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Research article

Development of analytical method and validation for determination of Lisinopril dihydrate in bulk drug and dosage form using HPTLC method

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Key words: Lisinopril, HPTLC, Validation

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Abstract
A simple, reproducible and efficient High Performance Thin Layer Chromatography method was developed for Lisinopril dihydrate in bulk drug and dosage form. A constant application rate of 0.1 ml/s with nitrogen aspirator was used, and the space between two bands was 6 mm. The slit dimension was 5 × 0.45 mm, and the scanning speed was 10 mm/s. The mobile phase consisted of n-butanol: methanol: ammonia in the ratio of 3.0: 1.0: 1.0 (v/v/v). The retention time (min) and linearity range (µl) for Lisinopril was (0.20) and (1-5) respectively. The method so developed was validated for its accuracy and precision. The LOD and LOQ were found to be 0.050237 and 0.152233 for Lisinopril respectively. The accuracy was found to be 98.88%. The developed method was found to be accurate, precise and selective for determination of Lisinopril in bulk and dosage form.

Introduction

Lisinopril (Figure 1) is an orally bioavailable, long-acting angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity [1]. Lisinopril, a synthetic peptide derivative, specifically and competitively inhibits ACE, which results in a decrease in the production of the potent vasoconstrictor angiotensin II and, so, diminished vasopressor activity. In addition, angiotensin II-stimulated aldosterone secretion by the adrenal cortex is decreased which results in a decrease in sodium and water retention and an increase in serum potassium [2]. Literature survey revealed that various analytical methods like spectrophotometric, HPLC, have been reported for the determination of Lisinopril but no method was available on HPTLC. The review of literature prompted us to develop an accurate, precise method for the estimation of Lisinopril in bulk and dosage forms by using mobile phase n-butanol: methanol: ammonia in the ratio of 3.0: 1.0: 1.0 (v/v/v) which was a unique method with better results [3-6].

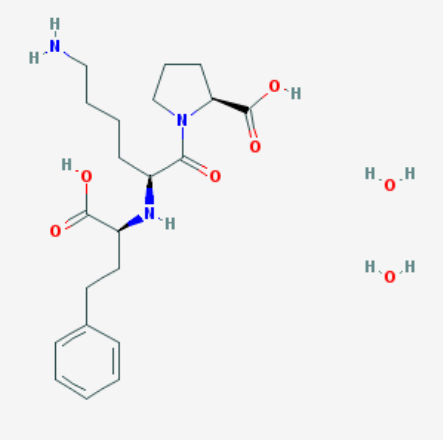

Figure 1. Structure of Lisinopril dehydrate.

Materials and methods

Lisinopril was procured as generous gifts from Wockhardt Pharma, Aurangabad, Maharashtra. Fixed dose tablets containing 10 mg of Lisinopril was procured from Local market brand of Lupin Pharma limited. All chemicals and reagents were of analytical-

grade and were purchased from Merck Chemicals, Mumbai, India.

Instrumentation and conditions

Table 1. Instrumentation

Instrument	**CAMAG TLC Scanner "Scanner_170422" S/N 170422 (2.01.02)**
Wavelength	210
Lamp	D2
UV-Visible Double beam spectrophotometer with single Monochromator	Jasco Model V-550

The samples were spotted in the form of bands of 6 mm width with a 100 µl sample syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated aluminum 60F254 plates, (20×10 cm with 250 mm thickness; E. Merck, Darmstadt, Germany) using a CAMAG Linomat 5 (Muttenz, Switzerland) sample applicator. A constant application rate of 0.1 ml/s with nitrogen aspirator was used, and the space between two bands was 6 mm. The slit dimension was 5 × 0.45 mm, and the scanning speed was 10 mm/s. The mobile phase consisted of n-butanol: methanol: ammonia in the ratio of 3.0: 1.0: 1.0 (v/v/v). Linear ascending development was carried out in an HPTLC twin-trough glass chamber (CAMAG) saturated with the mobile phase vapor. The optimized chamber saturation time was 20 min at room temperature (25 ± 2°C) at a relative humidity of 60 ± 5%. The length of each chromatogram run was 8 cm. Following the development, the HPTLC plates were dried in a current of air using an air dryer. Densitometric scanning was performed using a CAMAG TLC Scanner in the reflectance-absorbance mode at 210 nm for LISINOPRIL operated by WINCATS software (Version 1.4.4.6337, CAMAG). The radiation source used was the deuterium lamp emitting a continuous uv spectrum between 190 to 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was based on peak areas with linear regression.

