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Validated RP-HPLC Method Development for the Simultaneous Estimation of Lisinopril and Hydrochlorthiazide in Bulk and Pharmaceutical Dosage Form

Amarnath Mundra*, Harshini.S, Sireesha. D, Akifulhaque, Vasudha.B

Department of Pharmaceutical Analysis and Quality assurance, Anurag group of institutions (Formerly Lalitha college of pharmacy), Hyderabad.

A B S T R A C T

A simple and cost effective RP-HPLC method is described for the determination of lisinopril and Hydrochlorothiazide in pure form and in pharmaceutical formulations. The drug was highly soluble in acetonitrile, so it was selected as the solvent system for the drug. This ensured adequate drug solubility and maximum assay sensitivity. The linearity range for Lisinopril and Hydrochlorothiazide at its wavelength of detection of 230 nm was obtained as 20–30 µg/ml and 50-75 µg/ml. The absorbance was found to increase linearly with increasing concentration of Lisinopril and Hydrochlorothiazide, which is corroborated by the calculated correlation coefficient value of 0.9999. The limit of detection and limit of quantification of Lisinopril and Hydrochlorothiazide was found to be 0.10 µg/ml & 0.10 µg /ml and 0.32 µg /ml & 0.30 µg /ml respectively. The validity of the described procedure was assessed. Statistical analysis of the result shows high accuracy and good precision. The proposed method was successfully applied to the determination of Lisinopril and Hydrochlorothiazide in pharmaceutical formulations.

Keywords: Estimation, Lisinopril, Hydrochlorothiazide, RP-HPLC Method.

A R T I C L E I N F O

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***Corresponding Author**
Amarnath Mundra
Anurag Group of Institutions
(Formerly Lalitha College of Pharmacy)
Ghatkesar (M), Rangareddy (D).
Hyderabad, Telangana, India.
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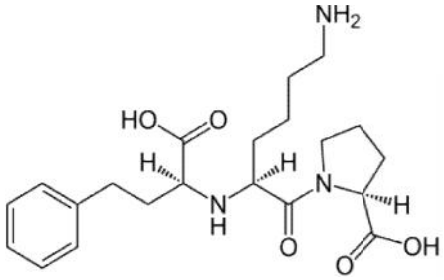
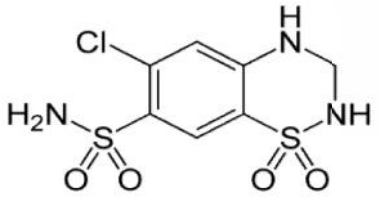
1. Introduction

Lisinopril is a drug of the angiotensin-converting enzyme (ACE) inhibitor class used primarily in treatment of high blood pressure, heart failure, and after heart attacks. Its indications, contraindications, and side effects are as those for all ACE inhibitors. It is a key component of renin-angiotensin-aldosterone system (RAAS). Used to improve prevention of renal disease in hypertensive patients and diabetes mellitus and microalbuminuria.



Hydrochlorothiazide is a diuretic often used to treat high blood pressure and swelling due to fluid buildup. Other uses include diabetes insipid us, renal tubular acidosis, and to decrease the risk of kidney stones in those with high calcium level in the urine. Hydrochlorothiazide is taken by mouth and may be combined with other blood pressure medications as a single pill to increase the effectiveness. A thiazide diuretic often considered the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension and hyperparathyroidism.



Chemicals and reagents

Methanol (HPLC grade) was obtained from Merck specialties private limited, Mumbai, India. Water (HPLC grade) was obtained from Merck specialties private limited, Mumbai, India. Pure drug Lisinopril and Hydrochlor-thiaizide obtained as gift samples from Chandra labs (Hyderabad, India). Listril Plus tablets manufactured by Torrent Pharmaceuticals purchased from local pharmacy is used for the analysis. The label claim states that this formulation contains 2.5 mg of Lisinopril and 12.5 mg of Hydrochlorothiazide of each tablet.

2. Materials and Methods

Instruments used

SHIMADZU double beam UV/Visible Spectrophotometer model UV 1800s was employed with a spectral band width of 1 nm and SHIMADZU Electronic balance model AX 200 and Ultra Sonicator (Fast clean) model 2k 811056 were also used during the analysis.

