] amino] octahydro 1H -indole-2-carboxylate L- arginine.[1] It inhibits the angiotensin converting enzyme.[2] Perindopril arginine is rapidly metabolized in 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 1 (AT 1) to angiotensin 11 (AT 11) AT 11 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 infraction and stable coronary disease.[3]

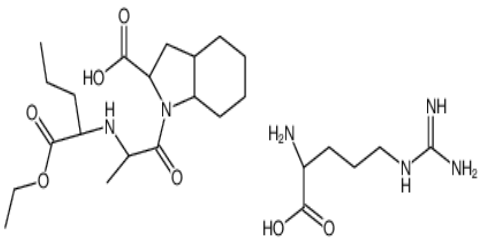

Fig. 1: Chemical structure of Perindopril arginine.[4]

On literature survey, No RP-HPLC method was reported for the estimation of Perindopril arginine by using above mobile phase. So we have developed a novel, simple, rapid, accurate, precise and highly sensitive RP-HPLC method for the estimation Perindopril arginine in tablet dosage form according to ICH guidelines. The developed method was validated as per ICH norms.[5,6]

MATERIALS AND METHODS
Materials and reagents
Pharmaceutical grade standard perindopril arginine was obtained from Shanghai Huirui Chemical Technology Co. Ltd. (China). The Pharmaceutical dosage form used in this study was Coversyl tablet and contains equivalent to 10 mg of perindopril arginine per tablet. All chemicals and reagents used were of HPLC grade and were purchased from Sudhagar Biological and Chemicals Pvt. Ltd., Chennai, India.

The instrument and chromatographic conditions
Shimadzu HPLC system (Shimadzu corporation Kyoto, Japan) consisted of a pump (LC -20AD solvent deliver module, SPD-20A UV- Visible detector) run under Lab solutions software, with manual injecting facility programmed at 20 µL capacity per injection was used. The column used was Phenomenex Luna C_{18} (150 mm × 4.6 mm, 5.0 µm particle size). Different mobile phases were tested in order to find the best condition for separation of Perindopril arginine. The mobile phase consisting of Methanol: Acetonitrile: Water in 0.1%

Triethylamine (40:30:30, v/v/v) and the flow rate was maintained at 1.0 ml/min. UV detection was carried out at 229 nm. The mobile phase and samples were filtered through a 0.45 µm membrane filter. Mobile phase was degassed by Sonica ultrasonic cleaner (model 2200 MH) prior to use.

Selection of detection wavelength
10 µg/ml solution of perindopril arginine was prepared in methanol and scanned in the range between 200 and to 400 nm and the spectrum was recorded. The drug showed maximum absorbance at 229 nm. Therefore, 229 nm was selected as the detection wavelength for the isocratic elution of drug.

Preparation of standard and sample solutions
Mobile phase
Methanol: Acetonitrile: Water in 0.1% Triethylamine (40:30:30, v/v/v) is programmed as RP HPLC method.

Preparation of standard stock solution
10 mg of perindopril arginine was weighed and transferred in to a 100 ml volumetric flask. Dissolved in Methanol and the volume was made up to 100 ml to get a concentration of 100 µg/ml of perindopril arginine with more methanol.

Preparation of working standard solution
From above standard stock solution of Perindopril arginine, 2 ml of solution was taken in to a 10 ml volumetric flask and was made to the mark with the methanol to get 20 μg/ml of perindopril arginine.

Preparation of sample solution
Twenty tablet (perindopril arginine 10 mg) were weighed accurately and the average value of each tablet was determined. The weighed tablet was crushed into a fine powder. The tablet powder equivalent to 10 mg of perindopril arginine was weighed and transferred into a 100 ml of standard flask. The drug was dissolved in methanol and sonicated for 30 minutes. The solution was made up to the mark with the same solvent and sonicated for 15 minutes. Then the solution was filtered through Whatman's filter paper no 41. From the filtrate solution, pipetted out 2 ml of filtrate and diluted to 10 ml in a standard flask with more methanol to get a concentration of 20 µg/ml of perindopril arginine. Sample solution were injected and the chromatograms were recorded. From the peak area observed in the chromatogram, the amount of drug was calculated.

Validation method for analysis of perindopril arginine
The proposed method was validated as per ICH guidelines.

Linearity
Different aliquots of 1 - 5 ml of standard stock solution was transferred into series of 10 ml volumetric flasks separately and the volume was made up to the mark with methanol to get concentrations 10, 20, 30, 40 and 50 µg/ml, respectively.

Accuracy (Recovery)
To the pre analyzed sample solution, a known amount of standard stock solution was added at different levels i.e. 50, 100 and 150 %. The solutions were reanalyzed by the] proposed method.

Precision
Repeatability
Analyzed multiple injections (n=6) at the same sample to determine intra-day precision.

