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Validated RP-HPLC Method for Simultaneous Estimation of Lisinopril and Hydrochlorothiazide in Combined Dosage forms

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Abstract

A rapid and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for simultaneous estimation of Lisinopril and Hydrochlorothiazide. Chromatographic separation was achieved on reverse phase Agilent LC 1100 series HPLC instrument on a Zorbax SB CN column (150 mm x 4.6 mm, 5µ) using the mobile phase consisting of buffer (p^H 6.0) and acetonitrile in the ratio of 70:30 v/v. The mobile phase was pumped at a flow rate of 1.5 ml/min and detection was done by UV detector at 215 nm. The proposed method was found to be simple, fast, accurate, precise and reproducible and could be applied for routine quality control analysis for simultaneous determination of Lisinopril and Hydrochlorothiazide in pharmaceutical dosage forms.

Keywords: Lisinopril, Hydrochlorothiazide, RP-HPLC, validation.

Contents

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1. Introduction

Lisinopril (Figure 1) is Angiotensin Converting Enzyme (ACE) inhibitor. It is an antihypertensive drug that act as vasodilator and reduce peripheral resistance. It inhibits ACE, which is involved in the conversion of angiotensin – I to angiotensin – II. Angitensin – II stimulates the synthesis and secretion of aldosterone and raises blood pressure via a potent direct vasoconstrictor effect. ACE is identical to bradykininase (kininase – II) and ACE inhibitors also reduce the degradation of bradykinin, which is a direct vasodilator and is also involved in the generation of prostaglandins. It is used in the treatment of hypertension and heart failure, prophylactically after myocardial infarction, and in diabetic neuropathy [1]. Hydrochlorothiazide (Figure 2) is used in the treatment of hypertension, either alone or with other antihypertensive such as ACE inhibitors and β-blockers. It also used to treat oedema associated with heart failure and with renal and hepatic disorders. Other indications have included the treatment of oedema accompanying the pre-menstrual syndrome, the prevention of water retention associated with corticosteroids and oestrogens, the treatment of diabetes insipidus, and the prevention of renal calculus formation in patients with hypercalciuria. It is a moderately potent diuretic and exert its diuretic effect by reducing reabsorption of electrolytes from the renal tubules, thereby increasing the excretion of sodium and chloride ions, and consequently of water. It acts mainly at the beginning of the distal tubules. [2].Literature survey revelats that these are few analytical methods

have been reported for the determination of Lisinopril and Hydrochlorothiazide in pure drug, pharmaceutical dosage forms and in biological samples using spcetrophotometry[3-16], liquid chromatography [17-22], high performance thin layer chromatography [23-27], gas chromatography [28], and polarography [29] either in single or in combined forms**.**

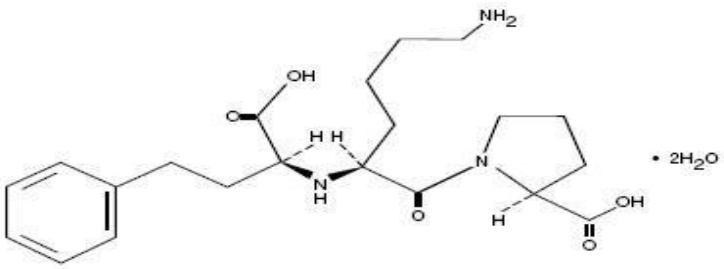

Figure 1. Molecular structure of Lisinopril

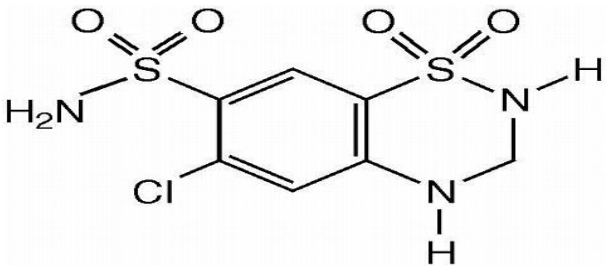

Figure 2. Molecular structure of Hydrochlorothiazide

2. Experimental

Chromatographic Conditions

The chromatographic separation was achieved on HPLC Agilent LC 1100 series consisting of isocratic binary pump and variable wavelength UV-Visible detector and 20µL Hamilton syringe was used for injecting the samples. Data was analysed by using Chemstation software. The analysis of the drug was carried out on Zorbax SB CN C_{18} column (150 x 4.6 mm; 5 µm), flow rate 1.5 mL/min, wave length 215 nm, injection volume 20 µL and run time 10 minutes.

