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Method Development And Validation Of Lisinopril And Hydrochlorothiazide In Comined Dosage Form By RP-HPLC

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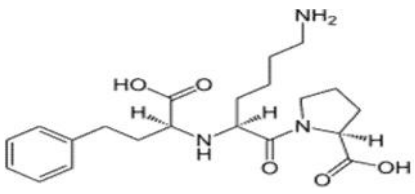
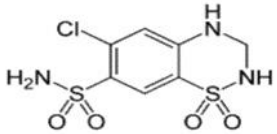
Abstract: A simple, specific,accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Lisinopril dihydrate and Hydrochlorothiazide from combined dosage form by Reverse phase C_{18} column (Neosphere C_{18},10µ,250mm x 4.6mm). The sample was analyzed using a mobile phase of sodium phosphate buffer solution: methanol (70:30 v/v adjust pH 4.5 with orthophosphric acid).The flow rate was 1.0 ml/ min with detection at 225 nm. The retention time for Lisinopril Dihydrate and Hydrochlorothiazide was found to be 7.0667 and 5.1000 min respectively, and recoveries from combined dosage form were between 98 and 102%. The method can be used for estimation of combination of these drugs in combined dosage form.
Keywords: Lisinopril, Hydrochlorothiazide, Reverse phase HPLC & validation.

Introduction:

Lisinopril [1] is a drug of the angiotensin-converting enzyme (ACE) inhibitor class primarily used in treatment of hypertension, congestive heart failure, and heart attacks and also in preventing renal and retinal complications of diabetes. Its indications, contraindications and side effects are as those for all ACE inhibitors. It is designated chemically N^2-[(1*S*)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline and It empirical formula is $C_{21}H_{31}N_3O_5$ and its structural formula is:



Hydrochlorothiazide[2] is white crystalline compound, soluble in water, but freely soluble in sodium hydroxide solution with molecular weight 297.74. It is a designated chemically is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide is an first line diuretic drug of the thiazide class that acts by inhibiting the kidney's ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus the cardiac output, is belived to lower peripheral vascular resitance. It emprical formula is $C_7H_8ClN_3O_4S_2$ and its structural formula is:



Literature survey reveals the availability of several methods for estimation of both Lisinopril[3-5] and Hydrochlorothiazide[6-8] includes spectrophoto metric, HPTLC- densitometry determination, spectrofluorimetric, HPLC as alone or in combination with other drugs. No method has been reported for the estimation of Lisinopril dihydrate and Hydrochlorothiazide in combined

dosage form. Present work emphasizes on the quantitative estimation Lisinopril dihydrate and Hydrochlorothiazide in their combined dosage form by RP-HPLC.

Experimental

Chemicals and Reagents

Lisinopril dihydrate (purity: 98.91%) & Hydrochlorothiazide (purity: 99.97%) were obtained from Lupin Pharma Ltd.(Aurangabad, India). Methanol was of HPLC grade and all other chemicals used were of analytical grade. Purified water from Milli-Q-system (Millipore, Bangalore, India) was used throughout the analysis.

Instrumentation and Analytical Conditions

Analysis was carried out using Younglin (Acme 9000) isocratic HPLC with Rheodyne manual sample injector using Autochrome 3000 software and the Analytical column used was Neosphere C_{18}, 10µ, 250mm x 4.6mm, using sodium phosphate buffer solution: methanol (70:30) adjust pH 4.5 with Orthophosphoric acid as mobile phase at a flow rate of 1.0 ml/min and detection and detection carried out at 225 nm. The mobile phase was filtered through a 0.45 µm membrane filter (Millipore®).

Preparation of Standard stock solution

Solution (A): Weighed accurately 100 mg of Lisinopril Dihydrate working reference standard and transferred carefully in to a 50ml volumetric flask. Added 35ml of mobile phase and sonicated for 15 min, cooled to room temperature and diluted 50ml with mobile phase. Mixed well.

Solution (B): Weighed accurately 100mg of Hydrochlorothiazide working reference standard and transferred carefully in to a 50ml volumetric flask. Added 35ml of mobile phase and sonicated for 15min, cooled to room temperature and diluted 50ml with mobile phase. Mixed well.

Mixtured standard solution: Diluted 5ml of Solution (A) and 5ml Solution (B) to 50ml with mobile phase.

Preparation of Sample solution

Twenty tablets, (Brand name: Lisoretic) each containing 20 mg Lisinopril Dihydrate, 12.5 mg Hydrochlorothiazide were weighed and average weight was calculated. One fourth of the average weight was accurately weighed and transferred to 100.00 ml volumetric flask, added 70ml of mobile phase. Sonicated for 15min and cooled to room temperature. Diluted to 100ml with mobile phase. Mixed well and filtered through Whatman No.1 filter paper. Discarded first few ml of the filtrate. Injected separately 20µl of the standard preparation in to the equilibrated HPLC system in 5 replicate and measured the response of the major peak due to Lisinopril Dihyrate and Hydrochlorothiazide. Then inject separately 20µl of the sample preparation in to duplicate and measured the response of the major peak due to Lisinopril Dihyrate and Hydrochlorothiazide and calculated the content of Lisinopril Dihyrate and Hydrochlorothiazide.

