JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Validated stability indicating reversed-phase high-performance liquid chromatography (RP-HPLC) for the estimation of Lisinopril in Pharmaceutical tablet dosage form

Smita A. Navale*, Mahesh N. Pingale*, Sonali M. Takale, Shubham S. Pawal

***Vidya Niketan College of Pharmacy, Lakhewadi. Indapur**

*Correspondence Address

Smita A. Navale, Mahesh N. Pingale

Vidya Niketan College of Pharmacy,

Lakhewadi, Indapur Pune

Pin Code - 413103

Abstract:

A dependable specific and stable RP-HPLC method was developed and accepted for the determination of lisinopril both in pharmaceutical doses form and bulk the detection was carried out at a wavelength of 237 nm the developed approach was validated in accordance with ICC recommendation

Kewords : Lisinopril , RP HPLC, Validation, Analytical Method, Method Development

Introduction :

The angiotensin converting enzyme is blocked by the synthetic peptide derivative lisinopril, a long-acting, potent ACE inhibitor that cleaves angiotensin I into angiotensin II. This lowers blood pressure by resulting in the blood vessels to enlarge or dilate. It is suggested for the treatment of hypertension and congestive heart failure.[1]] Lysine derivative lisinopril [1,2] is a potent inhibitor of the angiotensin converting enzyme that appears like the structure of its substrate. It is primarily employed to treat hypertension, congestive heart failure, and to prevent diabetic complications involving the kidneys and eyes..[2] It can be utilized to avoid kidney problems in diabetics. It is used together with a diet and taken orally. It is an off-white to solid-white crystalline powder that is slightly insoluble in water.[3] Angiotensin II stimulates the ACE cortex obstruction, which results in reduced plasma angiotensin I, vasopressor activity, and decreased aldosterone secretion. Finally, these reductions may lead to a temporary rising of serum potassium.[4]

The aim of the study is to develop a straightforward RPHPLC Assay technique. Review articles were made visible for method development and validation, and method validation was carried out in conformity with ICH and regulatory guidelines.[5] Therefore, the objective of this research is to develop and validate a slightly better and

dependable HPLC method for lisinopril tablet dosage estimation. Therefore, the objective of this research is to develop and validate a slightly better and dependable HPLC method for lisinopril tablet dosage estimation. [6]

Materials And Methods :

a) Material And Reagent :

All The chemicals and reagent where of analytical grade. Lisinopril was a kind gift from Lupin Private Ltd. Tarapur , Mumbai. Acetonitrile (HPLC Grade) , Methanol (AR Grade) , Trifluroacetic acid (AR Grade) And Water (HPLC Grade) Was a Research Lab Fine chemistry industries Mumbai

b) Instrumentation :

Name of Instrument	Manufacturer
Balance Mettler	Digital electronic balance Citizen & Contact (CY 220 & CY 223)
V-1800 UV-visible spectrophotometer(Software UV probe 2-33 version)	UV-Visible double beam spectrophotometer (Shimadzu 1800)
HPLC System	Agilent HPLC
pH Meter	Digital pH Meter

c) Description :
Lisinopril is a white to off-white, crystalline powder.

Solubility:
Solubility of Lisinopril was observed by dissolving them in different solvents.

Solvent	Solubility (Lisinopril)
Water	++
Methanol	++
Ethanol	-
Acetonitrile	+

(++) symbolizes Freely soluble , (+) symbolizes soluble , (-) symbolizes insoluble

Chromatographic Condition :

Oven Temp: 300C
Flow rate: 1 ml/min**.**
Mobile Phase : Acetonitrile :Type I Water (70:30, % v/v)
Runtime: 12 minutes
Injection Volume : 10 µl
Wavelength : 256 nm
Diluent: Type -1 water : Acetonitrile (50 : 50, % v/v)
Column : Agilent Zorbax Bonus RP(250 x 4.6 mm, 5 µ)

d) Standard Preparation:

a. Lisinopril Standard Stock Solution-I (LSSS-I):
Initially Prepare a Standard Stock Solution (SSS-I) of by adding 5 mg of Lisinopril in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Lisinoprilin LSSS-I= 500 µg/ml).