Preparation of standard stock solution

Standard stock solution was prepared by taking 10 mg of Lisinopril dissolved in 10 ml methanol to get concentration of 1000µg/ml.

Preparation of sample solution

The given 20 tablets were powdered using pestle & mortar to fine powder. The portion equivalent to 10 mg Lisinopril was transferred in a 10 ml volumetric flask, 5 ml of Methanol was then added, and sonication was done for 15 min with swirling. After sonication, the volume was made up to the mark with Methanol, and mixed well. The solution was filtered through 0.45µ Whatman filter. From this 1µl of Sample solution was applied on the pre-coated silica gel $60F_{254}$ plate and from the peak area obtained, the amount of Lisinopril in formulation was calculated using the calibration graph.

HPTLC method optimization and chromatographic conditions

The Chromatographic conditions were optimized for estimation of Lisinopril. For effective result of Lisinopril, the mobile phase containing a mixture of n-butanol: methanol: ammonia in the ratio of 3.0: 1.0: 1.0 (v/v/v) was found to be optimum. After chamber saturation, the plates were developed to a distance of 80 mm and then dried in hot air. Densitometric analysis was carried out using a Camag TLC Scanner (Camag) in the absorbance mode at 254 nm and 210 nm for Lisinopril but the best peak was achieved at 210 nm. The chromatograms were integrated using win CATS evaluation software as shown in Figure 2.

Method validation

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

Linearity and range

Standard stock solutions at a concentration of 1000µg/ml of Lisinopril was prepared using methanol. From the standard stock solution 1µL, 2µL, 3µL, 4µL and 5µL solution were spotted on the TLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves as shown in Figure 3, and Table 2.

Precision

The precision of the method was verified by intraday and interday precision studies. Intraday studies were performed on Lisinopril for six times with different concentrations on the same day i.e. Intra-day Precision as well Inter-day precision of the method was checked

by repeating studies on two different days. The relative Standard deviations obtained indicate the method is highly precise. The intraday and inter-day precision RSD (%) values for Lisinopril were found to be 1.48848 and 1.24354, respectively. The developed method was found to be precise as the RSD values for repeatability and inter-day precision studies were <2%, respectively, as recommended by ICH guidelines and Shown in Table 5.

Limit of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were determined as the amounts of the analyte for which the signal-to-noise ratios (S/N) were 3 and 10, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background (N), by spotting a blank, then calculating S/N for Lisinopril after application a series of solutions (prepared by dilution of the standard stock solution). The LOD and LOQ were found to be 0.050237 and 0.152233 for Lisinopril respectively and Shown in Table 5.

Specificity

The peak purity of Lisinopril was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the spot. A good correlation (r^2=0.986) was also obtained between the standard and sample spectra Lisinopril, respectively as shown in Figure 4 and Table 3. The data shows no interference of Mobile phase and diluent with Formulation and Standard which indicates the specificity of the results.

Accuracy

Accuracy of the method was obtained by performing recovery studies by the standard addition method at different levels of standard drug i.e. 80%, 100% and 120% of Lisinopril to analyzed tablet powder sample and mixture were reanalyzed by the proposed method. From the amount of drug percentage recovery was calculated. The relevant results are furnished in Figure 5 and Table 4.

Result and discussion

During the stage of method development different mobile phases were tried and the mobile phase comprising of n-butanol: methanol: ammonia in the ratio of 3.0: 1.0: 1.0 (v/v/v) was confirmed.

The results of validation studies on method developed for Lisinopril in the current study gives highest resolution, minimum tailing factor and Rf values of 0.20 for Lisinopril scanned at 210 nm. UV scanning at 200-400 nm shows that 210 nm is the suitable wavelength for detection of Lisinopril (Figure 2).

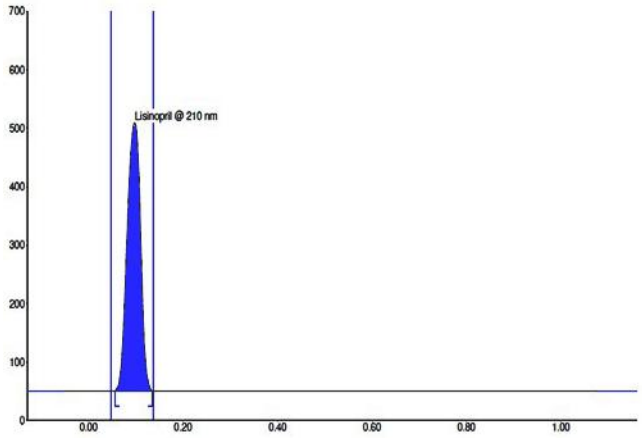

Figure 2. Spectrum of Lisinopril Dihydarate.