Validation of the Method[10]

The method was validated in terms of linearity, accuracy, precision of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of Lisinopril, Hydrochlorothiazide and measured the absorbance at 225nm. Calibration curves where constructed by plotting the area against the concentration. Lisinopril shows the linearity in the concentration range from 05-25 µg/ml with correlation coefficient of 0.9999 and Hydrochlorothiazide shows the linearity in the concentration range from 10-50 µg/ml with correlation coefficient of 0.9999. Recovery studies were carried out to study the accuracy of the proposed method and ascertained by standard addition method. A known amount of drug was added to preanalysed tablet powder, at three level and the percentage recoveries were calculated. Precision was found to be lower than 1%. Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions.

Results and Discussion

1. Estimation

A RP-HPLC method was developed for the simultaneous estimation of Lisinopril and Hydrochlorothiazide in combined dosage forms, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. The standard and sample solutions were prepared and chromatograms were recorded.

The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean and relative standard

deviations in formulation were calculated. The result of analysis shows that the amount of drugs present in the formulation has a very good correlation with the label claim of the formulation.

Figure 1: Typical chromatogram of Lisnopril and Hydrochlorothiazide

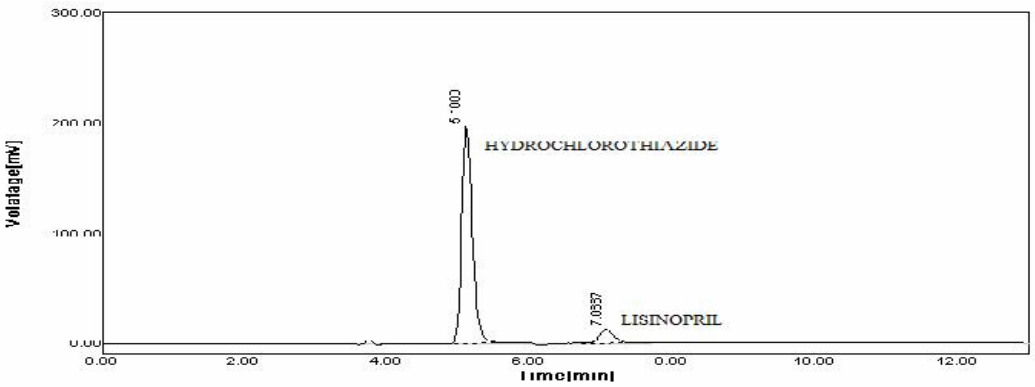


Table 1: Optimized Chromatographic condition

Parameter	Optimized condition
Instrument	Younglin (Acme 9000)
Column	Neosphere C_{18},10µ,250mm x 4.6mm
Mobile phase	Sodium Phosphate buffer : Methanol (70:30) pH 4.5 (dil.Orthophosphoric acid)
Flow rate	1.0ml/min
Detection	225nm
Injection volume	20µl
Temperature	Ambient

*Filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated

Table 2: System Suitability Parameters

Parameter	Lisinopril	Hydrochlorothiazide
Theoretical plates	4737.2	7281.6
Resolution	4.9177	
Tailing factor	1.3750	1.2500
LOD (µg/ml)	0.296	0.421
LOQ (µg/ml)	0.893	1.42

Table 3: Analysis of Formulation and Recovery studies

Drug	Label claim (mg/ml)	*Estimation	**Recovery	
		mg/tablet	Amount added(µg/ml)	% recovery
Lisinopril	20 mg	19.980	3.90	99.48%
			4.947	99.90%
			5.90	100.16%
Hydrochlorothiazide	12.5 mg	12.41	9.858	99.96%
			12.27	98.95%
			14.70	99.80%

*mean (%RSD) of five observations, **mean (%RSD) of three determinations

2.Validation of Methods

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulation at 80%, 100% and 120% levels. The recovery studies were carried out 6 times of each level and the percentage recovery and mean of the percentage recovery were calculated and given in Table 3. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.

The precision of the method was determined by studying repeatability and reproducibility. The area of drug peaks and percentage relative standard deviation were calculated. The results revealed that the developed method was found to be reproducible in nature.

The standard drug solutions in varying concentrations ranging from 80 to 120 % of the targeted level of the assay concentration were examined by the assay procedure. Lisinopril and Hydrochlorothiazide were found to be linear in the range of 80 to 120 µg/ml and 200-300 µg/ml respectively.

The slope, intercept and correlation coefficient values were also calculated. The correlation coefficient of Lisinopril and Hydrochlorothiazide were found to be 0.9999 and 0.9999 respectively. The calibration curves were ploted as peak area 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 o the concentration of the analytes. The range demonstrates that the method is linear outside the limits of expected use. The additional peaks were observed in the chromatogram of the formulation, which may be due to excipients present in the formulation. These peaks do not interfere with the standard peaks, which clearly confirm the assay method was found to be highly specific.

The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the develop methods. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). LOD of Lisinopril and Hydrochlorothiazide were found to be 0.296 µg/ml and 0.42 µg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Lisinopril and Hydrochlorothiazide were found to be 0.893 µg/ml and 1.42 µg/ml respectively.

The system suitability studies were performed for the standard solutions and were presented in Table 2. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the following advantages.

- The standard and sample preparation requires less time.
- No tedious extraction procedure was involved in the analytical process.
- Suitable for the analysis of raw materials. Run time required for recording chromatograms were less than 15 times.

Hence, the chromatographic method developed for Lisinopil and Hydrochlorothiazide were found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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