EPH - International Journal of Applied Science

ISSN (Online): 2208-2182

Volume 03 Issue 02-June,2017

DOI: https://doi.org/10.53555/eijas.v3i2.43

TITLE: - LISINOPRIL AN OVERVIEW

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Abstract:-

Lisinopril is an oral long-acting angiotensin converting enzyme inhibitor. It is a lysinederivative of Enalprilate and has structural similarity with its substrate. It differs from captopril by lacking the sulfhydryl group [1,2]. Lisinopril is chemically described as (S)-1-[N2-(1carboxy-3-phenylpropyl)-L-lysyl]-Lproline dihydrate. Its empirical formula is $C_{21}H_{31}N_3O_5.2H_2O$.The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the estimation of Lisinopril from bulk samples and tablet dosage forms. A non-polar C8 analytical chromatographic column was chosen as the stationary phase for the separation and determination of Lisinopril. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of buffer and methanol in the ratio of 35:65 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention time of Lisinopril was found to be 2.29 min. The linearity was found satisfactory for the drug in the range 20.0-60.0 µg/mL (Table 1.4). Precision of the method was studied by repeated injection of tablet solution and results showed lower % RSD values (Table 1.5-1.7). This reveals that the method is quite precise. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent individual recovery and the % RSD at each level were within the acceptable limit (Table 1.8). This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method is specific. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust (Table 1.9). The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor (Table 1.10). The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive (Table 1.11). The solution stability studies indicate that the drug was stable up to 24 hours (Table 1.12). The forced degradation studies indicate that the drug was stable in stability studies (Table 1.13).

I. INTRODUCTION

Lisinopril is an oral long-acting angiotensin converting enzyme inhibitor. It is a lysine-derivative of Enalprilate and has structural similarity with its substrate. It differs from captopril by lacking the sulfhydryl group [1, 2]. Lisinopril is chemically described as (S)-1-[N2 (1-carboxy-3-phenylpropyl)-L-lysyl]-Lproline dihydrate. Its empirical formula is $C_{21}H_{31}N_3O_5.2H_2O$. Lisinopril is used in high blood pressure (hypertension), congestive heart failure, and in heart attack, also in renal and retinal complications of diabetes [2]. It also exhibits haemodynamic effects [3]. It promotes natriuresis and useful in preventing diabetic retinopathy in the patients of type II diabetes [4, 5]. Its onset of action is 1-2 hours. Duration of action is 24 hours. Absorption of the lisinopril is slowly and moderately from GI tract (oral) and peak plasma concentration obtain after 7 hours.. The drug distribution is up to 25%. It is excreted unchanged in urine and does not undergo metabolism. The drug is given orally in case of hypertension. Adult dose is initially 5-10 mg daily given at bedtime. Dose of drug in renovascular hypertension, volume depletion, severe hypertension in the beginning 2.5-5 mg once daily. In diuretic patients 5 mg once daily. Maintenance dose is 20 mg once daily up to 80 mg daily can be given. In case of children ≥ 6 years: initially up to 0.07 mg/kg (up to 5 mg once daily) can be given. Bioavailability of the drug is about 25% [6].

II. DRUG PROFILE

1.1. Lisinopril

Lisinopril is an ACE inhibitor used in treatment of hypertension, heart failure & in diabetic nephropathy. It also exhibits haemodynamic effects [7]. It is alysine derivative of enalaprilate. It is an active site directed inhibitor [8]. They promote natriuresis & useful in preventing diabetic retinopathy in the patients of type II diabetes [9-10].

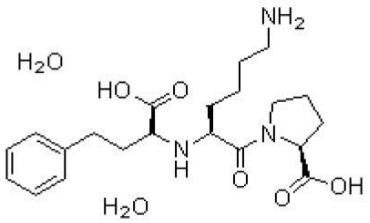

Figure 1.1: Molecular structure of Lisinopril

Molecular formula	:	$C_{21}H_{31}N_3O_5$
Molecular weight	:	405.48
Chemical name	:	(S)-1-[N2-(1-carboxy-3-phenylpropyl)-L-lysyl]-Lproline dehydrate
Solubility	:	Lisinopril is soluble 1 in 10 of water & 1 in 70 of methyl Alcohol

