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RESEARCH ARTICLE

A Sensitive RP-HPLC Method Development and Validation for the Simultaneous Estimation of Losartan Potassium and Hydrochlorothiazide

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ABSTRACT

A simple, accurate, sensitive and validated RP-HPLC method for simultaneous determination of Losartan and hydrochlorothiazide in combined tablet dosage form has been developed. Separation carried out on RP-HPLC system equipped with Inertsil ODS -3V Column (150 × 4.6 mm i.d., 5µm particle size) using mobile phase of Acetonitrile and phosphate buffer adjusted pH to2 with orthophosphoric acid at a flow rate of 1 mL/min in the Gradient program with run time 25 minutes and detection using UV/VIS detector was carried out at 226 nm. Results were linear in the range of 20–150 µg/mL and 5-40 µg/mL for losartan and hydrochlorothiazide respectively. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

KEYWORDS

RP-HPLC, Losartan, Hydrochlorothiazide, Tablet Dosage Form

INTRODUCTION

Losartan potassium, a non-peptide molecule, is chemically described as 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5ylphenyl) benzyl] imidazole-5-methanol monopotassium salt and is an angiotensin II receptor (type AT1) antagonist. Hydrochlorothiazide, a thiazide diuretic, inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter (SLC12A3) in the distal convoluted tubule and chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1$l^{6},2,4-benzothiadiazine-7-sulfonamide. Literature survey reveals High Performance Liquid Chromatographic (HPLC) for determination of losartan and hydrochlorothiazide combination are not official in Pharmacopeias of USP and BP. And their determination is official as single compound in Pharmacopeias.

Various analytical methods have been reported for the assay of Losartan and hydrochlorothiazide alone or in combination with other antihypertensive agents in pharmaceutical formulations.

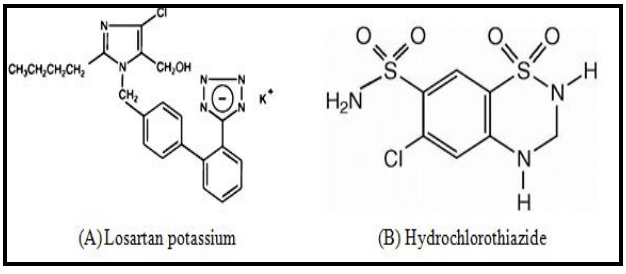

Figure 1: The Chemical Structures of Losartan potassium (A) and Hydrochlorothiazide (B)

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They include UV-VIS spectroscopy[5-13], high performance liquid chromatography[14-26], high performance thin layer chromatography[27] and LC - MS/ MS[28-30].

As on only few methods is available for their simultaneous determination, however, it is essential to develop a suitable analytical method for simultaneous estimation of Losartan potassium and hydrochlorothiazide in bulk and in pharmaceutical preparations, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity.

We have developed a simple, precise, Losartan potassium and hydrochlorothiazide in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC- UV detection method was validated in accordance with International conference on Harmonization (ICH).

MATERIALS AND METHOD

Chemicals and Reagents

Pharmaceutically pure samples of Losartan potassium and hydrochlorothiazide were obtained as a gift samples from Dr. Reddy's, Hyderabad used as such without further purification. A combination of Losartan potassium and hydrochlorothiazide 50/12.5 mg & 100/25 mg in tablet formulations (Hyzaar) was procured from Indian market, HPLC grade methanol, Acetonitrile, water and sodium dihydrogen phosphate dihydrate (AR grade) purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

Instrumentation and Chromatographic Conditions

Analysis was performed with a Waters 2695 separation module equipped with Empower-2 software and loop of injection capacity of 80µL, and waters-PDA detector set at 226 nm. Compounds were separated on a Inertsil ODS - 3V Column (150 × 4.6 mm i.d., 5µm particle size) under reversed phase partition conditions. The mobile phase was an Acetonitrile and pH -2 phosphate buffer (pH 2.0 ±0.05, adjusted with orthophosphoric acid). The flow rate was 1.0ml/min and the run time was 25 minutes with gradient elution. Samples were injected using Rheodyne injector with 10 µL loop and detection was carried out at 226 nm. Before analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.45µPVDF filter.