Chemicals and Solvents

The working standards of Lisinopril (API) and Hydrochlorothiazide (API) were obtained from Dr. Reddy's laboratories limited, Hyderabad. The branded formulations (tablets) (Lipril-H tablets containing 5 mg of Lisinopril and 12.5 mg of Hydrochlorothiazide) were procured from the local market. Acetonitrile, Water and Methane sulphonic acid used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

Preparation of mobile phase and diluents

700 mL of 0.1% Methane sulphonic acid (1.0 mL of methane sulphonic acid was added to 700 mL of water. This solution was mixed and volume made up to 1000 mL with water) was mixed with 300 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µm nylon filter. The same mobile phase was used as diluent.

Preparation of standard stock solution

Accurately weighed and transferred 20 mg of Lisinopril and 50 mg of Hydrochlorothiazide working standards into 50 mL volumetric flask, about 30 mL of diluent was added, sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Preparation of standard solution

Pipetted 5 mL of the standard stock solution into 50 mL volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution

20 tablets of Lisinopril and Hydrochlorothiazide were weighed and calculated the average weight. Accurately weighed and transferred the sample equivalent to 20 mg of Lisinopril and 50 mg of Hydrochlorothiazide into 50 mL volumetric flask. About 30 mL of diluent was added, sonicated to dissolve it completely and made volume up to the mark with diluent. Mixed well and filtered through 0.45 µm filter. Further pipetted 10 mL of the above stock solution into a 100 mL volumetric flask and dilute up to the mark with diluent.

Method-validation The developed analytical method was validated as per ICH guidelines[30] for the parameters like linearity, accuracy, precision, ruggedness, specificity and system suitability.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of drug in the samples within a given range. Linearity was performed by preparing mixed standard solutions of Lisinopril (20 to 60 µg/ml) and Hydrochlorothiazide (50 to 150 µg/ml) and at seven concentration levels. The plot of peak area of each sample against respective concentration of Lisinopril and Hydrochlorothiazide was found to be linear. Beer's law was found to be obeyed over these concentration ranges. The correlation coefficient shall not be less than 0.9998. Linearity results were presented in Table 1 and 2.

Accuracy

Accuracy indicates the deviation between the mean value found and the true value. The accuracy of the method was determined by standard addition method by means of recovery experiments. 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 50%, 100% and 150% level. 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 were presented in Table 3 and 4. Satisfactory recoveries ranging from 97.56 to 101.35 for Lisinopril and 97.56 to 101.15 for Hydrochlorothiazide respectively were obtained by the proposed method. This indicates that the proposed method was accurate.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions. Six replicate mixed standard solutions of Lisinopril and Hydrochlorothiazide were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.05and 0.11 for Lisinopril and Hydrochlorothiazide respectively, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 5 and 6.

Robustness

The robustness study was performed by slight modification in flow rate of the mobile phase, temperature of the column and composition of the mobile phase. Mixed samples of Lisinopril and Hydrochlorothiazide at a concentration of 40µg/mL and 100µg/mL respectively were 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 7 & 8.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.06µg/mL and 0.20 µg/mL for Lisinopril and 0.11 µg/mL and 0.25 µg/mL for Hydrochlorothiazide. The LOD and LOQ showed that the method is sensitive for Lisinopril and Hydrochlorothiazide.

System suitability test

The specificity of this method was determined by complete separation Lisinopril and Hydrochlorothiazide. The typical chromatogram of Lisinopril and Hydrochlorothiazide was shown in (Fig. 3) with parameters like retention time, resolution and tailing factor. Here tailing factor for peaks of Lisinopril and Hydrochlorothiazide was less than 2% and resolution was satisfactory. The average retention time for Lisinopril and Hydrochlorothiazide were found to be 2.973 and 7.672 respectively, for five replicates. The peaks obtained for Lisinopril and Hydrochlorothiazide were sharp and have clear baseline separation. Analysis was also performed for active Lisinopril and Hydrochlorothiazide, as well as placebo sample at different conditions. After analysis it was found that there is no interference of peak in the Lisinopril and Hydrochlorothiazide region for the placebo & active sample. Hence the developed method was specific for the analysis of this product**.** The system suitability parameters are given in Table 9.

