Development and validation of RP-LC method for lisinopril dihydrate in bulk and its pharmaceutical formulations

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Lisinopril in tablet formulations. The separation was achieved by using column Hypersil BDS C18, 250 × 4.6 mm, 5 µm, in mobile phase Phosphate buffer pH 5.0±0.05 and Acetonitrile in the ratio of 96:4 v/v. The flow rate was 2.0 mL.min-1 and the separated Lisinopril was detected using UV detector at the wavelength of 210 nm. The retention time of Lisinopril, was noted to be 3.68 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Liquid Chromatography; Lisinopril, Validation

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INTRODUCTION:

Lisinopril {(S)-1-[N2-(1-carboxy-3-phenylpropyl)-Lproline] dihydrate}[1-4] is a lysine analog of enalaprilat, the active metabolite of enalapril. It is a long-acting, nonsulfhydryl angiotensin-converting enzyme (ACE) inhibitor that is used for the treatment of hypertension and congestive heart failure in daily dosages of 10-80 mg. Very few analytical methods HPLC [5-8], LC-MS/MS [9], GC-MS [10], HPLC [11,12] have been reported for the determination of lisinopril in pharmaceuticals. HPLC method and the following procedure and chromatographic conditions were established for the determination of lisinopril in pure and pharmaceutical formulations.

Fig:1.01 Lisinopril dihydrate
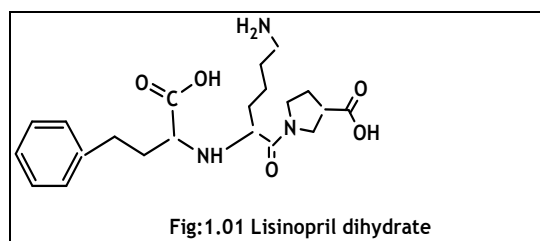
MATERIALS AND METHODS

2.1. Chemicals and Reagents

Milli-Q Water, Acetonitrile (HPLC Grade), Sodium hydroxide (AR Grade), Sodium dihydrogen phosphate (AR Grade) were obtained from Merck, Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

2.2 Instrumental description& Chromatographic conditions

The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Hypersil BDS C_{18}, 5 µ (250 mm x 4.6 mm) was used. The output of signal was monitored and integrated using waters Empower 2 software. A stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5µm diameter.

The mobile phase was delivered through the column at flow rate of 2.0mL/min. The column temperature was maintained at 50°C.The sample injection volume was 20µL.Waters dual λ absorbance detector or equivalent was set at wavelength of 210nm.

Buffer preparation

Weighed and dissolved 2.76g of sodium dihydrogen orthophosphate dihydrate in 1000 mL of water and adjusted the pH to 5.0±0.05 with 1N Sodium hydroxide. Filter the solution through 0.45µm membrane filter.

Mobile phase preparation

Prepare a filtered and degassed mixture of Buffer and Acetonitrile in the ratio of 96:4 v/v respectively.

Diluent preparation

Acetonitrile used as a diluent.

Standard solution: 60mg of Lisinopril working standard was accurately weighed and transferred into a 200mL volumetric flask, dissolved and diluted to volume with water. Filtered it through 0.45 µ or finer porosity membrane filter.

Sample solution: Weigh and transfer accurately 60mg of sample was accurately weighed and is transferred into a 200mL clean, dry volumetric flask, add 100mL of water and sonicate to dissolve. Make up to volume with water. Filter through 0.45 µ or finer porosity membrane filter.

Estimation of Lisinopril from commercial formulations by the proposed method:

Ten tablets are weighed to get the average weight and pulverized. The sample powder, claimed to contain 60mg of active ingredient was transferred into 200mL volumetric flask and dilute to volume with water. This solution was further diluted stepwise with water, as under preparation of standard solutions to get different required. The area under the curve, the drug content per each tablet was calculated.

<div align="center">

RESULTS AND DISCUSSION

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Method development

Different mobile phases were employed for developing proposed HPLC method for the determination of Lisinopril. Initially mobile phases consisting of buffer (pH 5.0) and acetonitrile in the ratio of 50:50 were tried. Hypersil BDS, C_{18}, 5.0µ (250mm x 4.6mm) was used. Early elution with tailing of peaks was observed. Then the composition of the mobile phase was changed to 96:4. Under these conditions broad peak shape and pronounced tailing was observed. For the same mobile phase, if pH was adjusted to 5.0 with 1N sodium hydroxide and used as eluent, lisinopril was eluted at around 3.68min with symmetric peak shape. A typical chromatogram for lisinopril using Hypersil BDS, C_{18}, 5.0µ (250mm x 4.6mm) with mobile phase, composed of buffer (pH adjusted to 5.0 with 1 N Sodium hydroxide): acetonitrile 96:4 at 2.0mL/min flow rate.