b. Then add 1 ml of LSSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Lisinopril in LSSS-II =50 µg/ml)

c. Then add 2 ml of LSSS-II in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Lisinopril =10 µg/ml)

e) Drug Product Sample Preparation for Assay:

i. 10 tablets were weighed and average weight was calculated. And tablets was crushed & mixed in mortar and pestle.

ii. Powder weight equivalent to 5 mg Lisinopril was weighed into 10 ml volumetric flask & add 5 ml diluent, sonicate for 5 minutes and make the volume to 10 ml with diluent. (Conc. of Lisinopril = 500 µg/ml).

iii. Further, pipette out 0.2 ml of above solution in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Lisinopril = 10 µg/ml).

Selection of Wavelength:

The sample was scanned from 190-400 nm with DAD detector. The Wavelength selected for analysis chosen was 256 nm for appropriate identification of Lisinopril.

e) Method Validation:

a Specificity & Assay:

i. Individual sample of Lisinopril of 10 µg/ml was prepared and peaks were for identified from Retention Time.
ii. Blank was injected to ensure there is no blank peak interfering with the main analyte peak.
iii. Assay was calculated by using following formula;

$$\% \ \boldsymbol{Assay} = \boldsymbol{Sample\ Area} / \boldsymbol{Standard\ Area} \ \boldsymbol{x}\ \mathbf{100}$$

Repeatability & System Suitability:

i. A single sample was prepared as described and 6 injections were made from same sample and checked for system suitability.
ii. System suitability parameters are as below:

1. Retention Time,
2. Theoretical plates
3. Asymmetry (Tailing factor),
4. Peak purity.

Linearity & Range:

i. 5 samples of varying concentrations ranging from 80-120% were made.

ii. The concentrations are given below

Table no. 5.6 Linearity of Lisinopril by HPLC

% Level	Lisinopril Conc. (µg/ml)
80	8
90	9
100	10
110	11
120	12

iii. The sample preparations are given as below;

iv. X ml of Lisinoprilwas added to 10 ml diluent to make up the concentrations given above:

X ml of LSSS-II	Diluted to
1.6	10 ml
1.8	10 ml
2.0	10 ml
2.2	10 ml
2.4	10 ml

Accuracy:

i. Samples were prepared of 80%, 100% and 120% concentration by spiking the same amount of concentration given above in table for both Lisinopril.

ii. Samples were injected in triplicate to calculate % RSD.

iii. % recovery was also calculated

LOD/ LOQ:

i. Was calculated by using ANOVA technique.

ii. Formula:

$$LOD = \frac{3.3 \times Std.\ Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

$$LOQ = \frac{10 \times Std.\ Error\ of\ Intercept}{Coefficients\ of\ X\ Variable\ 1}$$

Robustness:

i. The Robustness was performed by changing the column temperature by ± 2˚C.

ii. Each Sample was injected % Assay was calculated at each condition was calculated.

Condition	Increased	Normal	Decreased
Column Oven Temperature	32°c	30°c	28°c

Intra & Inter-day Precision:

i. Single mixture working standard and drug product samples were prepared and injected twice in a day at different time intervals to evaluate intra-day precision.

ii. Same mixture working standard and drug product samples were analysed on second day to evaluate the inter-day precision.

iii. % Assay was calculated at each interval and stability of solutions were estimated.

Lisinopril by UV

1. Chromatographic Conditions:

a. Wavelength: 244 nm

b. Diluent: Acetonitrile: Type -1 water (50: 50, % v/v).