The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in standard solutions. Chromatography was performed in column temperature maintained at 30±5◦c. The UV spectrum of Losartan potassium and hydrochlorothiazide for selecting the working wavelength of detection was taken using a shimadzu UV-1800, With UV Probe software UV-Visible spectrophotometer (shimadzu, Kyoto, Japan). All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of Analytical Solutions

Preparation of Standard Stock Solutions

Weigh accurately and transfer about 100 mg of losartan potassium working standard, 25 mg of hydrochlorothiazide working standard into a 100 ml volumetric flask. Add about 80 ml of diluent, sonicate to dissolve and dilute to volume with diluent and mix. Dilute 5 ml to 50 ml with diluent and mix. Filter the solution through the 0.45µ PVDF filter.

Calibration Curve of Losartan Potassium and Hydrochlorothiazide

Aliquots 1.0, 2.5, 4.0, 5.0 ml of stock solution of Working standard solution of Losartan and Hydrochlorothiazide were transferred in a series of 50 mL volumetric flasks for 20, 50, 80, 100% levels and Aliquots 3.0 ml of same stock solution were transferred in 25 and 20 ml volumetric flasks for 120 and 150% levels. Finally the volume was made up to the mark with the diluent.

Two replicates per concentration were injected and chromatograms were recorded. The peak area ratios of Losartan potassium and hydrochlorothiazide were calculated and

respective calibration curves were plotted of response against concentration of each drug.

Procedure for Analysis of Tablet Formulation

Accurately transfer ten (for50/12.5 mg) or five (for 100/12.5mg) intact tablets in to a 500 ml volumetric flask.

Add 100ml of diluent and sonicate to disperse the tablets completely. Add about 300 ml of diluent and sonicate for 30 min with intermittent vigorous shanking and stir with the aid of magnetic stirrer for 30 min and dilute to volume with diluent and mix and allow the sample solution to settle down.

Dilute 5 ml of supernatant solution to 50 ml with diluent and mix. Filter the solution through the 0.45N PVDF filter. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solutions were injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

Method Validation

The method was validated for specificity, linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

System Suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. Prepared the mixture solution of 100 ppm for losartan and 25 ppm for hydrochlorothiazide and taken the six chromatogram and observe the Retention time, Resolution, Theoretical Plate, and Asymmetry. %RSD was Calculated.

Linearity

The linearity response was determined by analyzing 6 independent levels of calibration curve in the range of 20-150 µg/ml and 5-40 µg/ml for losartan and hydrochlorothiazide respectively. Plot the calibration curve of Area versus respective concentration and find out correlation co-efficient and regression line equation for losartan and hydrochlorothiazide.

Precision

Intra-day precision

For Intraday precision, it was carried out by preparing 6 replicates injections of 100% concentrations, within the linearity range and measuring the peak area of solution on the same day. % RSD (% relative standard deviation) was calculated.

Inter-day precision

For Inter-day precision, it was carried out by preparing 6 replicates injections of 100% concentrations, within the linearity range and measuring the peak area of solution on different day. % RSD (% relative standard deviation) was calculated.

The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses. The low % RSD values indicated that drugs showed good agreement with the label claim ensures the precision of the method

Accuracy

Recovery was determined by spiking the formulation with standards of each drug equivalent to

25,100 and 150 % of the amount originally present.

Robustness

As defined by ICH, The robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed to injected the standard and samples by small variation in the

chromatographic conditions and found to be unaffected by small variations like ± 0.2 mL/min in flow rate of mobile phase, ± 0.5 variation in pH, different type of filters and ± 5 column temperature variation.

Specificity

Specificity was tested against standard compounds and against potential interferences. Specificity was determined by comparing the responses of standard and sample solution.

RESULTS AND DISCUSSION

Method Development

Several tests were performed in order to get satisfactory separation-resolution Losartan potassium and hydrochlorothiazide in different mobile phases with various ratios of buffers and organic phases by using different columns. The ideal mobile phase was found to be an Acetonitrile and phosphate buffer (pH 2.0 ±0.05, adjusted with ortho phosphoric acid). This mobile phase used under gradient elution gave a very satisfactory and good resolution of Losartan potassium and hydrochlorothiazide.

Increasing or decreasing pH of mobile phase by ± 0.2 did not show significant change in retention time of each analyte. The retention time of Losartan potassium and hydrochlorothiazide on the analytical column was evaluated at a flow rate of 1.0 ml/min. The injection volume was 10 µL.