III. EXPERIMENTAL

3.1. Instrumentation

The author had attempted to develop and validate a liquid chromatographic method for determination of Lisinopril using an isocratic Waters HPLC system on a Xterra C8 column (150 mm x 4.6 mm, 3.5 µm). The instrument is equipped with a 2695 binary pump with inbuilt degasser, 2487 Dual absorbance detector and Rheodyne injector with 20 µL sample loop. A 20 µl Hamilton syringe was used for injecting the samples. Data was analysed using Waters Empower 2 software. A double-beam Elico SL-159 UV -Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

3.2. Chemicals and Solvents

The reference sample of Lisinopril (API) was obtained from Sun Pharmaceutical Industries Ltd., Baroda, India. The branded formulation (tablets) (LISTRIL tablets containing 10 mg of Lisinopril) were procured from the local market. HPLC grade acetonitrile and analytical grade potassium dihydrogen phosphate was obtained from Qualigens Fine Chemicals Ltd, Mumbai, India. Hydrochloric acid, sodium hydroxide, hydrogen peroxide, triethyl amine and orthophosphoric acid of analytical grade were obtained from Merck Chemicals Ltd, Mumbai, India. Milli-Q water was used throughout the experiment dispensed through 0.22 µ filter of the milli-Q water purification system from Millipore, Merck KGaA, and Darmstadt, Germany.

3.3. The Phosphate buffer solution

Weigh about 7.0 grams of potassium dihydrogen phosphate and transfer to 1000 mL standard flask, add 400 mL of Milli-Q water mix and dilute to volume with Milli-Q water, sonicate for five minutes and cool to room temperature, measure the pH of above buffer solution and finally adjusted the pH to 3.0±0.05 with orthophosphoric acid solution and filtered through 0.45 µ nylon filter.

3.4. The mobile phase

A mixture of potassium dihydrogen phosphate buffer pH 3.0 and methanol in the ratio of 35:65 v/v was prepared and used as mobile phase.

3.5. The diluents
The potassium dihydrogen phosphate buffer pH 3.0 and methanol mixture in the ratio of 35:65 v/v was used as diluent.

3.6. Preparation of standard solution of the drug
About 40 mg of Lisinopril was accurately weighed and transferred into a 100 ml clean dry volumetric flask containing 50 ml of diluent. The solution was sonicated for 5 min and then volume was made up to the mark with a further quantity of the diluent to get a concentration of 400 µg/ml for Lisinopril (Stock solution). Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the diluent.

3.7. Preparation of sample (tablet) solution
Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 40 mg of Lisinopril was transferred to a 100 ml volumetric flask containing 50 ml of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of diluent. Then this mixture was filtered through 0.45 µ membrane filter. Pipette 1 ml of the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the diluent.

IV. METHOD DEVELOPMENT
For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

4.1. Detection wavelength
The spectrum of diluted solution of the Lisinopril in diluent was recorded on UV spectrophotometer. The peak of maximum absorbance was observed. The spectra of Lisinopril showed that a balanced wavelength was found to be 215 nm.

4.2. Choice of stationary phase
Preliminary development trials have performed with octadecyl columns and octyl columns with different types, configurations and from different manufacturers. Finally the expected separation and shapes of peak was succeeded in Xterra C8 column.

4.3. Selection of the mobile phase
To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like water, methanol and acetonitrile with or without different buffers in different combinations were tested as mobile phases on a C8 stationary phase. A mixture of potassium dihydrogen phosphate buffer pH 3.0 and methanol in the ratio of 35:65 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and almost free from tailing.

4.4. Flow rate
Flow rates of the mobile phase were changed from 0.5-2.0 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 0.8 ml/min flow rate was ideal for the successful elution of the analyte.

4.5. Run time
No interference in blank and placebo solutions for the drug peak in the trail injections with a runtime of 6.0 min.

4.6. Optimized chromatographic conditions
Chromatographic conditions as optimized above were shown in Table 1.2. These optimized conditions were followed for the determination of Lisinopril in bulk samples and its combined tablet formulations. The chromatogram of standard and sample solutions of Lisinopril was shown in Figure 1.2 and Figure 1.3. The chromatograms of stability studies of Lisinopril were shown from in Figure 1.7 to Figure 1.10.