Estimation of Lisinopril and Hydrochlorothiazide in tablet dosage forms

Twenty tablets were weighed and finely powdered. An accurately weighed portion of this powder equivalent to 20 mg of Lisinopril and 50 mg of Hydrochlorothiazide was transferred to a 50 mL volumetric flask containing 30 mL of the mobile phase. 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 the mobile phase. Then this mixture was filtered through whatman No.41filter paper 5.0 mL of this filtrate was further diluted to 50 mL with the mobile phase. The solution was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The assay results of tablet dosage formulation by the proposed method are presented in Table 10.

3. Results and Discussion

The RP-HPLC procedure was optimized with a view to develop accurate and stable assay method with the pure drugs Lisinopril and Hydrochlorothiazide. Chromatographic separation was achieved on reverse phase Agilent LC 1100 series HPLC instrument on a Zorbax SB CN column (150 mm x 4.6 mm, 5µ) using the mobile phase consisting of buffer (p^H 6.0) and acetonitrile in the ratio of 70:30, v/v. The mobile phase was pumped at a flow rate of 1.5 ml/min and detection was done by UV detector at 215 nm. The % recovery was found to be within limits of the acceptance criteria with recovery range 97.56 to 101.35% for Lisinopril and 97.56 to 100.15% for Hydrochlorothiazide. The high percentage of recovery indicates that the proposed method is highly accurate. The detection limit of the proposed method was 0.06µg/mL and 0.11 µg/mL and the quantification limit was 0.20 and 0.25 for Lisinopril and Hydrochlorothiazide respectively, which indicate the sensitivity of the method. The assay procedures were repeated for six times and the results were found to give 100.87% of Lisinopril and 100.22% of Hydrochlorothiazide. The number of theoretical plates calculated was 5524 and 7712 for Lisinopril and Hydrochlorothiazide respectively, which indicates efficient performance of the column. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the simultaneous estimation of the drugs Lisinopril and Hydrochlorothiazide by the proposed HPLC method.

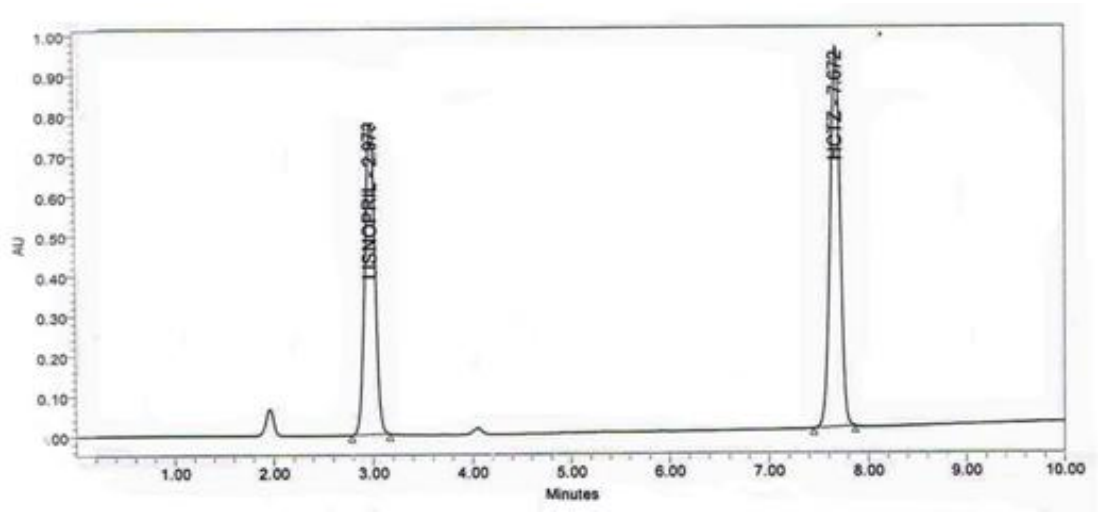

Figure 3. Typical chromatogram Lisinopril & Hydrochlorothiazide

Table 1. Linearity data of Lisinopril

Concentration (µg/mL)	Peak area (n=6)
20	697466
32	1110988
36	1241983
40	1391224
44	1496287
48	1669431
60	2083567

