

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF LISINOPRIL IN BULK AND PHARMACEUTICAL DOSAGE FORM*¹Kumaraswamy Gandla, ²Ravinder M., ¹K. Sushimitha, ²Narmada Gade¹Department of Pharmacy, Chaitanya (Deemed to be University), Hanumakonda, Telangana.²Department of Chemistry, Chaitanya (Deemed to be University), Hanumakonda, Telangana.***Corresponding Author: Dr. Kumaraswamy Gandla**

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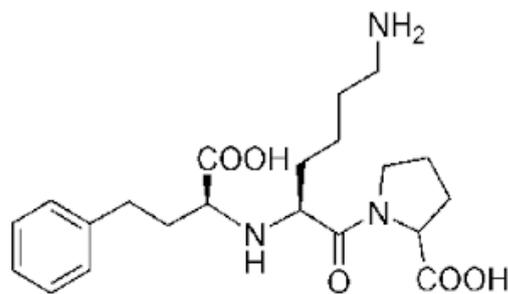
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ABSTRACT

An accurate, precise and reproducible RP-HPLC method was developed and validated for the estimation of lisinopril in bulk and pharmaceutical dosage form. The chromatographic separation was carried out on Phenomenex column C18 (250x4.6mm,5µm), column by using the mobile phase Acetonitrile:Buffer 0.1M (70:30 % v/v) at a flow rate of 1.0 mL/ min. The detection was carried out at a wave length of 237 nm. The retention time for Lisinopril was found to be 3.444 respectively. The developed method was validated according to ICH guidelines.

KEYWORDS: Lisinopril, RP-HPLC, UV-Visible Detector; ICH Validation.**INTRODUCTION**

Lisinopril is a medication of the angiotensin-converting enzyme inhibitor and is used to treat high blood pressure, heart failure, and after heart attacks. For high blood pressure it is usually a first-line treatment. It is also used to prevent kidney problems in people with diabetes mellitus. It is used together with diet and it is taken orally. It is a solid white to off-white, crystalline powder, which is soluble in acetonitrile, DMSO, slightly insoluble in water. The brand names of it are Prinivil (tablet film coated with strength of 20,30 and 40 mg), Zastrel (tablet film coated with strength of 10,15 and 20 mg), The structure of Lisinopril is

**Fig. 1: Structure of Lisinopril.**

Lisinopril is chemically 5-chloro n-[[[(5S)-2-oxo-3-[4-(3-xomorpholin-4-yl)phenyl]-1,3-oxozolidin-5-yl]methyl} thiophene-2-carboxamide. It is used as Antihypertensive Drug (Angiotensin-converting enzyme (ACE) inhibitors) After reviewing various literature survey, Literature reveals that various methods like GC-MS, HPTLC, RP-

HPLC and Spectrophotometric methods has been developed for the determination of Lisinopril. The main aim of our study is to develop a simple, accurate, precise RP-HPLC method and validate as per ICH Guidelines which should be followed for routine analysis of drugs in pure and pharmaceutical tablet dosage form..

EXPERIMENTAL**Instrumentation**

HPLC(Shimandzu Prominence Binary) equipped with Rheodyne injector, PDA Detector and LC-Solutions software. The column was Enable C₁₈-c(250x4.6mm,5µm) with column oven max limit of 80°C.

Chemicals and Reagents

The solvents used were of HPLC/AR grade. Pure drug sample of Lisinopril. was obtained as a gift sample from MSN PVT LTD, HYD.

Chromatographic conditions

The Mobile phase consisted of Methanol:Acetonitrile:Buffer0.1M (50:30:20) with adjusted pH to 3.2 by using phosphoric acid. Contents of mobile phase were filtered before use through a 0.22µm membrane filter and sonicatedfor 15min. The mobile phase was pumped with 1.0ml/min flow rate from the solvent reservoir to the column .the injection volume was 20µl. The column oven temperature was maintained at 30⁰c. The eluents were detected at 237nm.

Preparation of Phosphate Buffer

The buffer solution is prepared by weighing 6.84 g of potassium dihydrogen phosphate and added to 1000 mL of deionized water (HPLC grade). This weight of KH₂PO₄ was required to make 50 mM buffer.

Preparation of Mobile Phase

The mobile phase preparation is based on the aqueous: organic ratio of Acetonitrile: Buffer 0.1M (70:30) with adjusted pH to 3.2 by using phosphoric acid. Sonication is required to get rid of the air bubbles.

Method Development: Method development by using HPLC and UV Spectrophotometry was initiated by taking λ_{max} .