4.7. UV-visible spectroscopic methods
Quantitative estimation of Lisinopril in bulk and its marketed formulations by simple and sensitive UV spectrophotometric method have been developed which is accurate and economical. The diketone o-phenylendiamine complex formed is soluble in acidic and basic solvents. It has been determined by spectrophotometrically at λmax 399.5 nm in alkaline media and displays linearity with Beer's range of 2 µg to 30 µg, the factors like temperature, reaction duration, pH of solvent are optimized. The method has been validated according to ICH Guidelines.

4.8. Spectrofluorimetric methods of estimation by reaction modifications
Fluorimetric method is based upon the condensation reaction between primary amino group of Lisinopril and Fluorescien to form fluorescent derivative (LSFN) in methanol at 60°C for 5 min. The formation of fluorescent derivative can be determined by the UV (λmax 227 nm), NMR, Mass and IR spectra. Spectrofluorimetric methods are used to estimate

lisinopril present in small quantities i.e. in ng in blood or plasma. These are frequently based upon coupling reactons with fluorephores.

4.9. Combination of methods or techniques

By using HPLC (high-performance liquid chromatography) a new method was developed in which impurities in the bulk lisinopril drug was differentiate, In which mobile phase used in a ratio of 5% (v/v) acetonitrile and 95% (v/v) aqueous buffer and Detection was carried out at 210 nm later on by merge with the FT-ICRMS and NMR data, structures of unknown impurities were also found. In another method isomerization of lisinopril has been studied by using HPLC, NMR, and DFT. It shows that 77% Trans and 23% cis was eluted. Another sensitive and precise HPLC method with fluorimetric detection has been developed for the assay of lisinopril in human plasma and urine. The reaction mixture was chromatographed on C18- column with gradient elution, using methanol and 0.02 M phosphate buffer pH 3.2. The mean recovery of lisinopril from plasma and urine was 63.41 and 74.08%, respectively. Another simple and sensitive liquid chromatography tandem multiple-stage mass spectrometry (HPLC/MS/MS) method that is suitable for bulk lisinopril analysis was developed, by which lisinopril and its RSS isomer were separated and differentiated. LC–UV scan of lisinopril revealed the presence of an unknown impurity (`~0.14%) at a relative retention time RT of 3.26 m using phosphate buffer acetonitrile as binary gradient system. The impurity was isolated by HPLC employing a linear gradient of water and acetonitrile. The establishment of structures as well as quantification and characterizations of degraded products were carried out by sophisticated instrumental methods like UV, IR, and RP-HPLC.
Furthermore, the mechanism of degradation was verified by GC-MS studies.

4.10.Titrimetric methods

In 1835 Gay–Lussac invented the volumetric methods which actually lead to the foundation of the term titration. While the assay method is very old yet but certain signs of some renovation may including as dispersion to non-aqueous titrations, intensifying the field of application to weak acids and bases as well as to detection of potentiometric end point improving the accuracy of the methods. Simultaneously detection of group analysis procedures, in which titrimetric methods play a vital role in establish reaction rates. The main advantages of this method are time saving and labor with high precision and the fact that there is no need of using reference standards. Titrimetric methods have been used for the determination of lisinopril 1 in commercial dosage forms.

4.11. Miscellaneous methods

In polarographic method, dealing of the compound with nitrous acid as a result measuring the cathodic current produced by the resulting nitro so derivative. The polarographic behavior was calculated as adopting direct current (DCt), differential pulse (DP) and alternating current (ACt) polarography along with well define pH range of 1.0–8.0 was achieved in BrittonRobinson buffers (BRb). This method may also be performed for determination of lisinopril in spiked human urine and plasma.

By using Spectrophotometric and polarographic technique determination of lisinopril has been established using 2,4-dinitrofluorobenzene by formation of colored products and polarographically active derivatives. In this study methods have been validated and optimized by different experimental conditions which may be pertain to the determination of lisinopril in their commercial scale tablets. Comparative statistical analysis has been recognized by means of the authorized HPLC methods.

A novel method for the quantitative determination of the angiotensin-converting enzyme inhibitor lisinopril in human plasma have been established by mean of negative ion chemical ionization mass spectrometry and gas chromatography.
Other method with respect to chemical nature the drug may also act as photosensitive behavior as go through hydrolysis and oxidized in presence of oxygen. Due to respect of stress degradation of lisinopril certain studies have been conducted under different conditions recommended by International Conference on Harmonization (ICH) in which objective of study as to find out the pathway for stress degradation of Lisinopril in bulk.