Materials: Analytically pure samples of Lisinopril and Hydrochlorothiaizide were obtained as gift samples from Chandra labs (Hyderabad).Tablets of brand "LISTRIL PLUS" having Lisinopril and Hydrochlorothaizide (2.5 mg and 12.5 mg) manufactured by Torrent Pharmaceuticals. was purchased from local pharmacy.

Method

Selection of wavelength: In order to determine the wavelength of maximum absorption of the drug, qualitative solution of the drug was prepared in acetonitrile and scanned using UV spectrophotometer within the wavelength region of 200-400 nm against methanol as blank. The absorption curve showed characteristic absorption maximum at 230 nm, hence this wavelength is fixed for analysis of Lisinopril and Hydrochloorthaizide

- Preparation of standard solutions
- Preparation of working standard solutions
- Preparation of sample solutions
- UV spectrophotometric method

Estimation of Lisinopril and Hydrochloorthiazide was achieved by UV method. It shows maximum absorbance when dissolved in acetonitrile at 230 nm.

Method validation

Lisinopril showed linearity in the range of 20-30 µg/ml and Hydrochlorthiazide showed in the range of 50-75 µg/ml. The calibration graph was plotted with peak area in the Y axis and concentration of standard solution in the X axis. The slope, intercept and correlation coefficient values for Lisinopril were found to be 67.7142, 24.5148 and 0.9999 respectively. The slope, intercept and correlation coefficient values for Hydrochlorthiazide were found to be 31.7155, 9.3218 and 0.9999 respectively.

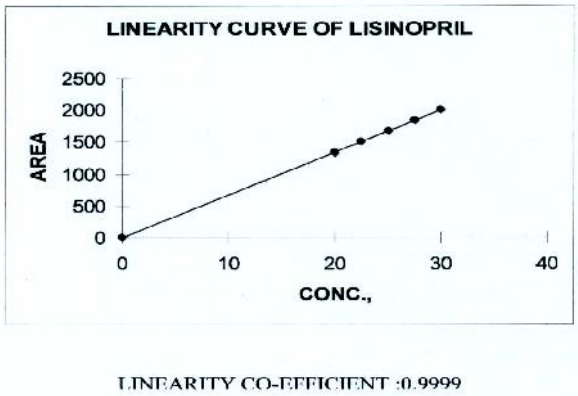


Figure 1: Linearity Plot for Lisinopril

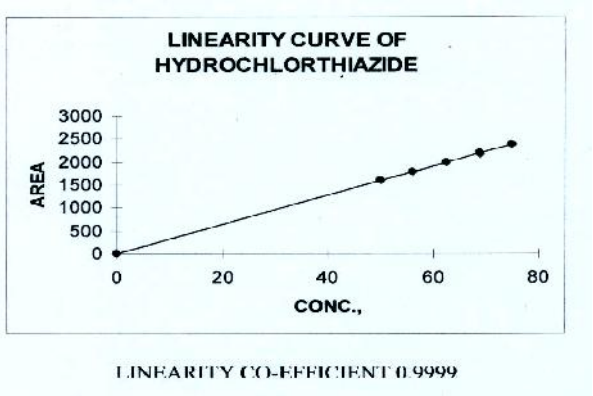


Figure 2: Linearity Plot for Hydrochlorthiazide

Accuracy

Accuracy expresses the closeness of agreement between the value, which is accepted either as conventional true value or and accepted reference value (International Standard e.g.pharmacopoeal standard) and the value found (mean value) by applying the test procedure a number of times.

To study reliability, suitability and accuracy of the method, recovery studies were carried out, by adding a known quantity of the standard to the preanalyzed sample and recovery study was done. The recovery was carried out at 80%, 100% and 120% level and the contents were determined from the respective chromatogram. From the results obtained we can conclude that the method was accurate.