Result And Discussion:

6.4.1. Selection of wavelength

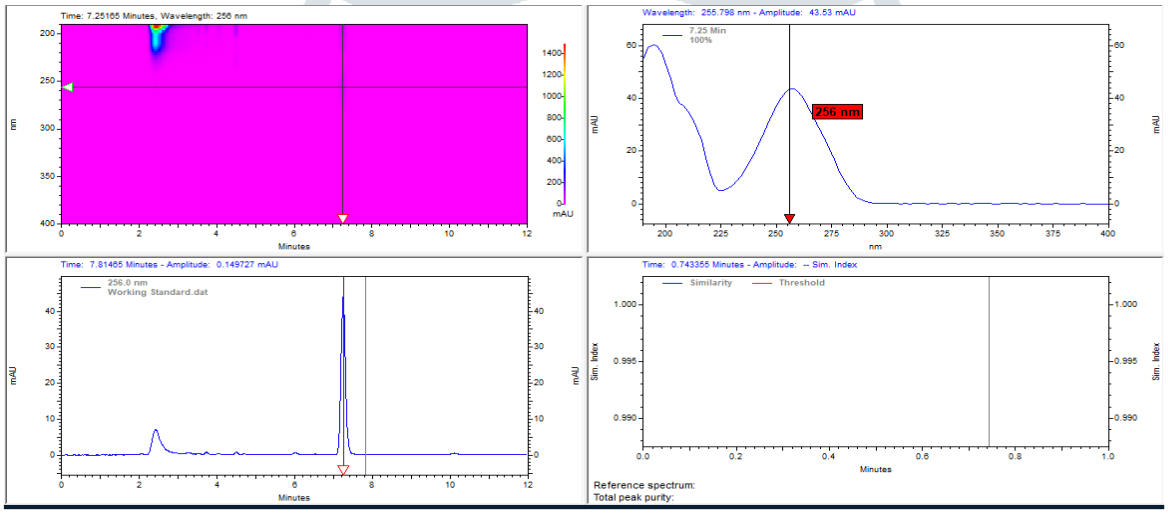


Figure no. 6.3 UV spectra of Lisinopril between 200-400nm in mobile phase

Lisinopril showed the absorbance at 296nm. Hence, HPLC analysis was carried out at 244nm

6.4.2. Selection of Mobile phase

Different mobile phases like Acetonitrile and Type-I water in varying proportions of mobile phases were tried for better resolution of the chromatogram.

Table no.6.11. Optimization of Chromatographic Conditions

Sr . No	Mobile Phase	Ratio	Wavelength(nm)	RT (min)	TP (N)
1	ACN: Type-I Water	50-50	250	13.96	19823
2	ACN: Type-I Water	40-60	250	7.26	18775
3	ACN: Type-I Water	70-30	250	5.1	17424
4	ACN: Type-I Water	60-40	256	6.31	18854

After several combinations of mobile solvents such as Acetonitrile and type I Water was selected in ratio 70:30 respectively using C18 column (Zorbax Bonus-RP.) which has given good resolution, capacity factor, acceptable system suitability parameters. The drug eluted within 7mins which will reduces the overall analysis time and cost. [Detection wavelength is 244nm]

a. Assay:

Table no. 6.11. Assay of Lisinopril

Sample	Lisinopril		
	RT	Area	% Assay
WS	7.25	739908	-
Drug Product	7.25	732507	99.00