Table 1.2: Optimized chromatographic conditions for the estimation of Lisinopril in tablet dosage form

Mobile phase	:	Potassium dihydrogen phosphate buffer pH 3.0:methanol, 35:65 v/v
Pump mode	:	Isocratic
pH of Buffer	:	3.0±0.05
Diluent	:	Potassium dihydrogen phosphate buffer: methanol, 35:65 v/v
Column	:	Xterra C8 column, 150 mm x 4.6 mm, 3.5 µm
Column Temp	:	Ambient
Wavelength	:	215 nm
Injection Volume	:	20 µl
Flow rate	:	0.8 mL/min
Run time	:	6 min
Lisinopril	:	2.298±0.5 min

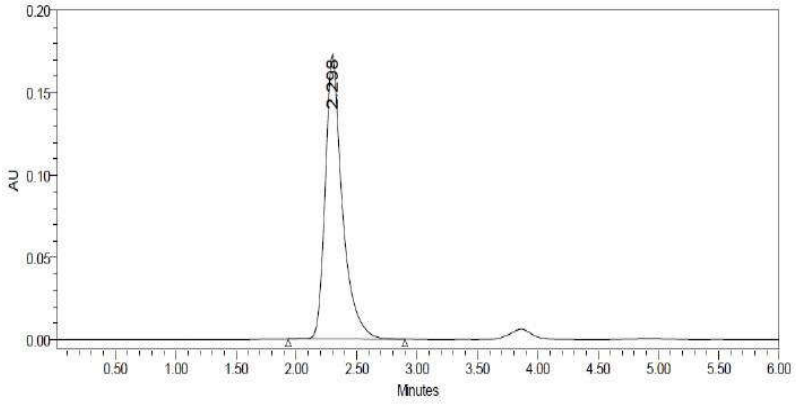

Figure 1.2: Chromatogram of standard solution of Lisinopril

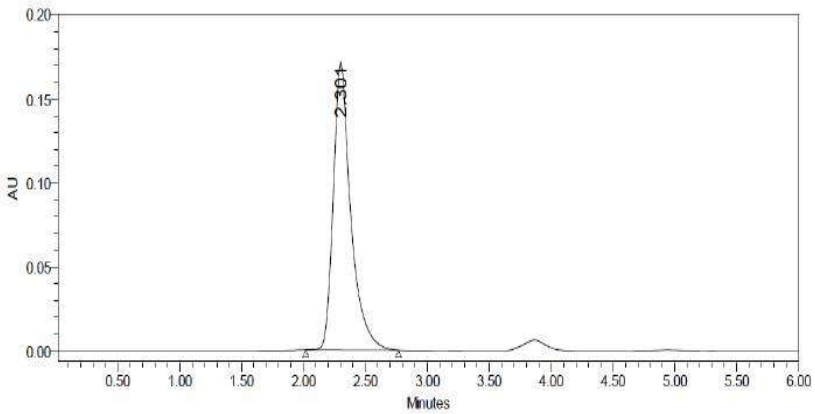

Figure 1.3: Chromatogram of sample solution of Lisinopril

V. VALIDATION OF THE PROPOSED METHOD

The proposed method was validated as per ICH [19-20] guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

5.1. Specificity

A study conducted to establish specificity of the proposed method involved injecting blank and placebo using the chromatographic conditions defined for the proposed method. It was found that there was no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results were shown in Table 1.3. The chromatograms of blank and placebo for Lisinopril were shown in Figure 1.4 and Figure 1.5.

