Research Article

RP – HPLC Method for the Simultaneous Determination of Lisinopril and Hydrochlorothiazide in Pharmaceutical Formulation

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Accepted on: 17-07-2013; **Finalized on:** 31-08-2013.

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 C18 column (LiChrosorb C18,10µ,250mmx 4.6mm). The sample was analyzed using a mobile phase of potassium dihydrogen phosphate buffer solution:acetonitrile (30:70 v/v adjust pH 3.4 with orthophosphoric acid).The flow rate was 1.5 ml/ min with detection at 215 nm and the column was maintained at 40ºC temperature The retention time for Lisinopril Dihydrate and Hydrochlorothiazide was found to be 3.4 and 6.9 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, Method Validation, HPLC Determination.

INTRODUCTION

Lisinopril (LIZ) (Figure 1) is a potent, competitive inhibitor of angiotensin-converting enzyme (ACE), the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin aldosterone system (RAAS). Lisinopril may be used to treat hypertension and symptomatic congestive heart failure, to improve survival in certain individuals following myocardial infarction and to prevent progression of renal disease in hypertensive patients with diabetes mellitus and microalbuminuria or overt nephropathy. Lisinopril is chemically (2S)-1-[(2S)-6-amino-2 {[(1S)-1-carboxy-3-phenylpropyl]amino}hexanoyl] pyrrolidine-2-carboxylic acid[1,2]. Various analytical methods for the estimation of lisinopril in the given dosage form were reported in literature which includes high performance liquid chromatography with UV detection[3], capillary electrophoresis[4] and aqueous and non-aqueous titration[5].

Hydrochlorothiazide (HCT) (Figure 2), 6-chloro-3, 4-dihydro-7-sulfamoyl-2H-1, 2, 4-benzothia-diazine-1,1-dioxide, is a thiazide diuretic[6] (Figure 2). It increases sodium and chloride excretion in distilled convoluted tubule. Many analytical methods were reported for the analysis of HCT alone and combination with other drugs by stability indicating method[7], RP-HPLC methods [8,9] and Spectrophotometric methods[10,11].

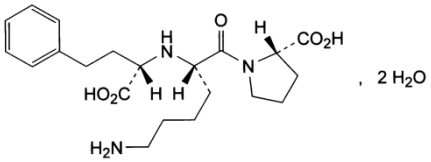

Figure 1: Structure of Lisinopril dihydrate

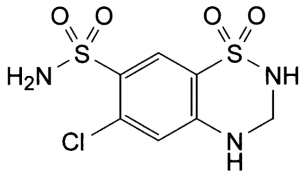

Figure 2: Structure of Hydrochlorothiazide

The two drugs are used in association in the treatment of hypertension. Several methods are available in the literature for the determination of HCT and LIZ. Most of these methods are for the determination of either LIZ or HCT separately. For instance, HCT has been determined in urine samples and tablet formulation by HPLC[12, 13] and by non-aqueous titration[14]. LIZ and HCT were determined simultaneously in pharmaceutical tablets using first-derivative ultraviolet spectrophotometry[15] and by HPLC with either programmable UV-detectors at two different wavelengths or with electrochemical detection[16,17]. This manuscript describes the development and subsequent validation (ICH 1996) of RP-HPLC method for the simultaneous determination of LIZ and HCT in commercial tablets. No interference from excipients of tablet formulation was found. The linearity of response, accuracy and intermediate precision of the described method has been validated. The proposed method was successfully applied for simultaneous determination of LIZ and HCT in combined dosage forms that are available in market.

MATERIALS AND METHODS

Reagents

All chemicals and reagents used were of HPLC-grade. Lisinopril dihydrate and Hydrochlorothiazide reference standards were obtained from Sigma Aldrich. Tablet formulation containing HCT 12.5 mg and LIZ 20 mg were

obtained commercially. HPLC grade Acetonitrile was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Instrumentation

A Shimadzu HPLC system was utilized consisting of the following components: LC – 20 AD pump, vacuum degasser unit DGU – 20 A_5 and a UV/VIS variable detector SPD – 20 A. Separation was carried out on a LiChrosorb C 18 column (250 x 4.6 mm, particle size 10 µm) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC installed properly with the chromatographic software.

Chromatographic Conditions

The mobile phase was 70:30 % v/v mixture of Acetonitrile:Phosphate buffer (136 mg potassium dihydrogen phosphate in 1000 ml water, adjusted with orthophosphoric acid, pH=3.4±0.1). The mobile phase was filtered through 0.45 µm membrane filter and degassed by using sonicator for about 10 min before use. The sample solutions were also filtered using 0.45 µm membrane filters. The mobile phase was delivered isocratically at a flow rate 1.5 ml/min. The column was maintained at 40°C temperature. The injection volume was 20 µl and the total run time was 8 minutes. The detection was carried out at 215 nm.

Preparation of the Standard Solution

Accurately weighed quantity of 40 mg LIS and 25 mg HCT is transferred in 100 ml volumetric flask, dissolved with 50 ml mobile phase and made up with phosphate buffer having the concentration of 400 µg/ml of LIZ and 250 µg/ml of HCT. From the stock solutions further dilutions were prepared by diluting required volume of mobile phase.