The Lisinopril showed a good correlation coefficient (r2 = 0.986) in the given concentration range 1µl to 5µl the equation was y = 1291x + 3939 and linearity data was shown in Table 2 and Figure 3.

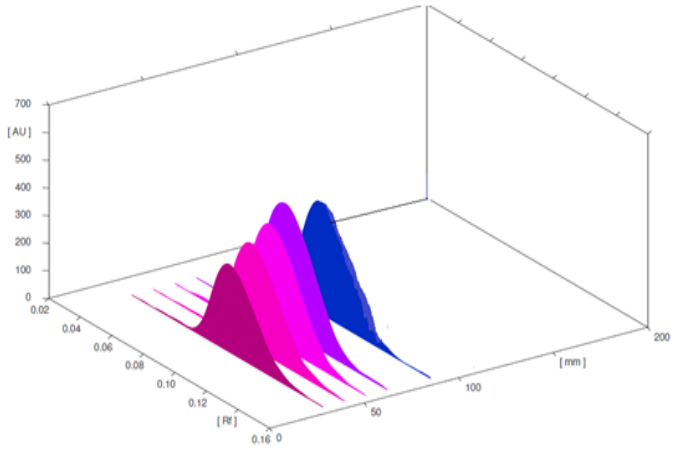

Figure 3. Chromatogram for Linearity of Lisinopril Dihydarate.

Table 2. Linearity data of Lisinopril Dihydarate.

Standard Concentration (µl)	Peak Area
1	4172
2	6335
3	8046
4	9193
5	10256

The LOD and LOQ were found to be 0.050237 and 0.152233 for Lisinopril respectively (Table 5).

The intraday and inter-day precision RSD (%) values for Lisinopril were found to be 1.48848 and 1.24354, respectively. The developed method was found to be precise as the RSD values for repeatability and inter-day precision studies were <2%, respectively, as recommended by ICH guidelines and Shown in Table 5.The peak purity of Lisinopril was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the spot. The data shows no interference of mobile phase and diluent with formulation and standard which indicates the specificity of the results Figure 4.

Accuracy of the method was obtained by performing recovery studies by the standard addition method at different levels of standard drug i.e. 80%, 100% and 120% of Lisinopril to analyzed tablet powder sample and mixture were reanalyzed by the proposed method. From the amount of drug found percentage recovery was calculated. The relevant results are furnished in Figure 5 and Table 4.

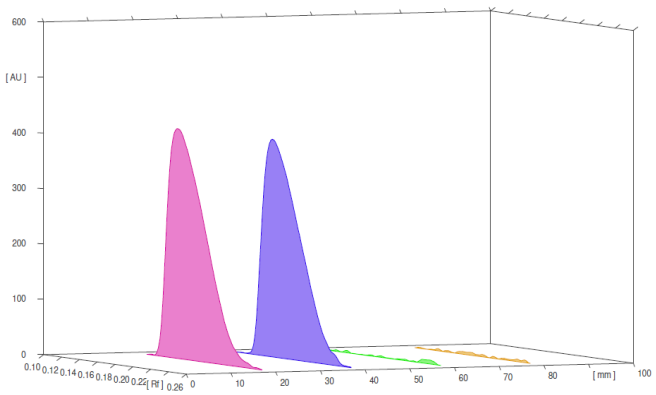

Figure 4. Chromatogram of Lisinopril Dihydarate for Specificity Study.

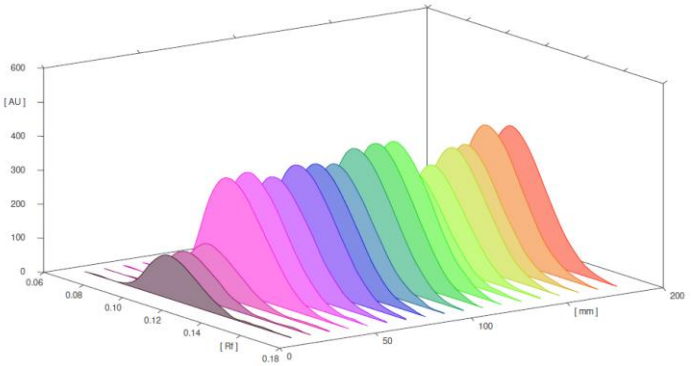

Figure 5. Chromatogram of Lisinopril Dihydarate for Accuracy Study.