Table 1.3: Specificity study

Name of solution	Retention time (min)
Blank	No peaks
Lisinopril	2.29

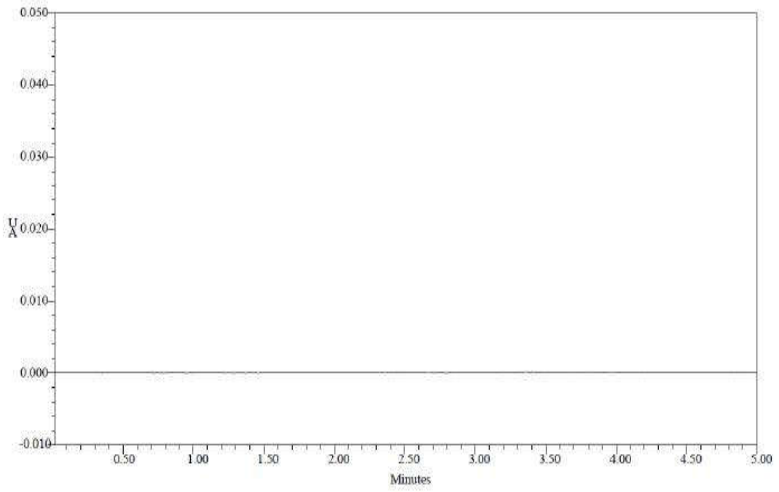

Figure 1.4: Chromatogram showing no interference of blank for Lisinopril

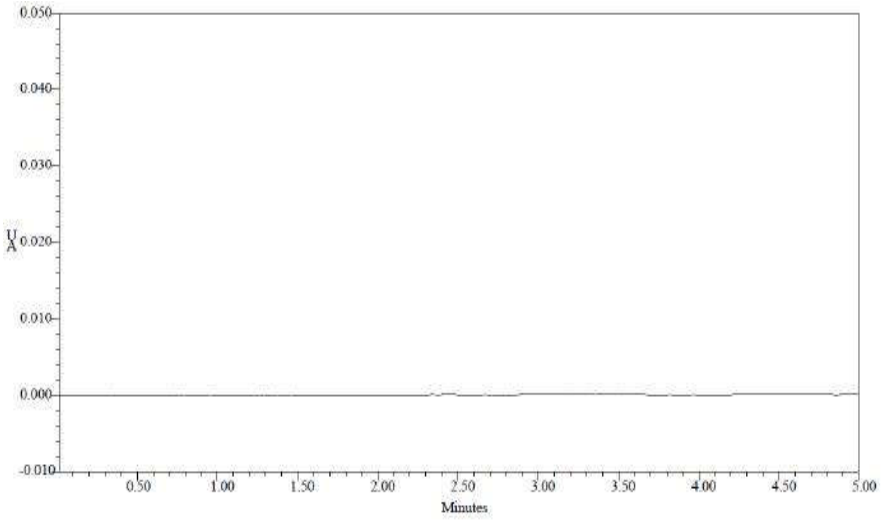

Figure 1.5: Chromatogram showing no interference of placebo for Lisinopril

5.2. Linearity

Linearity was performed by preparing standard solutions of Lisinopril at different concentration levels including working concentration mentioned in experimental condition from 20.0 to 60.0 µg/ml. twenty micro litres of each concentration was injected in duplicate into the HPLC system. The response was read at 215 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. Linearity results were presented in Table 1.4 and linearity plots are shown in Figure 1.6

Table 1.4: Linearity study of Lisinopril

Level	Concentration of Lisinopril (µg/mL)	Mean peak area
Level-1	20	862515
Level-2	30	1282509
Level-3	40	1699129
Level-4	50	2045597
Level-5	60	2426870
Slope		**38918**
Intercept		**106605**
Correlation Coefficient		**0.9992**

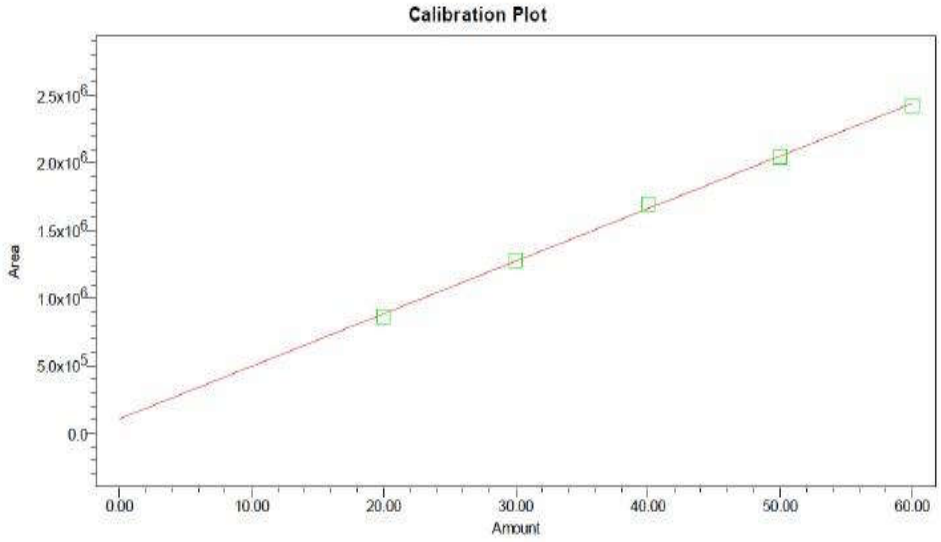

Figure 1.6: Linearity plot of Lisinopril

5.3. Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as system precision, method precision and intermediate precision.