Mean Recovery of Lisinopril and Hydrochlorthiazide for Accuracy

Table 1

Accuracy level	Mean Recovery of Lisinopril	Mean Recovery of Hydrochlorthiazide
Accuracy 80%	99.66	99.36
Accuracy 100%	99.40	99.41
Accuracy 120%	99.70	100.97

Precision

Repeatability of Injection

The precision of test method was done by performing assay on six replicate determination of sample preparation at test concentrations level (as per method of analysis) and calculated relative standard deviation of assay results. Six 20 µl injections from a standard solution were injected on to the analytical column and the peak area data obtained and %RSD were calculated. The System Precision and Method Precision were also calculated.

System Precision of Lisinopril and Hydrochlorthiazide

Table 2

S.No	Area of Lisinopril (mV)	Area of Hydrochlorthiazide (mV)
1	1672.139	1971.786
2	1677.812	1977.126
3	1678.182	1982.724
4	1677.391	1983.621
5	1679.121	1979.216
6	1678.714	1980.867
Mean	1677.237	1979.223
S.D	2.567	4.336
R.S.D	0.1530	0.2190

Acceptance Criteria: The RSD should be NMT 1.0%.

Robustness

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. Following optimized conditions were slightly varied to check the robustness:

Effect of flow rate

Robustness of assay method was carried out with variation of flow rate (±0.2ml/minute of set value, i.e. 1.1ml and 1.5 ml/min). Standard preparation was prepared and analysis was performed analysis as per the test method and evaluated the system suitability parameters.

Effect of pH

Robustness of assay method was checked with the system suitability parameters by injecting standard preparation with mobile phase having altered pH (i.e. one is pH 4 and other one is pH 6), then evaluated the system suitability parameters.

Effect of flow rate

Table 3

Flow Rate	Retention time of Lisinopril (min)	Retention time of Hydrochlorthiazide (min)
1.1 ml/min	3.921	3.229
1.3 ml/min	3.795	3.020
1.5 ml/min	3.638	3.002

Ruggedness

The degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials.

Table 4: Variation of Analyst

Analysts	Area of Lisinopril (mV)	Area of Hydrochlorthiazide (mV)
Analyst I	1631.389	1981.322
Analyst II	1659.299	1955.457

Limit of Detection (LOD)

Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions.

The minimum concentration at which the analyte can be detected is determined from the linearity curve by following formula.

$$\text{Limit of detection} = \frac{\sigma}{S} \times 3.3$$

The lowest concentration of Lisinopril that can be detected was determined from standard curve was 0.1068 µg/ml. The lowest concentration of Hydrochlorthiazide that can be detected was determined from standard curve was 0.1009 µg/ml.

Limit of Quantitation (LOQ)

Limit of quantitation is the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug, Limit of quantitation can be obtained from linearity curve by applying the following formula.

$$\text{Limit of quantitation} = \frac{\sigma}{S} \times 10$$

Table 5: LOD and LOQ

Sample	LOD	LOQ
Lisinopril	0.10 µg/ml	0.32 µg/ml
Hydrochlorthiazide	0.10 µg/ml	0.30 µg/ml

The lowest concentration at which peak can be quantified is called LOQ was found to be 0.3237 µg/ml for Lisinopril and for Hydrochlorthiazide was found to be 0.3060 µg/ml.

Stability studies

Stability of the sample and standard used in HPLC method is required for a reasonable time to generate reproducible and reliable results. The stability of the sample spiked with drug was subjected to short term stability at room temperature after 8 hours.

Table 6: Stability Studies

Stability at room temperature	Area of Lisinopril(mV)	Area of Hydrochlorthiazide (mV)
At zero hour	1677.391	1983.621
After 8 hour	1659.299	1955.457

Assay Procedure for the Proposed Method

Sample preparation: For estimating the tablet, dosage form 20 tablets from a batch were selected randomly and powdered. About 990 mg of powdered formulation was accurately weighed and taken in a 100 ml volumetric flask; 15 ml of mobile phase was added. The mixture was subjected to sonication for 10 min with intermediate shaking for complete extraction of drugs. Cooled to room temperature and the solution was made up to the mark with mobile phase. Then 5 ml of clear solution was transferred into a 50 ml volumetric flask and diluted with mobile phase and 20 µl of this solution was injected for HPLC analysis.

Procedure: Separately Blank, Standard, and test preparation were injected into liquid chromatographic system and the areas for major peaks were recorded by using the following formula.