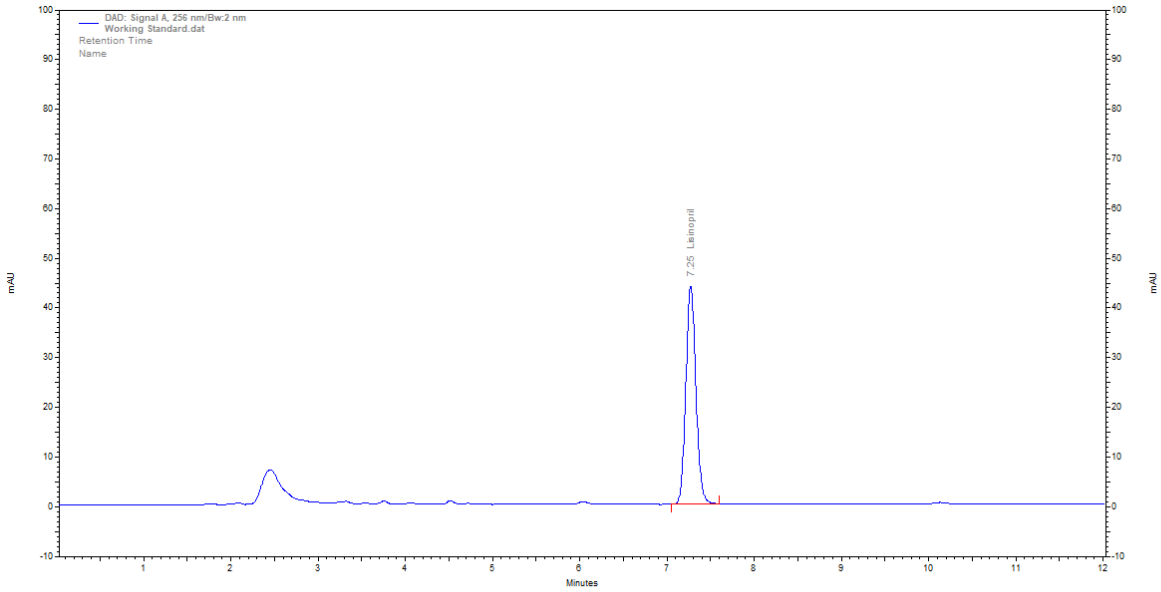
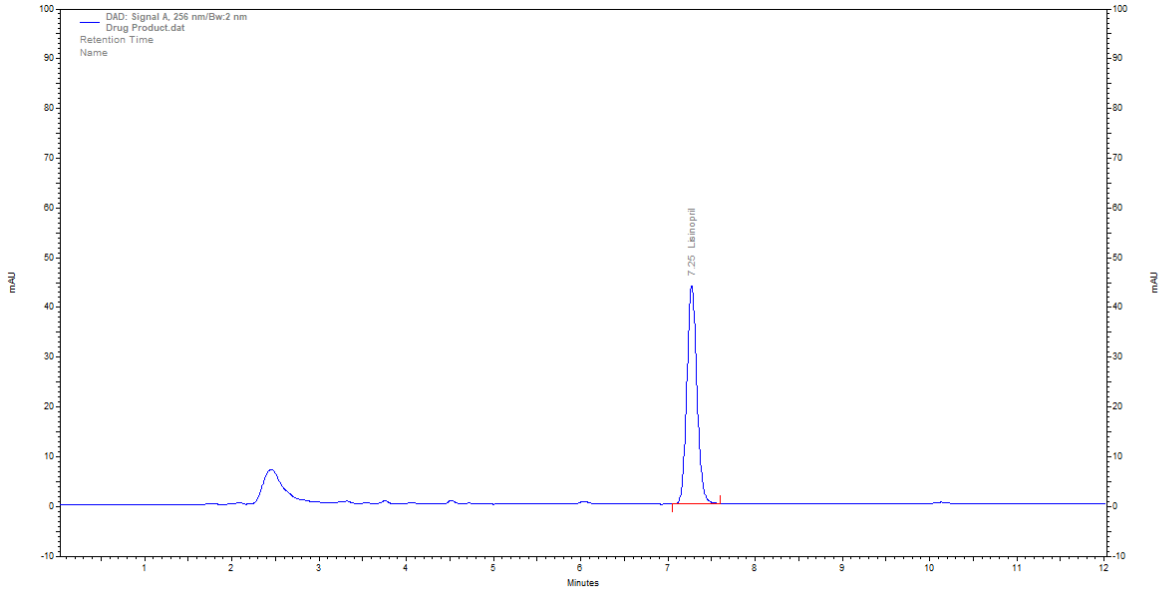