Table 2. Linearity data of Hydrochlorothiazide

Concentration (µg/mL)	Peak area (n=6)
50	3056562
80	4892616
90	5612836
100	6113152
110	6694467
120	7335612
150	9063918

Table 3. Recovery study for Lisinopril

Level	Amount of Lisinopril spiked (µg)	Amount recovered (µg)	%Recovery
50%	20	20.27	101.35
	20	20.03	100.1
	20	19.96	99.82
100%	40	39.80	99.49
	40	39.92	99.80
	40	39.80	99.49
150%	60	59.60	99.32
	60	59.38	98.97
	60	58.54	97.56

Table 4. Recovery study for Hydrochlorothiazide

Level	Amount of Hydrochlorothiazide spiked (µg)	Amount recovered (µg)	%Recovery
50%	50	49.93	99.87
	50	50.07	100.15
	50	49.91	99.82
100%	100	98.62	98.62
	100	99.07	99.07
	100	98.88	98.88
150%	150	148.0	98.61
	150	147.2	98.12
	150	146.3	97.56

Table 5. Precision study of Lisinopril

Injection number	Peak area
1	1399112
2	1397678
3	1398527
4	1397866
5	1399302
6	1398891
Mean	1398563
%RSD	0.05

Table 6. Precision study of Hydrochlorothiazide

Injection number	Peak area
1	6161924
2	6164658
3	6173584
4	6173176
5	6174789
6	6180506
Mean	6171441
%RSD	0.11

Table 7. Robustness study for Lisinopril

Condition	Mean area	% assay	% difference
Unaltered	1356598	98.04	-
Flow rate at 1.3 mL/min	1377156	98.67	0.63
Flow rate at 1.7mL/min	1368150	98.92	0.88
Mobile phase:			
• (Buffer(72):Acetonitrile(28))	1383705	98.70	0.66
• (Buffer(68):Acetonitrile(32))	1393609	98.80	0.76
Temperature of column at 43°C	1368400	98.59	0.55
Temperature of column at 47°C	1367680	98.98	0.94

Table 8. Robustness study for Hydrochlorothiazide

Condition	Mean area	% Assay	% Difference
Unaltered	6229950	98.80	-
Flow rate at 1.3 mL/min	6120960	99.57	0.77
Flow rate at 1.7mL/min	6054828	99.91	-1.11
Mobile phase:			
• (Buffer(72):Acetonitrile(28))	6152764	98.92	0.12
• (Buffer(68):Acetonitrile(32))	6134004	98.52	0.28
Temperature of column at 43°C	6039020	98.46	0.34
Temperature of column at 47°C	6057222	98.73	0.07

Table 9. Analytical validation parameters

Parameter	Lisinopril	Hydrochlorothiazide
Linearity (µg/mL)	25-150	25-150
Slope	34602.33	60654.82
Intercept	286.534	38848.79
Correlation coefficient	0.9998	0.9998
LOD (µg/ml)	0.06	0.11
LOQ (µg/ml)	0.20	0.25
Theoretical Plates	5524	7712
Tailing Factor	1.52	1.35
Retention Time (min)	2.973	7.672

Table 10. Assay studies

Drug	Label claim (mg)	Amount found (mg)	%Assay
Lisinopril	5	4.98	99.6
Hydrochlorothiazide	12.5	12.51	100.8

4. Conclusion

A simple, specific, sensitive, rapid, accurate and precise RP-HPLC method has been developed for simultaneous estimation of Lisinopril and Hydrochlorothiazide. The result of the study follows the protocol of ICH guidelines and it can be successfully applied for the simultaneous estimation of the marketed products of Lisinopril and Hydrochlorothiazide and in combined tablet formulations.

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