Determination of λ_{max}

A standard solution of Lisinopril of concentration 10 μ g/ml was prepared and scanned in the UV region i.e., 200 to 400 nm using photodiode array detector to detect the maximum wavelength. The spectrum was recorded and shown in fig no.2.

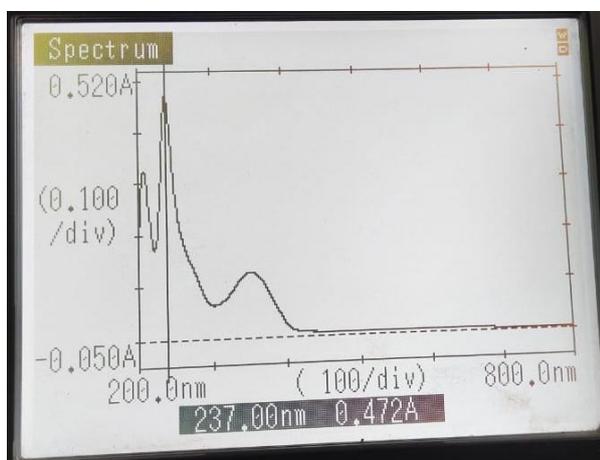


Fig.2: Maximum absorption spectrum of Lisinopril.

Preparation of Lisinopril Standard solution

Accurately weighed 10mg of Lisinopril was transferred to 10ml volumetric flask. About 4ml of acetonitrile was added and degassed to dissolve. The volume was made up to mark with same solvent to 10ml (1000 μ g/ml). Then 1ml of the above solution was diluted to 10ml with the solvent system (100 μ g/ml). The resultant standard solution (10 μ g/ml) was filtered through a 0.45 μ m membrane filter and degassed under ultra-sonic bath prior to use. From the above standard solution several working standard solutions are made by serial dilution technique.

Preparation of sample solution

The average weight of tablets were determined by weighing 20 tablets and powdered. Tablet powder was equivalent to 10 mg of Lisinopril was weighed and transferred into 10 ml volumetric flask about 5ml of acetonitrile solution was added and degassed for 15 min for the complete dissolution of drug, volume was made up to 10 ml with acetonitrile and mixed. Above solution was filtered through What'smann filter paper number 41 i.e., primary sample stock solution (1000 μ g/ml). From this above solution 1 ml is pipetted out and made to 10ml (100 μ g/ml) i.e., secondary stock solution and further serial dilutions were made for accuracy and assay studies.

RESULTS AND DISCUSSION

The solution of 10 μ g/ml of Lisinopril in diluent (acetonitrile) was prepared and the solution was scanned in the between 200-400nm. The drug showed absorption maximum at 237 nm with 2hr and 30min stability. Hence this was selected as detection wave length. After considering all system suitability parameters, Acetonitrile: Buffer (70:30 %v/v) with adjusted pH to 3.2 by using phosphoric acid. Was selected for analysis, 1ml flow rate was optimised for advanced studies. The retention time of Lisinopril was found to be 3.444 min.

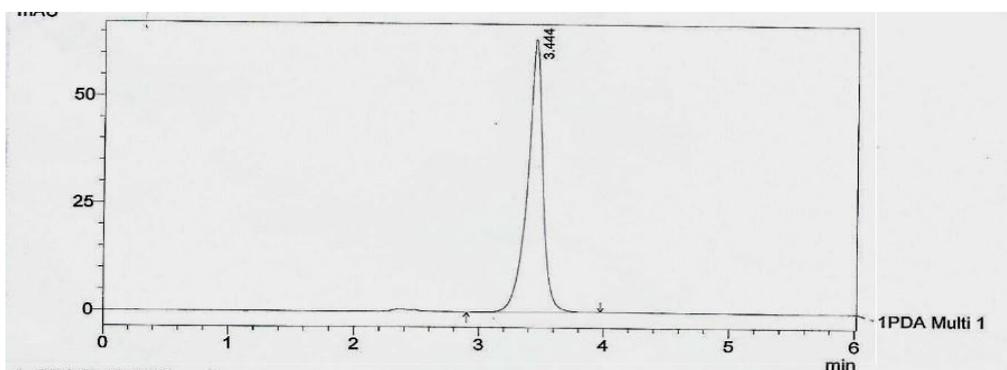


Fig. no. 3: Chromatogram showing the retention time of Lisinopril.