Figure no. 6.4. Chromatogram of Working Standard



6.4.3. Identification of Peaks

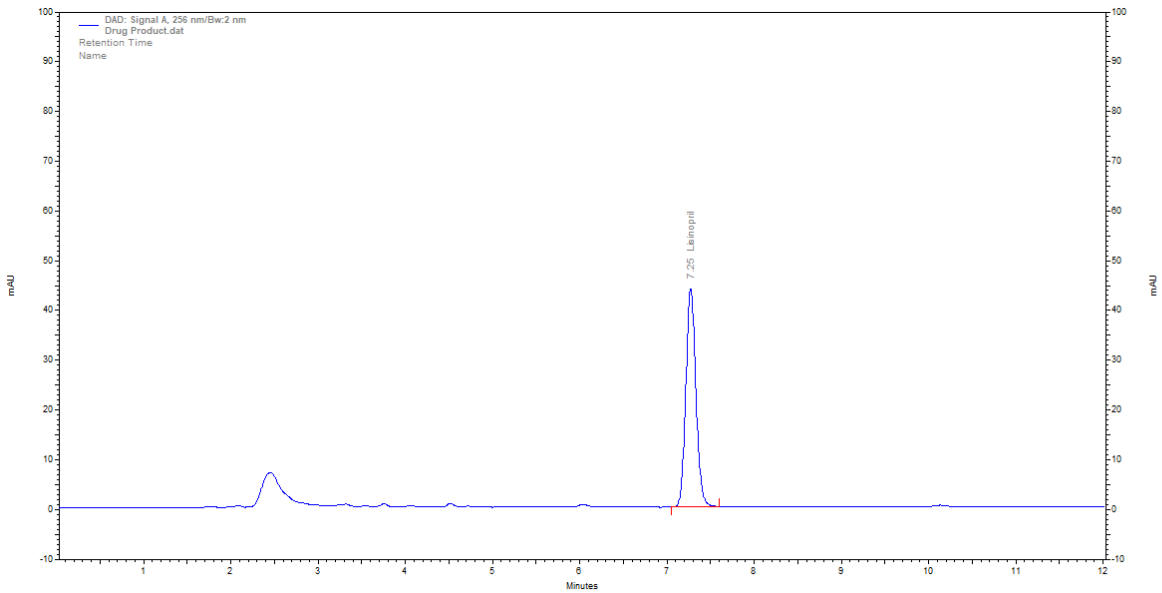


Figure no. 6.6 Chromatogram of Drug product in optimized chromatographic conditions

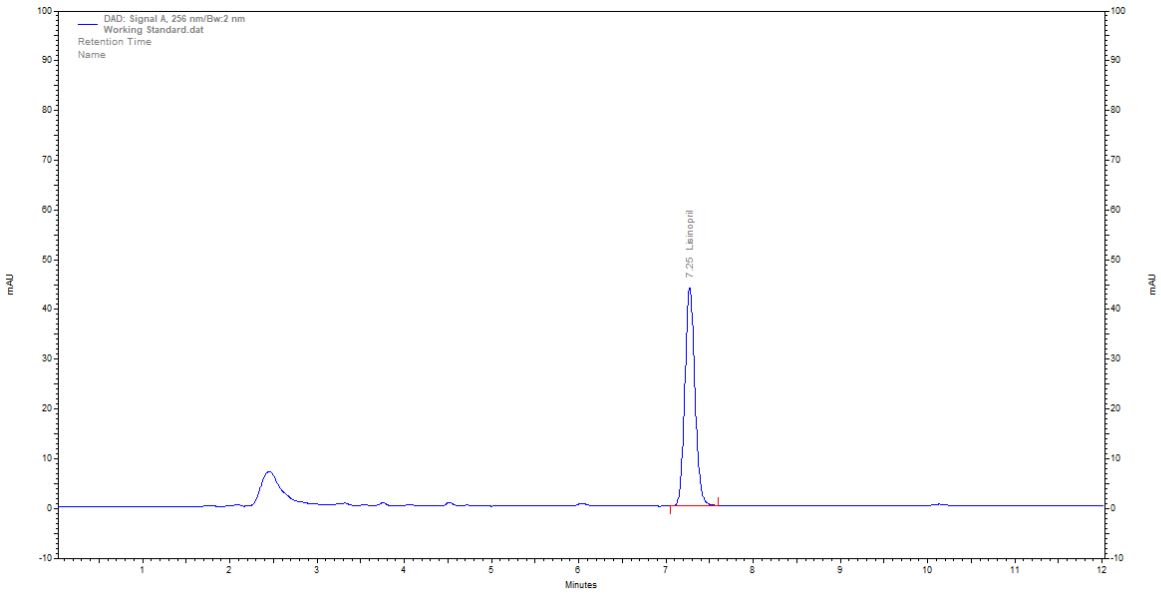


Figure no. 6.7 Chromatogram of working standard in optimized chromatographic conditions.