Method validation:
Specificity
Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution **Fig: 1.02** showed no peak at the retention time of Lisinopril peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Lisinopril in Lisinopril tablets. Similarly Chromatogram of Placebo solution **Fig: 1.03** showed no peaks at the retention time of Lisinopril peak. This indicates that the Placebo used in sample preparation do not interfere in estimation of Lisinopril tablets.

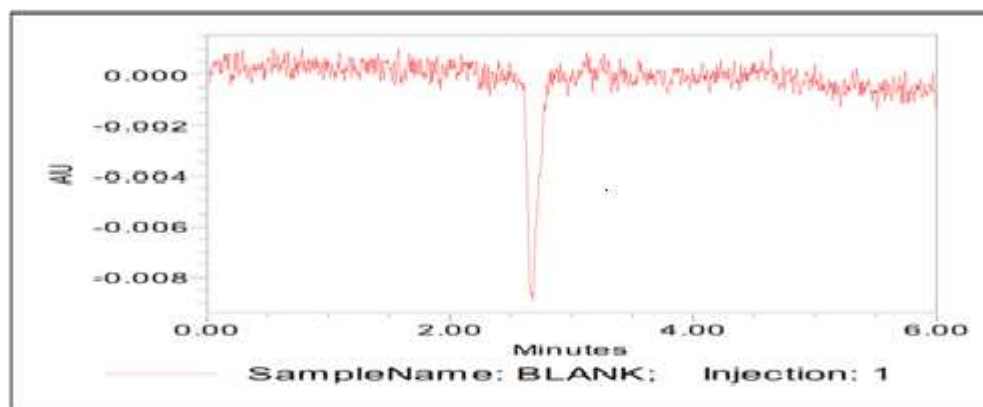**Fig: 1.02 typical chromatogram of Blank**
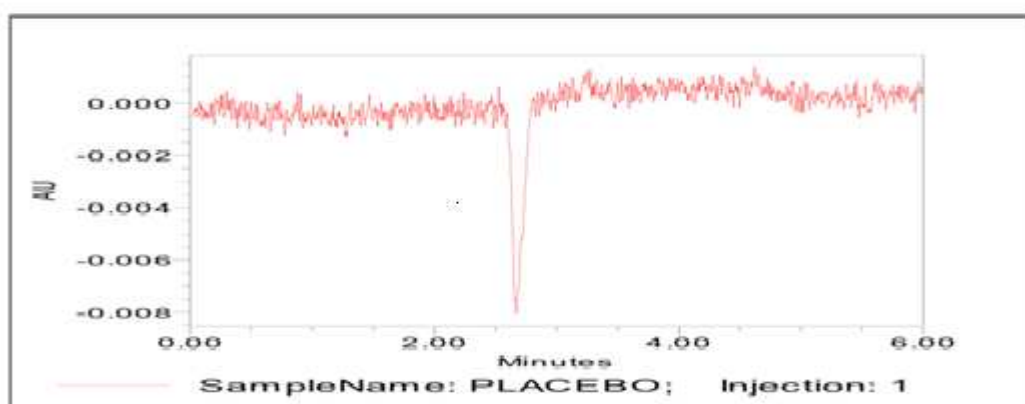

Fig: 1.03 typical chromatogram of Placebo

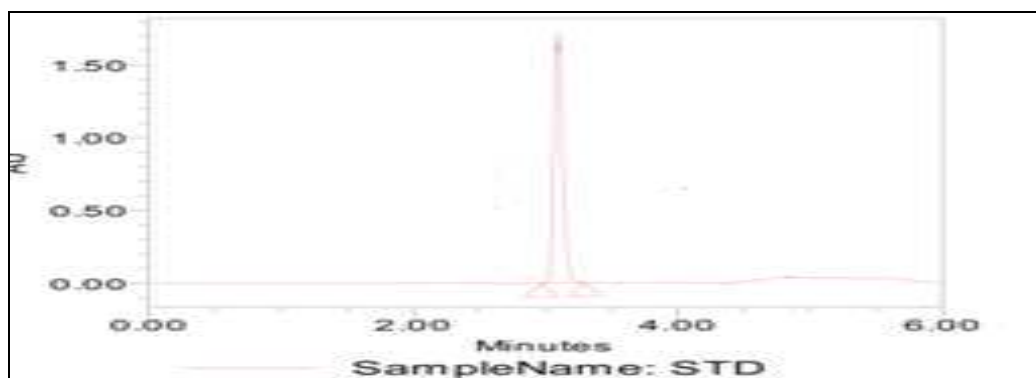**Fig: 1.04 typical chromatogram of Lisinopril Standard**