Intermediate precision
Performed the analysis on different day and or by different analysis on different analyst to evaluate inter-day precision.

Limit of Detection and Limit of Quantitation
Limit of Detection (LOD)
From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline

LOD= 3.3 X Intercept/ Slope

Limit of Quantitation (LOQ)
The limit of quantification (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline

LOQ= 10 X Intercept/ Slope

Robustness
The robustness of the method was established by making deliberate minor variations in the following method parameters

a) Flow rate: ± 0.1 ml/min
b) Change in the ratio of component in the mobile phase: ±3 %

RESULTS AND DISCUSSION

Method development and optimization
The HPLC procedure was optimized with a view to develop a suitable HPLC method for the determination of Perindopril arginine in tablet dosage form. Initially, methanol and water in different ratios were attempted. But Perindopril arginine gave broad peak with tailing, so acetonitrile was added with methanol and water. Mixtures of different ratios were tried. It was found that methanol: acetonitrile: water in 0.1% Triethylamine (40:30:30, v/v/v) gave acceptable retention times (4.784 min) with flow rate of 1.0 ml/min as shown in Fig. 2. The calibration curve as shown in fig. 3

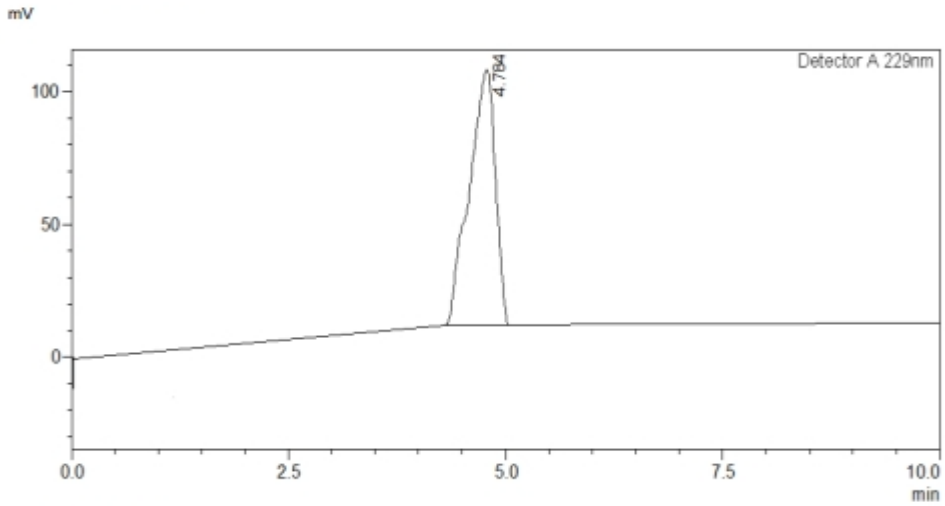

Fig. 2: Chromatogram of perindopril arginine obtained at optimum chromatographic conditions.

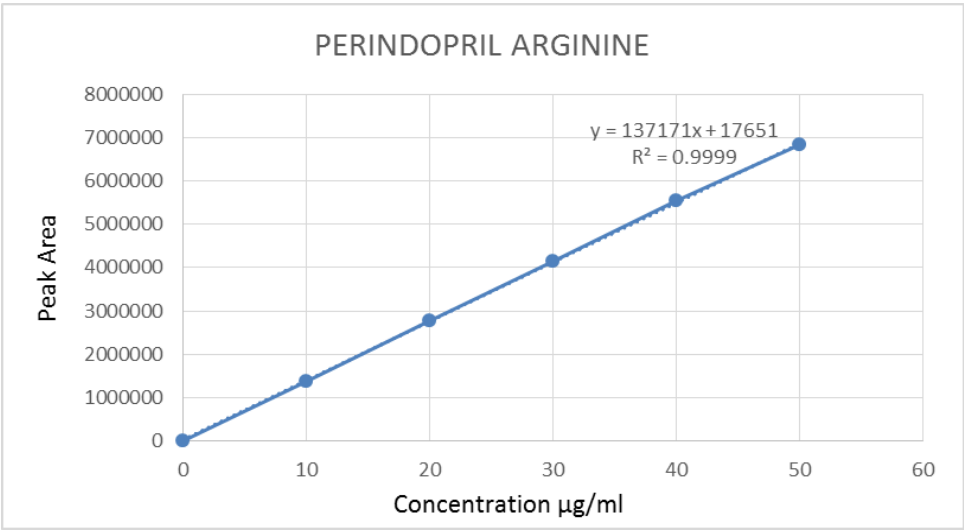

Fig. 3: Calibration curve for perindopril arginine by RP-HPLC method.

Method validation

The described method has been validated which include parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System suitability

System suitability and chromatographic parameters were validated such as tailing factor and theoretical plates was calculated. The results are given in table 1.