5.3.1. System precision

To study the system precision, five replicate standard solutions of Lisinopril was injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 1.20 for Lisinopril, which is well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 1.5.

Table 1.5: System precision

Injection number	Area of Lisinopril	Acceptance criteria
1	1713758	The %RSD of peak area of Lisinopril should not be more than 2.0
2	1700467	
3	1704762	
4	1716273	
5	1752656	
Mean	**1717583**	
SD	**20638**	
%RSD	**1.20**	

5.3.2. Method precision

The method precision study was carried out on five preparations from the same tablet samples of Lisinopril and percent amount of both were calculated. The % RSD of the assay result of five preparations for Lisinopril in method precision study was found to be 1.12 which was well within the acceptance criteria of not more than 2.0. The results obtained for assay of Lisinopril are presented in Table 1.6.

Table 5.6: Method precision

Sample number	%assay
	Lisinopril
1	100.9
2	99.5
3	99.2
4	101.6
5	99.1
Mean	**100.06**
SD	**1.1238**
%RSD	**1.12**

5.3.3. Intermediate precision

The intermediate precision study was carried out by different analysts, different columns, different reagents using different HPLC systems from the same tablet of Lisinopril and the peak area of Lisinopril was calculated. The % RSD of the peak areas of five preparations in intermediate precision study of Lisinopril was 0.63 which was well within the acceptance criteria of not more than 2.0. The results of intermediate precision study are reported in Table 1.7.

Table 1.7: Intermediate precision study of Lisinopril

Injection number	Area of Lisinopril	Acceptance criteria
1	1759603	The %RSD of peak area of Lisinopril should not be more than 2.0
2	1765945	
3	1773629	
4	1778465	
5	1788460	
Mean	**1773220**	
SD	**11161**	
%RSD	**0.63**	

Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels of 50%, 100% and 150%. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD at each level was calculated and results are presented in Table 1.8. The percent recovery of Lisinopril was 99.65 to 101.4 and the mean recovery was found to be 100.02 by the proposed method. This indicates that the proposed method was accurate.

Table 1.8: Recovery study for Lisinopril

%Concentration (at specification Level)	Mean peak area	Amount of Lisinopril spiked (mg)	Amount of Lisinopril recovered (mg)	%Recovery	Mean Recovery
50%	1044253	21.0	21.12	101.4%	100.02%
100%	1697389	40.0	39.94	99.85%	
150%	2440586	59.50	59.30	99.65%	

5.5. Robustness

The robustness study was performed by slight modification in flow rate of the mobile phase and composition of the mobile phase. Sample of Lisinopril at 40 µg/mL concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in Table 1.9.

Table 1.9: Robustness study for Lisinopril

Condition	Mean Peak area	%assay	%difference
Unaltered	1697854	99.8	-
Flow rate at 0.6 mL/min	1722354	101.4	1.6
Flow rate at 1.0 mL/min	1716241	101.1	1.3
Mobile phase:			
• Buffer(45):Methanol(55)	1705468	100.4	0.6
• Buffer(25):Methanol(75)	1714215	101.0	1.2

5.6. System suitability

System suitability was studied under each validation parameters by injecting six replicates of the standard solution. The system suitability parameters are given in Table 1.10.

Table 1.10: System suitability for Lisinopri

Parameter	Tailing factor	Theoretical plates
Specificity study	1.50	2348
Linearity study	1.22	2296
Precision study	1.30	2486
Robustness study		
Flow rate at 0.6 mL/min	1.45	2059
Flow rate at 1.0 mL/min	1.32	2187
Mobile phase:		
• Buffer(45):Methanol(55)	1.16	2318
• Buffer(25):Methanol(75)	1.25	2254

5.7. Limit of detection and Limit of quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ of Lisinopril are given in Table 1.11.