Calculation: Calculated the amount of Lisinopril and Hydrochlorthiazide using the formula:

$$\frac{\text{Sample Area X Sample dilution X Purity of Working Standard X Average weight}}{\text{Standard Area X Standard dilution X Label claim}} \text{ X } 100$$

Result: The mean recovery for assay results was found to be 98%-100.09% for Lisinopril and Hydrochlorthiazide as well.

3. Results and Discussion

Estimation:

Estimation of Lisinopril and Hydrochlorthiazide in dosage forms by the developed RP-HPLC method was carried out. The standard and sample solutions were prepared and the chromatograms were recorded. The assay procedure was performed and the assay percentage was calculated. The assay percentage of individual drugs and amount present per tablet were calculated and presented in Table.

Validation of the method

The suitability of the system was studied by the values obtained for Theoretical plate, Resolution and tailing factor of the chromatogram of standard drugs and presented in Table. The selectivity of the method was revealed by the repeated injection of mobile phase and no interference was found. The recovery studies were carried out by preparing 6 individual samples with same procedure from the formulation and injecting. The percentage recovery was calculated and presented in Tables. From the data obtained, added recoveries of standard drugs were found to be accurate.The system and method precision of the method were demonstrated by inter day, intraday and repeatability of injection studies. All the solutions were injected into the chromatographic system. The peak area and percentage relative standard deviation were calculated and presented in Tables. From the data obtained, the developed HPLC method was found to be precise. The standard drug solutions of varying concentrations ranging from 80 % to 120 % of the targeted level of the assay concentration (i.e.) 50 µg/ml to 75 µg/ml of Hydrochlorthiazide and 20 µg/ml to 30 µg/ml of Lisinopril, were examined by the proposed method. The response factor, slope, intercept, correlation co-efficient and Residual sum of squares values were calculated. The slope, intercept and correlation coefficient(r) were found to be 67.7142, 24.5148 and 0.999 respectively for Lisinopril and 31.7155, 9.3218 and 0.999 respectively for Hydrochlorthiazide, which was presented in Table 4. The calibration curves were plotted using response factor Vs concentration of the standard solutions. The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the concentrations of the analytes. The range demonstrates that the method is linear outside the limits of expected use. The robustness of the method was studied by carrying out experiments by changing conditions discussed earlier. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters (Table) and hence developed method is said to be robust. The ruggedness of the method was checked by analyzing the prepared solution with different analysts and the data were presented in Table. The developed HPLC method for the simultaneous estimation of Lisinopril and Hydrochlorothiazide in dosage forms is accurate, precise, linear, robust, simple and rapid.

Table 7: Linearity data for Lisinopril and Hydrochlorthiazide

Concentration of Lisinopril (µg/ml)	Peak Area Lisinopril (mV)	Concentration of Hydrochlorthiazide (µg/ml)	Peak Area Hydrochlorthiazide (mV)
20	1323.168	50	1592.519
22.5	1504.636	56.25	1795.587
25	1673.719	62.5	1995.935
27.5	1836.511	68.75	2184.428
30	2003.657	75	2389.205

Table 8: Analytical Performance Parameters for Lisinopril and Hydrochlorthiazide

Parameters	Lisinopril	Hydrochlorthiazide
Linearity Dynamic Range	20-30µg/ml	50-75µg/ml
Correlation Coefficient	0.9999	0.9999
Slope (m)	67.7142	31.7155
Intercept	24.5148	9.3218

Table 9: Recovery Studies for Lisinopril and Hydrochlorthiazide

S. No.	Inj. Sample	Spike level	Amount Present (mg)	Amount Recovered (mg)	% Recovered
1	**Lisinopril**	80 %	20.21	20.14	99.66
2		100 %	25.04	24.89	99.40
3		120 %	30.08	29.99	99.70
4.	**Hydrochlorthiazide**	80 %	50.07	49.75	99.36
5		100 %	62.59	62.22	99.41
6		120 %	75.12	75.85	100.97

Table 10

Sample No:	Area of Lisinopril (mV)	% Label Claim	Area of Hydrochlorthiazide (mV)	% Label Claim
1	1678.512	99.24	1975.731	99.48
2	1659.299	98.12	1955.457	98.46
3	1683.318	99.52	1987.786	100.09
4	1670.662	99.54	1980.389	99.72
5	1674.717	99.02	1963.103	98.86
6	1671.389	98.84	1981.322	99.77
Mean	1672.983	99.05	1973.965	99.39
S.D	8.1923	0.5305	12.2480	0.6160
R.S.D	0.4897	0.5355	0.6204	0.6197

Acceptance Criteria: The RSD should be NMT 1.0%.