Table 1: System suitability parameters.

Parameters	Perindopril arginine	Recommended limits
Retention time	4.784	RSD ≤2
Peak area	2749409	RSD ≤2
Tailing factor (T)	0.192	T <2
Theoretical plate (N)	4352	> 2000

Linearity

Linearity of this method was evaluated by linear regression analysis calculated by least square method and studied by preparing standard solution of perindopril arginine at different concentration in the range of 10 -50 µg/ml with correlation coefficient (r^2) of 0.9999. Results are given in table 2.

Table 2: Linearity data for Perindopril arginine.

Drug	Concentration (µg/ml)	Area
Perindopril arginine	10	1384300
	20	2772258

	30	4150628
	40	5543737
	50	6830632

Accuracy

Accuracy of the proposed method was determined by performing the recovery experiment. The recovery experiment was studied by adding known amount of standard perindopril arginine to the Pharmaceutical product and calculating the recovered standard amount. At 50%, 100% and 150% standard addition level and mean recovery of perindopril arginine found to be 100.14%, 100.30% and 100.36% respectively. The results of recovery experiment are given in table 3.

Table 3: Results of the accuracy study.

Accuracy Level	Amount of drug taken (µg/ml)	Amount of drug spiked (µg/ml)	Estimated amount (µg/ml)	Recovered amount (µg/ml)	Recovery %	RSD% (n=3)
50	20.014	10	30.15	10.14	101.40	0.1914
100	20.014	20	40.31	20.30	101.50	0.5002
150	20.014	30	50.37	30.36	101.20	0.5295

Precision

Precision was evaluated at the repeatability and intermediate precision levels. For repeatability analysis, six independent portions of a tablet dosage form were processed through the full analytical method and results was evaluated and obtaining a % RSD value of 0.7097 as shown in table 4.

Table 4: Precision result of the proposed method.

Sample No.	Perindopril arginine	
	Peak area response	Assay (%)
1	2749409	99.17
2	2748709	99.15
3	2767709	99.83
4	2744703	100.98
5	2793709	100.77
6	2745909	99.04
Average	2758358	99.95
% RSD	0.7065	0.7097

Robustness

Robustness study was conducted by deliberate changes in mobile phase composition and flow rate, revealed that there was no significant variation in % assay as shown in table 5.

Table 5: Robustness study.

Percent assay of the drug	Mobile phase, Acetonitrile: Methanol: Water		Flow rate, ml/min	
Perindopril arginine	39 : 29 : 32 (v/v/v)	41 : 31 : 28 (v/v/v)	0.9	1.1
	100.14	100.18	100.16	99.43

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ was found to be 0.798 (µg/ml) and 2.417 (µg/ml) for Perindopril arginine estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration. The results are given in table 6.

Table 6: LOD and LOQ data for Perindopril arginine.

Drug	LOD	LOQ
Perindopril arginine	0.798 (µg/ml)	2.417 (µg/ml)

CONCLUSIONS

The RP-HPLC method was developed, validated according to ICH guidelines and was applied for the determination of perindopril arginine in tablet formulation. The result obtained from validation studies revealed that, the developed method was found to be rapid, simple, accurate, precise, specific, selective and economical. Hence, this method can easily and

conveniently adopt for routine analysis of perindopril arginine in bulk and tablet dosage form.

ACKNOWLEDGEMENT

The management of ACMEC trust is hereby thanked by one of the author Dr. T. Vetrichelvan for their support in doing this research. The authors would like to acknowledge the Shanghai Huirui Chemical Technology Co. Ltd. (China), for providing drug as gift sample.

REFERENCE

1. Priya K. Rajput, Atul Bendale, Shailesh V. Luchar, Dr. Sachin B.Narkhede. Method development and validation of stability indicating RP-HPLC method for perindopril arginine and amlodipine besylate in synthetic formulation., 2016; 6(3): 347-355.
2. Kalaiyarasi Duraisamy, K.S. Jaganathan, Marothu Vamsi Krishna. Method development and validation of HPLC tandem/Mass spectrometry for qualification of Perindopril arginine and Amlodipine besylate combination in bulk and pharmaceutical formulations, 2017; 12(4): 307-314.
3. https://go.drugbank.com/salts/DBSALT001144.
4. Bhagirath K. Patel, Subharchandra K. Patel. Swati R. Patel. Analytical method development and validation for simultaneous estimation of amlodipine besylate and perindopril arginine in combined pharmaceutical dosage from, 2016; 3(1): 343-350.
5. Code Q2A: Text on Validation of Analytical Procedure Methodology Step-3 Consensus Guideline. ICH Harmonised Tripartite Guideline, 2005.
6. Code Q2B: Text on Validation of Analytical Procedure Methodology Step-3 Consensus Guideline. ICH Harmonised Tripartite Guideline, 1994.