The retention time of standard and sample for Losartan potassium and hydrochlorothiazide were satisfactory with good resolution. This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column or mobile phase to obtain satisfactory results.

Solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference from solvent and excipients. Finalized chromatographic conditions was mentioned on below Table-1.

Table 1: Finalized chromatographic conditions

Flow rate:1.0 ml/min	**Wave length**:226 nm	**Injection Volume**:10µL
Column temperature: 30±5˚C	**Sample temperature**: Ambient	**Run time**:25 minutes
Gradient program		
Time (in mints)	**Mobile phase-A (%v/v)** (pH 2.0 phosphate buffer)	**Mobile phase-B (%v/v)** (Acetonitrile)
0.0	80	20
10.0	50	50
15.0	30	80
20.0	30	70
21.0	80	20
25.0	80	20

To inject the standards on above finalized chromatographic conditions and their results was mentioned on below Table-2.

Table 2: Results from system suitability study of Losartan and Hydrochlorothiazide

System Suitability Parameters	Results		Acceptance Criteria
	Losartan	Hydrochlorothiazide	
Retention time	10.5	4.5	
%RSD for area of Losartan and Hydrochlorothiazide for five replicate injections of standard solution	0.04	0.08	NMT 2.0
Tailing factor for Losartan and Hydrochlorothiazide peak	1.0	1.1	NMT 2.0
Theoretical plates for Losartan and Hydrochlorothiazide	11875	60751	NLT 2000

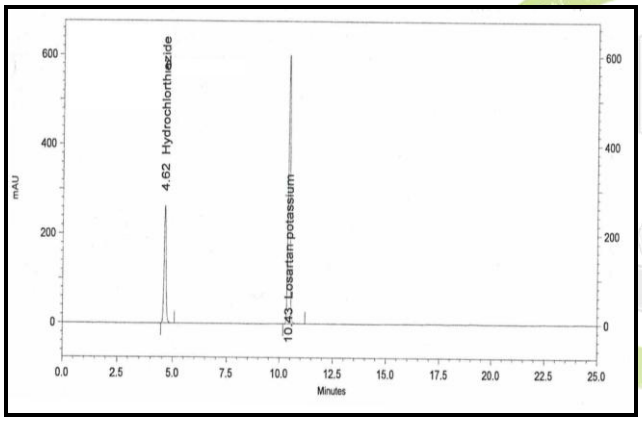

Figure 2: Optimized chromatogram for Losartan (100 ppm) and hydrochlorothiazide (25ppm)

Linearity

Calibration curves for Losartan and Hydrochlorothiazide were plotted separately of response against respective concentration of Losartan and Hydrochlorothiazide. The slope and intercept value for calibration curve were y = 38561.8667x – 10754.4910 (R^2 = 0.9999) for losartan and y = 68224.8965x + 8020.9581 (R^2 = 0.9999) for hydrochlorothiazide, where Y represents the peak area of analyte and X represents analyte concentration. The results are satisfactory, because there is a significant correlation between response factor and concentration of drugs within the concentration range. The calibration curves of losartan and hydrochlorothiazide are given in Figures 3 and 4 respectively.