Table 11: Effect of pH

pH	Area of Lisinopril (mV)	Retention time of Lisinopril (min)	Area of Hydrochlorthiazide (mV)	Retention time of Hydrochlorthiazide (min)
4.8	1692.162	3.987	1996.243	3.243
5.0	1631.389	3.797	1981.322	3.020
5.2	1669.531	3.614	1969.612	2.935

Table 12: Assay result for replicate injections of standard

Inj No.	Area of Lisinopril (mV)	Area of Hydrochlorthiazide (mV)	Amt of Lisinopril Per tab.(mg)	% of Lisinopril	Amt of Hydrochlorthiazide per tab.(mg)	% of Hydrochlorthiazide recovered
1	1678.512	1975.731	4.962	99.24	12.435	99.48
2	1659.299	1955.457	4.906	98.12	12.308	98.46
3	1683.318	1987.786	4.976	99.52	12.512	100.09
Mean	1673.71	1972.991	4.948	98.96	12.419	99.35
S.D	12.720	16.338	0.037	0.740	0.103	0.824
%R.S.D	0.760	0.828	0.748	0.748	0.829	0.829

4. Conclusion

From the reported literature, there were few methods established for the determination of Lisinopril and Hydrochlorthiazide in individual and in combination with other drugs. HPTLC, LC/MS/MS, Derivative spectroscopy, Fluorimetric, Voltametric methods were established to determine the above mentioned drug in formulations and in plasma samples. RP-HPLC method have been established for determining the other ACE inhibitors along with Hydrochlorthiazide. It was concluded that there was no method reported for the simultaneous estimation of the above selected multi-component dosage form by RP-HPLC method, which promote to pursue the present work. The scope and objective of the present work is to develop and validate a new simple HPLC method for simultaneous estimation of Lisinopril and Hydrochlorthiazide in pharmaceutical dosage

form. In simultaneous RP-HPLC method development, the mobile phase selected after optimization was Ammonium acetate Buffer (0.02 M) and Acetonitrile with the ratio of 75:25 v/v, with pH 5.0 adjusted with dil. Potassium hydroxide was found to be ideal. The flow rate was found to be optimized at 1.3 ml/min. Detection was carried out at 230 nm. Quantitation was done by calibration curve method with the above mentioned optimized chromatographic condition. This system produced symmetric peak shape, good resolution and reasonable retention times for Lisinopril and Hydrochlorthiazide were found to be 3.783 and 3.010 minutes respectively.

The Lisinopril and Hydrochlorthiazide showed linearity in the range of 20 to 30 µg/ml and 50 to 75 µg/ml respectively. The slope, intercept and correlation coefficient(r) values were found to be 67.7142, 24.5148 and 0.999 respectively for Lisinopril and 31.7155, 9.3218 and 0.999 respectively for Hydrochlorthiazide, which indicates excellent correlation between response factor Vs concentration of standard solutions. Precision of newly developed method was studied under system precision, method precision, and intermediate precision. Intermediate precision was studied under intraday and repeatability. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The %RSD value for percentage recovery of Lisinopril and Hydrochlorthiazide was found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for simultaneous estimation of the above mentioned drugs. From the above experimental data and results, the developed HPLC method is having the following advantages:

- The standard and sample preparation requires less time.
- No tedious extraction procedure was involved in the analysis of formulation.
- Run time required for recording chromatograms were less than 10 minutes.
- Suitable for the analysis of raw materials, applicable to dissolution studies and can be used for the content uniformity studies.

Hence, the chromatographic method developed for Lisinopril and Hydrochlorthiazide is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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