Sample preparation

Twenty tablets were accurately weighed (to obtain the average mass of tablets) and finally powdered. Weight equivalent to 40 mg of LIZ and 25 mg of HCT (2 tablets) was taken and transferred into a 200 ml volumetric flask. Approximately 50 ml of diluent (pH 3.4 buffer) were added and the mixture was sonicated for 15 minutes. After that 50 ml mobile phase were added and the mixture was sonicated again for 15 minutes. The mixture was then diluted to volume with phosphate buffer. The solution was then filtered off through a 0.45 µm filter discarding the first few ml of filtrate.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines as follows.

Linearity

The Table 1 presents the equation of the regression line, correlation coefficient (r^2) values of the slope and intercept for each compound between the peak areas and concentrations of 50-400 µg/ml with r^2=0.999, 25-250 µg/ml with r^2=0.995 for LIZ and HCT respectively. For all the two drugs, r^2<1 and the calibration curve equation showed a good linearity curve which means that the linearity test is validated.

Table: 1: Linearity Results, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Compounds	r^2	Calibration curve equation	LOQ ng	LOD ng
Lisinopril	0.999	Y=19595143-35458X	20	10
Hydrochlorothiazide	0.995	Y=4676005-1949X	100	50

Suitability of the method

The specificity of this method was determined by complete separation of LIZ and HCT as shown in Fig. 3. The peaks obtained for LIZ and HCT were sharp and have clear baseline separation. The retention time is good for the drugs separation (3-7 min) and no overlapping between peaks obtained from resolution data which indicate precise system.

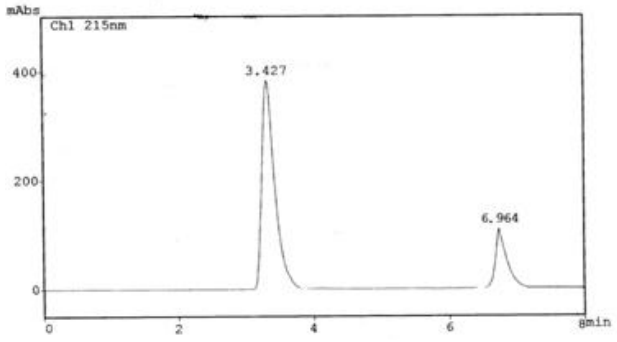

Figure 3: Chromatogram of Lisinopril and Hydrochlorothiazide

Precision

Table 2 shows the results of the test that obtained by running the samples in two days by using different analyst. In second day, the same chromatographic conditions applied and the concentration was 100 %. Assay % and RSD % values obtained are within range 98 %- 102 % (±2), which indicate a valid method. These precision tests were applied for the two drugs and the data observed are gave a precise and valid method of analysis.

Accuracy

The accuracy test was applied in different levels of concentrations for the two drugs in one sample solution with triple injections for each sample (Table: 3-4). The % of recovery equation is:

% Accuracy = [(recovered amount / actual amount) X 100]

The accepted limits of recovery are 98 %-102 % according to USP and all observed data are within the required range that indicates good recovery values.

Table 2: Results of Intermediate Precision

Sample #	Assay %	
	Lisinopril	Hydrochlorothiazide
1	99.15	100.2
2	100.9	100.5
3	99.05	98.88
4	100.1	98.74
5	98.85	100.1
6	100.3	99.46
Average	99.93	99.61
RSD %	0.831	0.852

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 100 µg/mL and 62.5 µg/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.

Table 3: Accuracy of Lisinopril

Parameters	% Taken	Mass taken (mg/1 tabl.)	Mass found (mg/1 tabl.)	% Found	% Recovery
	50.00	10.25	10.23	49.90	99.80
		10.25	10.13	49.41	98.83
		10.25	10.30	50.15	100.3
	100.0	20.50	20.41	99.65	99.65
		20.50	20.55	100.2	100.0
		20.50	20.52	100.1	100.1
	150.0	30.75	30.71	149.8	100.1
		30.75	30.78	150.2	100.1
		30.75	30.62	149.6	99.72
X					99.84
SD					±0.434
% RSD					0.435

Table 4: Accuracy of Hydrochlorothiazide

Parameters	% Taken	Mass taken (mg/1 tabl.)	Concentration found (µg/ml)	% Found	%
	50.00	6.3	6.30	50.00	100.0
			6.25	49.60	99.21
			6.40	50.79	101.6
	100.0	12.7	12.60	99.37	99.37
			12.70	100.0	100.0
			12.78	100.6	100.6
	150.0	19.0	18.93	149.4	99.63
			19.02	150.2	100.0
			19.10	150.8	100.5
X					100.1
SD					±0.683
% RSD					0.684

CONCLUSION

An accurate, sensitive and precise HPLC method with ultra violet detection was developed and fully validated for quality control analysis of LIZ and HCT in their combined tablets. The proposed method is very rapid, where the total analytical run time for both drugs is less than 8 min.

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Source of Support: Nil, **Conflict of Interest:** None.