6.5. Method Validation for Lisinopril

I. Specificity:

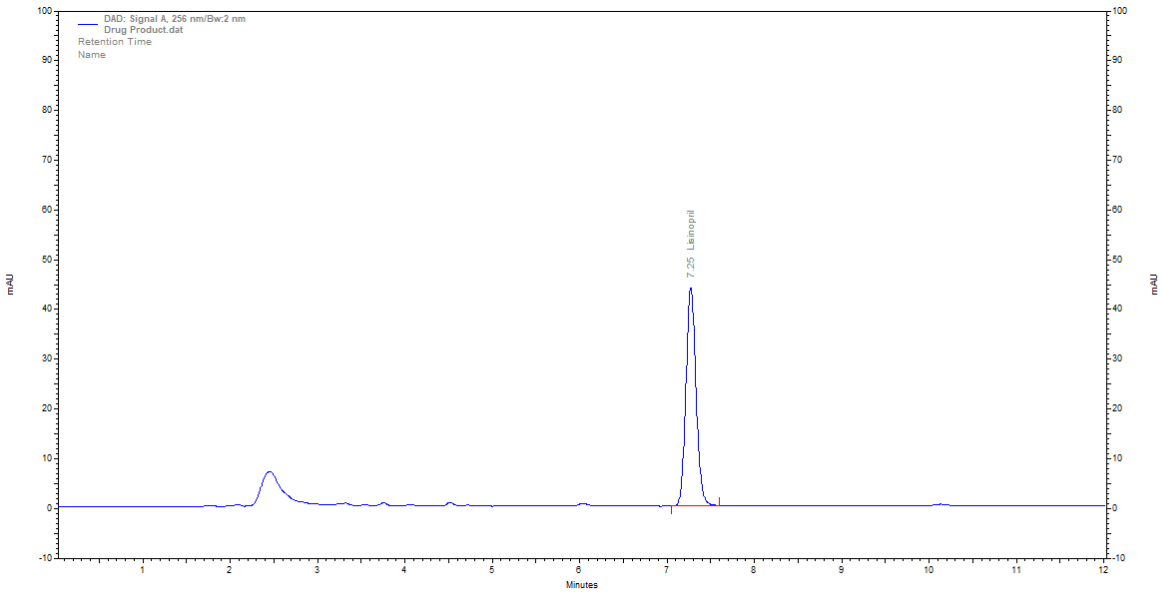


Figure no. 6.8 Chromatogram of Lisinopril

With above optimized conditions Lisinopril was eluted at 6.31min. Peak was sharp, and with good resolution. It eluted within 7 minutes, which reduced the analysis time and cost.