METHOD VALIDATION

Linearity

The calibration was done by using external calibration method, with the optimum chromatographic conditions. Standard stock solutions of Lisinopril were prepared by using acetonitrile and various concentrations has been

prepared in the range of 2-10 μ g/ml of Lisinopril in diluent. 20 μ l of each solution was injected individually and corresponding chromatogram was recorded at 237nm. The calibration curve has plotted using concentration against peak area. The procedure has repeated for three times. The R² was found to be 0.9993 indicates the concentration of Lisinopril has good

linearity. The calibration graph was shown in **fig**; values are tabulated in **table: 1**

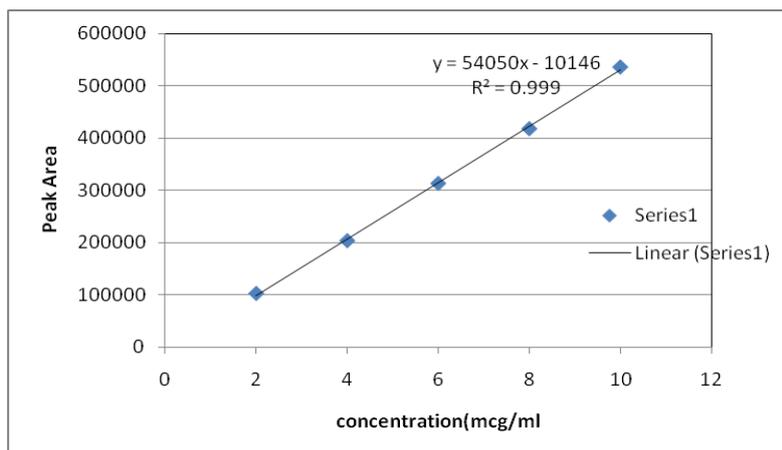


Fig. 4: Calibration curve of Lisinopril.

Table.no.1: Linearity of Lisinopril.

S.No	Concentration (µg/ml)	Mean Area*
1	2	102063
2	4	203142
3	6	312719
4	8	417439
5	10	535418
		Y = 54050X-10146 R ² = 0.9993

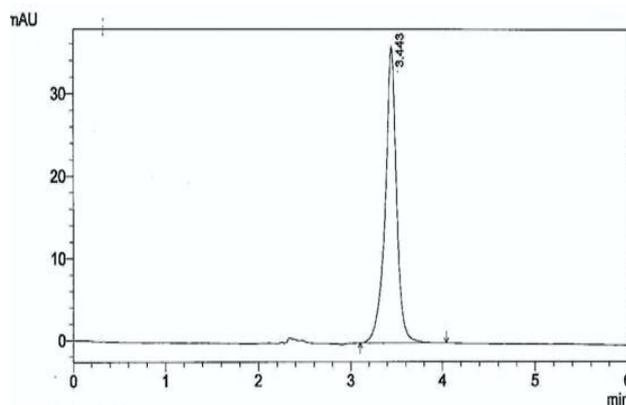


Fig.no.5: Chromatogram showing intraday precision of lisinopril.

Precision

The precision of the method was confirmed by repeatable injection of the standard solution for 6 times. The % RSD value was found to be 0.354. It shows that the method has good intra and interday precision.

Table 2: Intraday Precision Studies of Lisinopril.

S.No	Amount (µg/ml)	Amount found (µg/ml)	Percentage %	% Mean	SD **	% RSD
1.	6.0	6.011	100.1	100.1	0.021	0.354
2.		6.04	100.4			
3.		5.99	99.8			
4.		6.03	100.3			
5.		6.00	100.0			
6.		6.04	100.4			

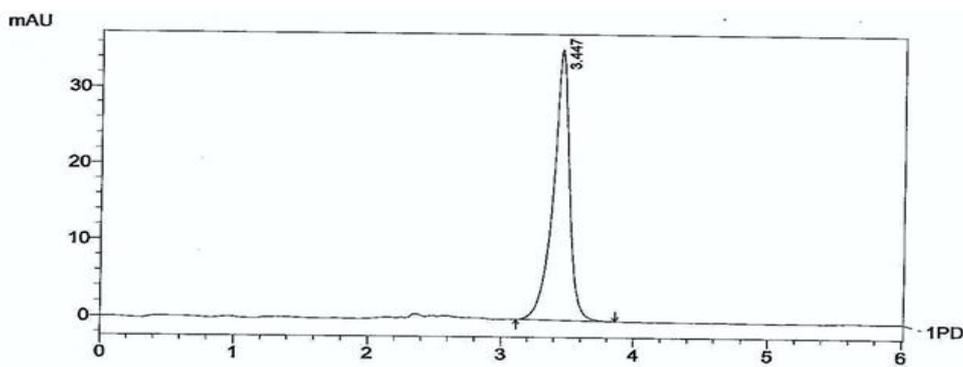


Fig. no.6: chromatogram showing interday precision of lisinopril.