Table 3. Specificity study of Lisinopril Dihydarate.

Sr. No.	Appl Vol	Sample ID	Start Rf	Start Height	Max Rf	Max Height	End Rf	End Height	Area
1	4.0 µl	Formulation	0.12	0.4	0.15	414.3	0.23	2.3	12857
2	4.0 µl	Standard	0.12	0.2	0.15	392.6	0.24	1.9	11830
3	4.0 µl	Mobile Phase	0	0	0	0	0	0	0
4	4.0 µl	Diluent	0	0	0	0	0	0	0

Table 4. Accuracy study of Lisinopril Dihydarate.

Accuracy level	Sample concentration (µl)	Standard concentration (µl)	Total amount added (µl)	% Recovery	Mean % Recovery
80	0.5	1.6	2.1	99.40	98.88
100	0.5	2.0	2.5	98.10	
120	0.5	2.4	2.9	99.14	

Table 5. Summary of validation parameters.

Parameter	Result of Lisinopril
Linearity (µl)	1-5
Correlation coefficient	0.986
Regression equation	Y=1291X+3939
Rf value	0.10
LOD	0.050237
LOQ	0.152233
Precision	Intra-day 1.48848
	Inter-day 1.24354
Specificity	Specific
Accuracy	98.88%

Conclusion

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today, HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput, and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC; thus reducing the analysis time and cost per analysis. Statistical analysis proves that the method is suitable for the analysis of Lisinopril Dihydrate as bulk drug and pharmaceutical formulation. The developed HPTLC technique is precise, specific, and accurate.

References

1. S. Budavari. The Merck Index: An encyclopedia of chemicals, drugs and biologicals,. Merck Research Laboratories, Division of Whitehouse Station, Merck and Co. Inc, NJ. 13th ed. 2001; 86.
2. S.C. Sweetman, Martindale. The Complete Drug Reference, Royal Pharmaceutical Society of Great Britain, London. 34th ed. 2005; 862.
3. Dhoka M.V. Validated HPTLC Method for determination of cefixime. Res J Pharma and Tech 2012; 342-345.
4. K.H. Gopani S.S. Havele, S.R. Dhaneshwar. Application of high performance thin layer chromatography densitometry for the simultaneous determination of amlodipine besylate and Lisinopril in bulk drug and tablet formulation. IJPT 2011; 3(2):2353-2367.
5. Sethi, P.D. HPTLC 'High Performance Thin Layer Chromatography',Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers and Distributors, New Delhi: 1stEdn. 2001; 52-58.
6. Mendham J, Denny RC, Barnes JD, Thomas M. Vogel's Text Book of Quantative Chemical Analysis. Pearsons Education, New Delhi: 6th edn. 2000; 156-158, 168-172.
7. Cazes J, Ewing's. Analytical Instrumentation Hand Book. Marcel Dekker Inc., NY: 2005; 295-345.
8. Stahl E, Thin Layer Chromatogrphy, 2nd edn. Springer Heidenberg: 2007; 1-86.
9. Obaid Shaikh, Sarfaraz Khan, Zahid Zaheer, Younus Shaikh, Madhukar Tajne. A validated HPTLC method for estimation of Nitazoxanide in bulk and formulation. Journal of Innovations in Applied Pharmaceutical Sciences 2016; 1(2):1-7.
10. Naved Ahmed, Obaid Shaikh, Aateka Barrawaz, Sarfaraz Khan, Zahid Zaheer. Development and validation of rapid HPLC method for determination of Aripiprazole in bulk drug and pharmaceutical formulation. Journal of Innovations in Pharmaceutical and Biological Sciences 2017; 4(3): 15-19.
11. Sushil D. Patil, Sunil V. Amurutkar, Chatpalliwar V. A., Chandrashekhar D. Upasani. Development and validation of RP-HPLC method for Empagliflozin and Metformin HCL. Journal of Innovations in Pharmaceutical and Biological Sciences 2017; 4(4): 185-189.
12. Haritha Gali, Vishwanadham Yerragunta. Development and validation of RP-HPLC method for simultaneous estimation of Naproxen and Esomeprazole in pharmaceutical dosage form. Journal of Innovations in Pharmaceutical and Biological Sciences 2016; 3(3): 39-42.
13. Desabattina Sai Jahnavi, Desabattina Venkata Narayana Rao, Yeluri Ramachandra Reddy. RP-HPLC method for the quantification of Metformin and Rosiglitazone in bulk and combined tablet dosage form: method development and validation. Journal of Innovations in Pharmaceutical and Biological Sciences 2016; 3(4): 55-60.