II. Linearity:

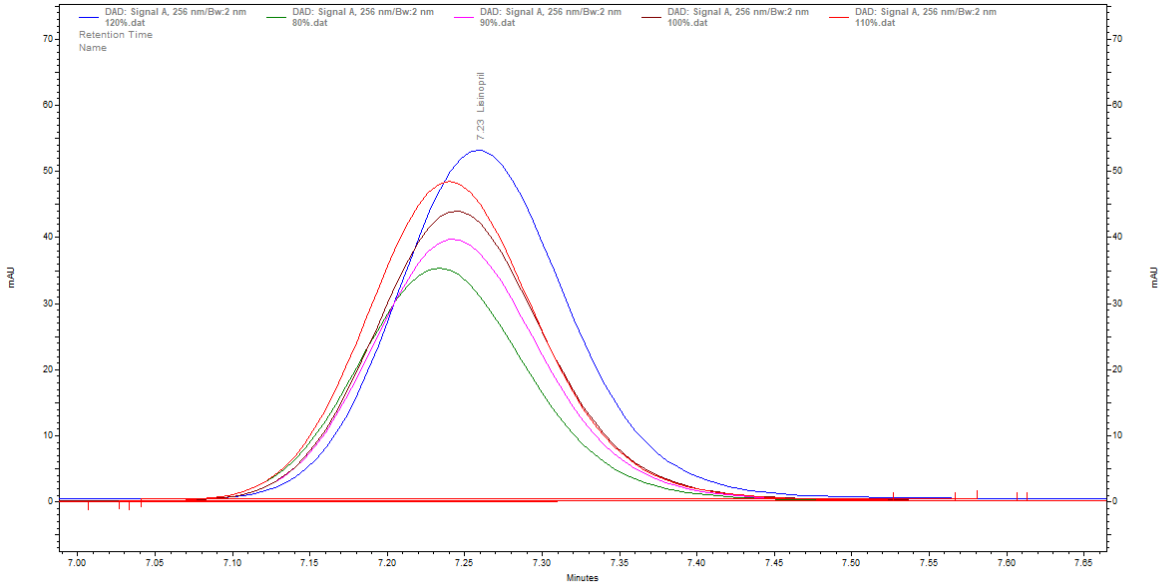


Figure no. 6.9 Overlay Chromatograms of serial dilutions of Lisinopril in optimized chromatographic conditions.

The peak response is directly proportional to the concentration of drug and was found to be linear in the range of 80-120µg/ml (**Figure no. 6.9**). The correlation coefficient was found to be 1 which is well within the acceptance criteria.

Table no. 6.12 Response of Lisinopril at various linearity levels

% Level	Con. **(µg/ml)**	Area
80	80	590496
90	90	666729
100	100	739908
110	110	814792
120	120	886610

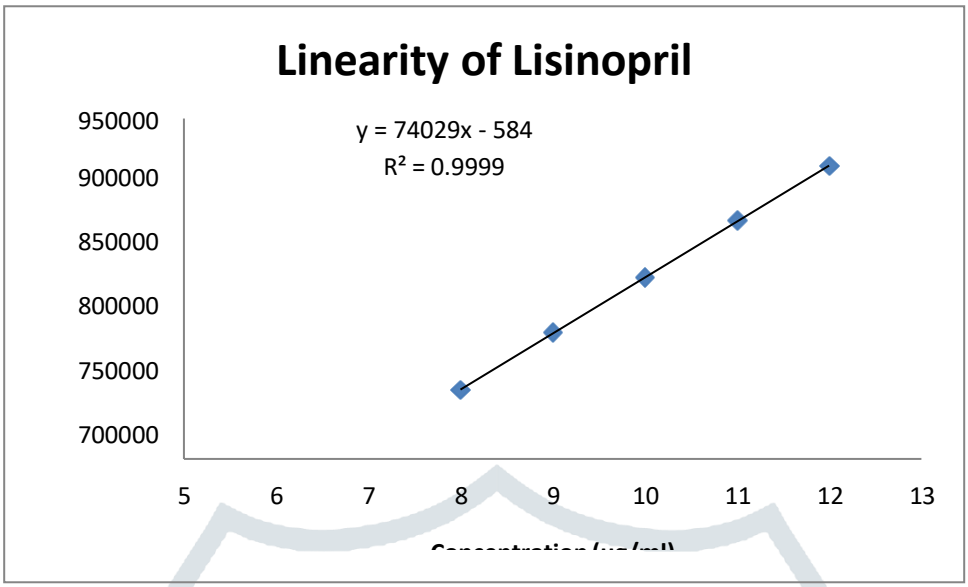


Figure no. 6.10 Calibration curve of Lisinopril of RP-HPLC method

Table no. 6.13 Linear Regression Analysis of Calibration Curves for Lisinopril

Parameter	**Values**
Absorption maxima	244nm
Beers law range	80-120
Regression coefficient(r^2)	0.9998
Regression equation	Y= 74029x – 584
Intercept	74029
Slope	584