Table. no.3: Interday Precision Studies of Lisinopril.

S.No	Amount (µg/ml)	Amount found (µg/ml)	Percentage %	Mean	SD*	% RSD
1.	6 µg/ml	6.00	100.0	100.11	0.525	0.08
2.		6.06	100.6			
3.		6.02	100.2			
4.		6.08	100.8			
5.		6.03	100.3			
6.		5.93	98.8			

Accuracy

Accuracy was confirmed by recovery studies by adding known amount of pure drug to the previously analyzed formulation by a proposed method. The % recovery of Lisinopril present in formulation was found between 99.2-100.0. The % RSD values were found to be 0.21, it

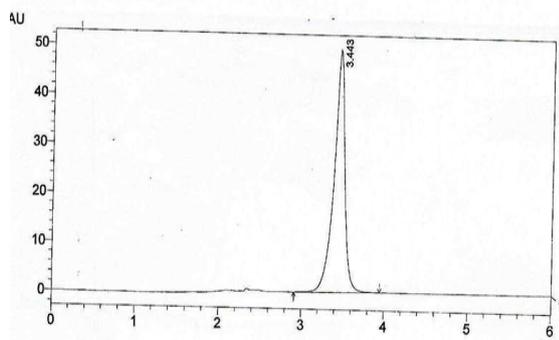
was extremely low when compared to the normal value. The high % recovery studies specify that there is no interference by the excipients present in the formulation, hence the described method was found to be accurate. The values were given in **table.no.4**.

Table. no.4: Results for Accuracy of Lisinopril.

S. No	% Spike Level	Amount (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery	SD	% RSD	
1.	80%	5.99	4.8	10.78	4.79	99.79	99.58	0.21	0.21	
2.				10.77	4.78	99.58				
3.				10.76	4.77	99.37				
4.	100%		6.0	6.0	11.94	5.95	99.1	99.23	0.32	0.32
5.					11.93	5.94	99.0			
6.					11.97	5.98	99.6			
7.	120%		7.2	7.2	13.16	7.17	99.5	100.0	0.55	0.55
8.					13.24	7.25	100.6			
9.					13.20	7.21	100.1			

Assay

The tablet formulation was selected for analysis. The nominal concentration 10µg/ml from calibration curve has been prepared by using diluent. 20µl of formulation was injected and the chromatogram has been recorded in **fig.no.7**, tabulated in **table.no.5**. The amount of Lisinopril present in the formulation was found to be 99.93.

**Fig.7: Assay of Lisinopril.****Table no.5: Assay of Lisinopril.**

S. No	Label claim	Amount Found (n=6)	Assay	SD*	% RSD
1	10mg	9.99mg	99.9%	0.150	0.150

Limit of Detection and Limit of Quantification

The LOD and LOQ were determined from the linearity studies and calculated by using average of slope and standard deviation of intercept. The limit of detection was found to be 0.08 µg /ml and the limit of quantification was found to be 0.248µg/ml. The values have been shown in table.no.6.

Table.no.6: Limit of Detection and Limit of Quantification.

S.No	Name of the drug	LOD (µg/ml)	LOQ(µg/ml)
1	Lisinopril	0.04	0.803

Robustness

Robustness of the method was done by the deliberate changes in flow rate, column oven temperature, mobile phase composition and wave length.

Table.no.7: Robustness studies for Lisinopril.

Parameter	Normal	Variation	Rt (Minutes)	Tailing factor	Theoretical plates	%RSD
Wave length variation	250	248	3.439	0.891	4563	0.122
		252	3.450	0.922	4732	0.173
Flow Rate variation	1.0	0.8	4.336	0.894	4971	0.389
		1.2	2.900	0.896	4213	0.139
Column oven Temperature variation	30° c	25°c	3.472	0.904	4734	0.040
		35°c	3.441	0.898	4527	0.183
Mobile phase variation	70:30	60:40	3.910	0.989	4313	0.134
		80:20	3.401	0.997	4282	0.164

CONCLUSION

The proposed HPLC was found to be specific, precise, accurate, rapid and economical for estimation of lisinopril in bulk and pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and results were validated statistically according to ICH and USP guidelines.

Hereby concluded that the method showed tremendous sensitivity, reproducibility, accuracy and repeatability, which proved the low percentage relative standard deviation. The results of recovery studies, determines that there is no interference from the excipients used in formulation. RP-HPLC method can be effectively applied for the routine analysis of Lisinoprilin pure and table formulation in quality control analysis.

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