Table 1.11: LOD and LOQ of Lisinopril

5.8. Solution stability

To determine the stability of Lisinopril in solution, the standard and sample solution were observed under room temperature. Any change in the retention time, peak shape and variation in response was compared to the pattern of chromatogram of freshly prepared solution. The solution stability results are shown in the Table 1.12.

Table 1.12: Solution stability of Lisinopril

Standard solution			Sample solution		
Time (hours)	Response	%variation	Time (hours)	Response	%variation
Initial	1776984	-	Initial	1775689	-
12	1771286	0.4	12	1763549	0.7
24	1756548	1.2	24	1755121	1.2

5.9. Stability studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hours at room temperature. The results show that for both solutions, the retention time and peak area of Lisinopril (%RSD less than 2.0) has no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hours, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of proposed method.

The results of the degradation studies are shown in the Table 1.13.

Table 1.13: Forced degradation study results for Lisinopril

Stress Conditions	Degradation Time (hrs)	Lisinopril	
		%Assay	%Degradation
Control	--	99.9	--
Acid	1	93.0	-6.9
Base	1	91.0	-8.9
Peroxide	1	82.8	-17.1
Thermal	48	87	-12.9

Control sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 40 mg of Lisinopril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of diluent. Then this mixture was filtered through 0.45 µ membrane filter. 5.0 mL of this filtrate was further diluted to 50 mL with mobile phase.

Acid degradation sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 40 mg of Lisinopril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug. Then 10 mL of 5N acid (Hydrochloric acid) was added, refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralized with 5N base (Sodium hydroxide) and diluted to volume with diluent. Filter about 25 ml of the above sample solution through 0.45 µ membrane filter Pipetted 5 ml of the above filtered sample solution into a 50 mL volumetric flask and diluted to volume with diluent. Typical chromatogram of acid degradation for Lisinopril is shown in Fig. 1.7.

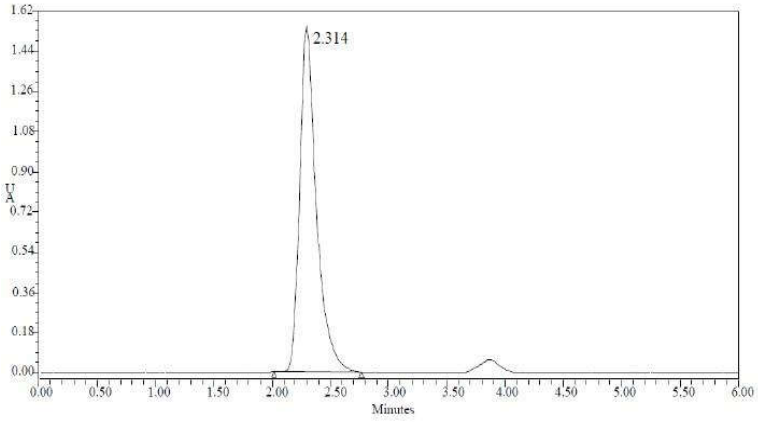

Figure 5.7: Chromatogram of acid degradation showing Lisinopril

Base degradation sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 40 mg of Lisinopril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug. Then 10 mL of 5N base (Sodium hydroxide) was added, refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralized with 5N acid (Hydrochloric acid) and diluted to volume with diluent. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipetted 5 mL of the above filtered sample solution into a 50 mL volumetric flask and diluted to volume with diluent. Typical chromatogram of base degradation for Lisinopril is shown in Fig. 1.8.

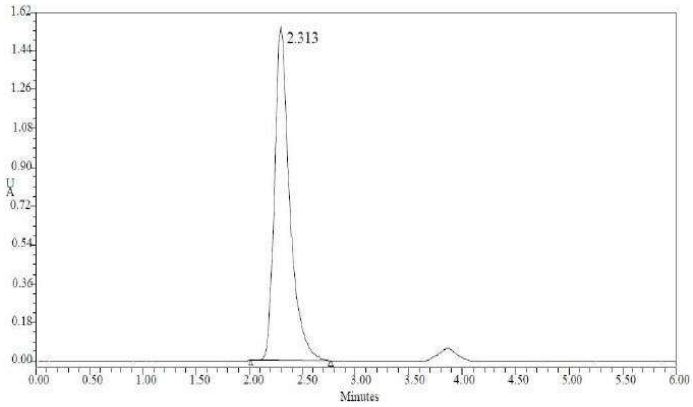

Figure 1.8: Chromatogram of base degradation showing Lisinopril

Peroxide degradation sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 40 mg of Lisinopril was transferred to a 50 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug. Then 4 mL of 30% hydrogen peroxide was added, refluxed for 60 minutes at 60°C, then cooled to room temperature and diluted to volume with diluent. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipetted 5 mL of the above filtered sample solution into a 50 mL volumetric flask and diluted to volume with diluent.