IV. Accuracy

Table no.6.15 Accuracy data for RP-HPLC Method

Sample ID	**Reps**	**Spiked Conc.**	**Area**	**Amt. Recovery**	**% Recovery**	**Average**	**STDEV**	**RSD**
80%	Rep 1	8.00	590496	7.98	99.78	99.77	0.015733	0.02
	Rep 2	8.00	590581	7.98	99.79			
	Rep 3	8.00	590395	7.98	99.76			
100%	Rep 1	10.00	739908	10.00	100.02	100.01	0.020737	0.02
	Rep 2	10.00	739979	10.00	100.03			
	Rep 3	10.00	739685	10.00	99.99			
120%	Rep 1	12.00	886610	11.98	99.87	99.87	0.017724	0.02
	Rep 2	12.00	886785	11.98	99.89			
	Rep 3	12.00	886471	11.98	99.86			

individual and mean value (n=3) at each level was recorded in (**Table no.6.15)**. It should be between 99.0 % to 102.0 % recovery as per ICH guidelines.

The percentage recoveries of the results indicate that the recoveries are well within the acceptance range, therefore, method was found to be accurate. **(Table no.6.15)**

V . Repeatability:

Table no.6. 16 Repeatability data for RP-HPLC Method

Reps	Area
Rep 1	739908
Rep 2	739979
Rep 3	739685
Rep 4	739789
Rep 5	739748
Avg.	739587
STDEV	73.97
RSD	0.873

The % RSD for peak area was found to be 0.87 % for Lisinopril, which indicate that themethod is precise and in accordance with ICH guidelines (i.e. <2).

Table no. 6.17 Intraday precision data of Lisinopril

Intra Day precision			
Day 1	**Sample ID**	**Lisinopril**	
		Area	**Assay**
Morning	**WS**	739908	-
	DP	732507	99.00
Evening	**WS**	736589	-
	DP	735849	99.90
STDEV			99.61
RSD			0.530625
Inter Day precision			
Day	**Sample ID**	**Lisinopril**	
		Area	**Assay**
Day 2	**WS**	738987	-
	DP	738521	99.94
AVG			99.61
STDEV			0.530625
RSD			0.53

Intra Day precision			
Day 1	**Sample ID**	**Lisinopril**	
		Area	**Assay**
Morning	**WS**	739908	-
	DP	732507	99.00
Evening	**WS**	736589	-
	DP	735849	99.90

Table no. 6.18 Interday precision data of Lisinopril

Inter Day precision			
Day	Sample ID	Lisinopril	
		Area	Assay
Day 2	WS	738987	-
	DP	738521	99.94

The percentage RSD (<2) values obtained showed that the method developed was precise atrepeatability and intermediate precision level.

Conclusion :

The preceding findings show that the developed RP-HPLC method is simple, sensitive, accurate, precise, and selective. As indicated by the percentage recovery, the technique is devoid of interference from excipients included in the formulation.

Acknowledgments

The authors deeply appreciate to Vidya Niketan College Of Pharmacy, Lakhewadi. Pune, India,

Reference :

1.M. Saeed Arayne, Safila Naveed , Najma Sultana , RP-HPLC Method for the Determination of Lisinopril in Active Pharmaceutical Ingredients, Dosage Forms and Human Serum

2. Najma Sultana, M. Saeed Arayne, Rubina Siddiqui, Safila Naveed, RP-HPLC Method for the Simultaneous Determination of Lisinopril and NSAIDs in API, Pharmaceutical Formulations and Human Serum

3. Kumaraswamy Gandla, Ravinder M., K. Sushimitha, Narmada Gade , DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF LISINOPRIL IN BULK AND PHARMACEUTICAL DOSAGE FORM

4. Vijaykumar T. Pawar , Harinath N. More , Manish S. Bhatia ,Development And Validation Of RP-HPLC Method For The Determination Of Lisinopril And Amlodipine In Bulk And Multicomponent Pharmaceutical Cardiovascular Dosage Form.

5. K. Raghu Babu, E.S.R.S.Sarma, N.Aruna kumari, G.M.J.Raju1 and V.Mallikarjuna Sarma Simple and stability indicating RP-HPLC assay method development and validation lisinopril dihydrate by RP-HPLC in bulk and dosage form.

6. Vikas Chander, Manindra Mohan, Richa Seth , Prashant Singh , Rakesh Singh, Sanjay Gupta Development and Validation of RP-HPLC Method for the Estimation of Lisinopril in Tablet Dosage Form