Table 1.01: System suitability parameters for Lisinopril by proposed method

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Lisinopril	3.68	8941	1.14

Linearity

Linearity studies were carried out by analyzing five separate solutions of drug prepared from stock solution in concentration range of 6.0 -30.0µg/mL. In this HPLC method the calibration curve was set up by plotting the lisinopril peak area ratios vs. function of drug concentrations. The curves were linear over the range of 6.0–30.0µg/mL **Fig.1.05** to get the target concentration of lisinopril in 20µL injected at a flow rate of 2.0mL/min.The correlation coefficient was 0.9999. The results obtained are presented in **Table: 1.02.**

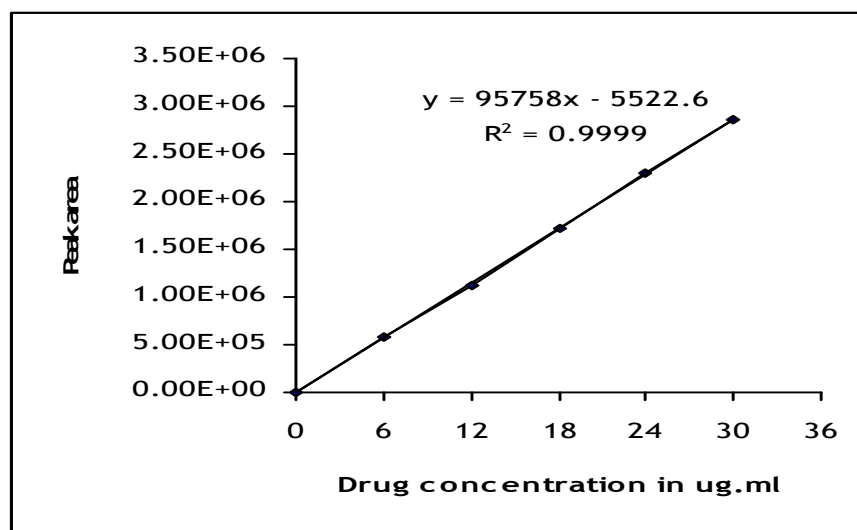**Fig: 1.05. Standard calibration graph of Lisinopril**

Table-1.02 Linearity studies for Lisinopril by proposed method

Parameter	Method
Detection wavelength (nm)	210
Linearity range(μg/mL)	6.0–30.0
Detection limits	0.016
Regression equation (Y=a+bc); Slope (b)	95758
Standard deviation on slope (S_b)	9.217×10^{-3}
Intercept (a)	5522.6
Standard deviation on intercept (S_a)	2.664×10^{-3}
Standard error on estimation (S_e)	6.324×10^{-2}
Correlation coefficient (r)	0.9999

Precision
Reproducibility of the proposed method was studied by five individual injections of the standard. The percent relative standard deviation was found to be 0.11 (n=5).

Precision of the method was determined by replicate analysis of five individual sample preparations in a similar manner as described earlier and the percent label claims were found to be 99.5%to 99.8% for lisinopril.

Accuracy
Recovery was determined by adding known amounts of the drug to the placebo. The recovery study was conducted in three different levels. Each solution was injected in triplicate. The percent recovery was calculated from the average of three replicates. In placebo preparations the percent recoveries were found between 99.6 to 101.2 for lisinopril. The results obtained are presented in **Table - 1.03.**

Table 1.03: Recovery studies for Lisinopril by proposed method

% Level	Recovery Range
50	99.6-99.7
100	99.3-100.3
150	99.3-101.2

Ruggedness and Robustness
Ruggedness test was determined between two analysts, instruments and columns. Robustness of the methodwas determined by small deliberate changes in flowrate, mobile phase pH and mobile phase ratio. Thecontent of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

CONCLUSION

An RP-HPLC method for estimation of lisinopril was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed

343

over a concentration range of 6.0-30.0µg/ml. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the drug or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed.

We have developed a fast, simple and reliable analytical method for determination of lisinopril in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of lisinopril. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of lisinopril in its different pharmaceutical dosage forms.

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