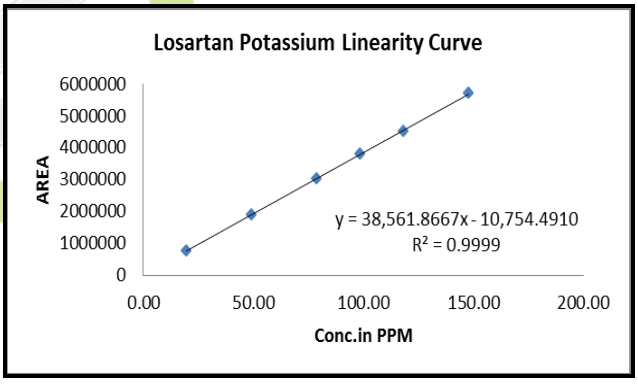

Figure 3: Linearity curve for Losartan

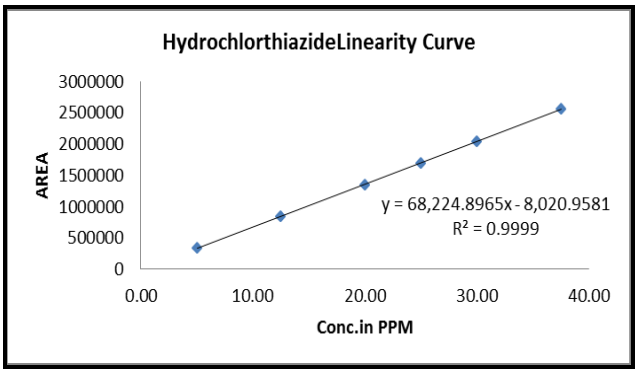

Figure 4: Linearity curve for hydrochlorothiazide

Precision

Intraday and Interday precision was determined by preparing six (n=6) replicate samples and analyzed on same day for intraday and on different days for interday precision. (Table3). For strength 100/25mg of intraday precision %RSD of losartan and hydrochlorothiazide are 0.5, 0.3 and Interday precision %RSD of losartan and hydrochlorothiazide are 0.3, 0.3 respectively. For strength 50/12.5mg of intraday precision %RSD of losartan and hydrochlorothiazide are 0.6, 0.5 and Interday precision %RSD of losartan and hydrochlorothiazide are 0.8, 0.6 respectively and overall %RSD for losartan 0.5, 0.6 and hydrochlorothiazide are 0.4, 0.5 for 100/25 mg and 50/12.5 mg strength respectively (Table 3).

Accuracy

% Recovery was calculated by comparing the area before and after the addition of the working standard. The percentage of individual drugs Found in formulation, mean, standard deviation in formulation were calculated and presented in Table 4**.** The results of the recovery analysis were found to be 99.96 to 100.69 for Losartan and 100.02 to 100.36 for Hydrochlorothiazide, and reported in Table 4. The results of analysis showed that the amounts of drugs found were in good agreement with the label claim of the formulations.

Table 3: Precision studies

S.No	% Assay							
	Losartan				Hydrochlorothiazide			
	Tab-1(n=6)		Tab-2(n=6)		Tab-1(n=6)		Tab-2(n=6)	
	Intraday precision	Interday precision	Intraday precision	Interday precision	Intraday precision	Interday precision	Intraday precision	Interday precision
1	100.4	99.6	101.1	99.8	100.9	101	101.2	101.1
2	100.4	99.4	100	100.1	100.4	100.4	101.4	101.3
3	101.1	100.1	100.7	101	100.5	100.8	101.3	100.9
4	100.2	99.8	99.9	99.5	100	100.6	101.1	100.7
5	100.3	99.9	99.7	100.9	100.5	101.1	100.6	100.2
6	99.5	100.2	99.8	99.1	100	101	100.2	99.8
Mean	100.3	99.8	100.2	100.1	100.4	100.8	101	100.7
%RSD	0.5	0.3	0.6	0.8	0.3	0.3	0.5	0.6
Over all % RSD (n=12)	0.5		0.6		0.4		0.5	

Tab-1 is 100/25 mg and Tab – 2 is 50/12.5 mg of Losartan and hydrochlorothiazide respectively

Table 4: Recovery studies of Losartan and Hydrochlorothiazide

Level of % Recovery	% Mean Recovery*		% R.S.D.*	
	Losartan	Hydrochlorothiazide	Losartan	Hydrochlorothiazide
25	99.96	100.02	0.15	0.03
100	100.21	100.36	0.18	0.07
150	100.69	100.04	0.20	0.08

**Avg. of six determinations for 25& 150, three determinations for 100%, R.S.D. is relative standard deviation*

Table 5: Summary of validation parameters of proposed RP-HPLC method

Parameters	Losartan		Hydrochlorothiazide	
Linearity range (µg/mL)	20-150		5-40	
Correlation co-efficient	0.9999		0.9999	
LOD [a] (µg/mL)	4.26		0.83	
LOQ [b] (µg/mL)	1.40		0.27	
Accuracy (% Recovery)	99.6-100.69		100.02-100.36	
Precision (% RSD)[c]				
Strength	100/25 mg	50/12.5 mg	100/25 mg	50/12.5 mg
Intraday (n[d]= 6)	0.5	0.6	0.3	0.5
Interday (n[d]= 6)	0.3	0.8	0.3	0.6

[a] LOD = Limit of detection. [b]LOQ =Limit of quantitation. [c]RSD = Relative standard deviation.

[d]n = Number of determination

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness

It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Specificity

No interference was detected at the retention times of both Losartan and Hydrochlorothiazide in sample solution.

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Losartan and Hydrochlorothiazide in combined tablet dosage form.

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