Typical chromatogram of peroxide degradation for Lisinopril is shown in Fig. 1.9.

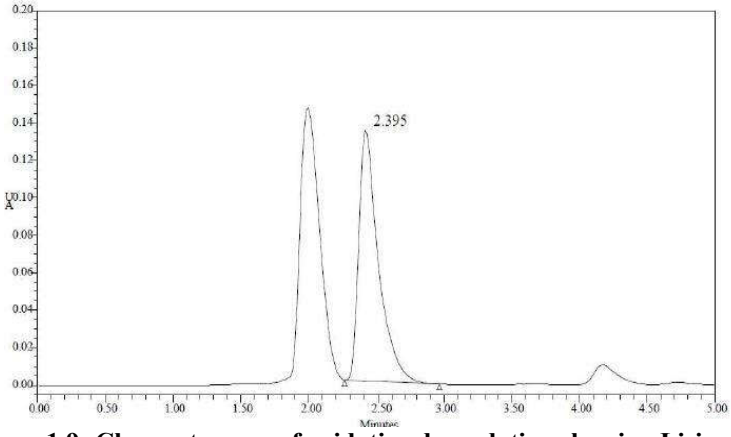

Figure 1.9: Chromatogram of oxidative degradation showing Lisinopril

Thermal degradation sample

Twenty tablets were weighed and finely powdered. The powder is exposed to heat at 105°C for about 2 days. An accurately weighed portion of powder sample equivalent to 40 mg of Lisinopril was transferred to a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 10 min for complete solubility of the drug. Filter about 25 mL of the above sample solution through 0.45 µ membrane filter. Pipetted 5 mL of the above filtered sample solution into a 50 mL volumetric flask and diluted to volume with diluent.

Typical chromatogram of thermal degradation for Lisinopril is shown in Fig. 1.10.

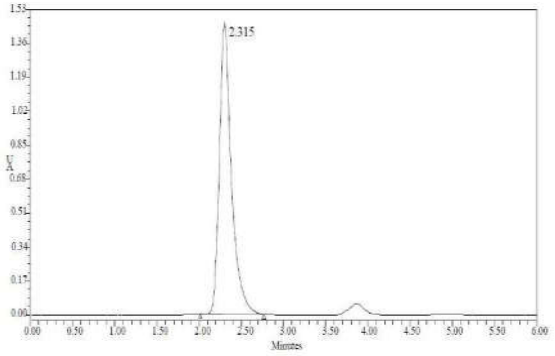

Figure 1.10: Chromatogram of thermal degradation showing Lisinopril

VII. RESULTS AND DISCUSSION

The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the estimation of Lisinopril from bulk samples and tablet dosage forms. A non-polar C8 analytical chromatographic column was chosen as the stationary phase for the separation and determination of Lisinopril. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of buffer and methanol in the ratio of 35:65 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention time of Lisinopril was found to be 2.29 min. The linearity was found satisfactory for the drug in the range 20.0-60.0 µg/mL (Table 1.4). The regression equation of the linearity curve of Lisinopril between concentrations over its peak areas was found to be Y= 38918X+106605 (where Y is the peak area and X is the concentration of Lisinopril in µg/mL). Precision of the method was studied by repeated injection of tablet solution and results showed lower % RSD values (Table 1.5-1.7). This reveals that the method is quite precise. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent individual recovery and the % RSD at each level were within the acceptable limit (Table 1.8).

This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method is specific. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust (Table 1.9). The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor (Table 1.10). The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive (Table 1.11). The solution stability studies indicate that the drug was stable up to 24 hours (Table 1.12). The forced degradation studies indicate that the drug was stable in stability studies (Table 1.13).

VIII. CONCLUSION

The proposed stability-indicating RP-HPLC method was simple, specific, sensitive, accurate and precise and can be used for analysis of Lisinopril in bulk samples and its tablet dosage forms.

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