Research Article

RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF LOSARTAN AND CHLORTHALIDONE IN PHARMACEUTICAL DOSAGE FORM

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Article Received on: 24/05/15 Revised on: 26/06/15 Approved for publication: 07/07/15

DOI: 10.7897/2230-8407.06793

ABSTRACT

A simple, precise, accurate and reproducible high performance liquid chromatography method was developed for the simultaneous quantitative determination of antihypertensive drugs Losartan and Chlorthalidone from their combined drug product. The separation was achieved by a simple isocratic method using phenomenex C18 column. The mobile phase contains a mixture of acetonitrile and water in the ratio 80:20 percentage v/v at a flow rate of 1.0 ml/minute and the column was maintained at normal temperature. The detector wavelength was 284 nanometer. The retention times of Losartan and Chlorthalidone were 1.72 minutes and 2.64 minutes respectively with a total runtime of 10 minutes. The described method was validated with respect to system suitability, specificity, linearity, precision and accuracy. A low percentage RSD indicated the method was accurate and precise and can be used for the quantification of these drugs in pharmaceutical dosage forms and biological fluids.

Keywords: Losartan, Chlorthalidone, HPLC, Method validation.

INTRODUCTION

The use of analytical sciences in the discovery, development and manufacture of pharmaceuticals is wide-ranging. From the analysis of minute amounts of complex biological materials to the quality control of the final dosage form, the use of analytical technology covers an immense range of techniques and disciplines. Analytical methods are developed according to ICH guidelines. The combined dosage form of any pharmaceuticals is for the synergistic effect or to give longer time effect. Chlorthalidone and Losartan combination is used as antihypertensive to decrease blood pressure. Chlorthalidone is a thiazide-like diuretic known as (*RS*)-2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isindol-1-yl)benzene-1-sulfonamide. Losartan [2-butyl-4-chloro-1-[p-(*o*-1*H*-tetrazol-5-yl-phenyl) benzyl]imidazole-5-methanolmonopotassium salt] was the first nonpeptide imidazole to be introduced as an orally active Angiotensin II antagonist. Literature studies show various HPLC methods reported for the estimation of individual, binary or tertiary combination of antihypertensive drugs or in combination with diuretics. However so far, no method was reported for the simultaneous determination in combination for Losartan and Chlorthalidone and its application to pharmaceutical samples. An attempt was made in this study to develop a rapid, economical, precise and accurate method for simultaneous estimation of Losartan and Chlorthalidone in tablet formulation. The proposed method is rapid, simple, accurate, and reproducible, and can be successfully employed in the routine analysis of both these drugs simultaneously, in tablet dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Drug substances were provided as a gift samples by IPCA, Mumbai, India. All the chemicals and reagents methanol, acetonitrile, isopropylalcohol and water of HPLC grade. The commercially

available drug product was procured from local market which contains Losartan 50mg and Chlorthalidone 6.25mg.

Instrumentation

Chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD – 20A photo diode array detector and injector with 20 µl loop volume. LC solution data station was applied for data collecting and processing (Shimadzu, Japan). A Phenomenex C18 column (250 mm x 4.6 mm, 5µ) was used for the separation.

Method Development

A variety of mobile phases were investigated in the development of a HPLC method for the analysis of Chlorthalidone and Losartan drug substances. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay.

Chromatographic Conditions

The mobile phase containing acetonitrile in water (80:20), found to resolve Losartan and Chlorthalidone. The mobile phase was filtered on a 0.45 micron membrane filter and then ultrasonicated for 30 min. The flow rate was set to 1.0 ml/min. Both the drugs showed good absorbance at 284 nm, which was selected as wavelength for further analysis. All determinations were performed at room temperature. The individual drug substance solution at concentration of 100µg mL⁻¹ in methanol was scanned from 200 nm to 400 nm. UV overlay spectra of the two drugs solutions showed that losartan and chlorthalidone standard solutions absorbed appreciably at 238nm and 284nm.

Preparation of Standard Stock Solution (2mg/ml)

Accurately 100 mg of Chlorthalidone, 100 mg of Losartan standards were weighed and taken into two 50 ml volumetric flask. Dissolved by sonication in sufficient quantity of methanol and then diluted up to the mark.

Preparation of Working Standard Solution (100 microgram/ml)

5 ml each of the above standard stock solution was pipetted out into a 100 ml volumetric flask and made up to mark with methanol to get a concentration of 100µg/ml for both Losartan and chlorthalidone. Working standard solutions of mixtures were prepared by serial dilution of the stock standard solutions (100 µg ml⁻¹) using mobile phase over a concentration range of 10-100 µg ml⁻¹.

Calibration curve

Calibration curve was prepared by taking appropriate aliquots of standard Losartan and Chlorthalidone stock solutions in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 20, 40, 60, 80, 100 µg/ml of Losartan and Chlorthalidone. Standard solutions (n=5) were injected through loop injection system and chromatograms were obtained. The effluent was monitored at 284 nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

Validation of the method

The developed method was validated in terms of linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precision, repeatability of measurement and system suitability parameters.

Assay of market formulation

20 tablets were accurately weighed and crushed into a fine powder. The powder equivalent to 50 mg was taken in 100 ml volumetric flask. About 50 ml methanol was added and then sonicated for 8 mins. Then the volume was finally made up to the mark (100ml) with methanol. The solution was filtered through 0.45 micron whatmann filter paper. Then 0.6 ml of solution was further diluted to 10ml with mobile phase to get final sample concentration. Standard and sample solutions were injected to get the chromatograms.

RESULT AND DISCUSSION

To develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of Losartan and Chlorthalidone, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. (Figure 1 & 2)

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Losartan and Chlorthalidone were found to be 20-100 µg/ml for both. The regression equation for Losartan and Chlorthalidone were found to be $y=30882x+6280$ and $y=25948x-21883$ with coefficient of correlation, (r) 0.9999 and 0.9999, respectively. (Table 1 & Figure 3 & 4)

Precision

Precision was evaluated by carrying out five independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table 2 - 5.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120% respectively. Known amounts of standard Losartan and Chlorthalidone were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table 6.

Specificity

The peak purity of Losartan and Chlorthalidone were assessed by comparing the retention time of standard Losartan and Chlorthalidone. Good correlation was obtained between the retention time of standard and sample of Losartan and Chlorthalidone.

System Suitability parameters

To ensure the validity of the analytical procedure, a system suitability test was established. System suitability parameters for the developed HPLC method were determined by injecting six replicates of the standard solution (40µg/ml). Parameters such as a number of theoretical plates (N), tailing factor and retention time were calculated. (Table 7)

Robustness of method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, wavelength and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes in wavelength, flow rate and mobile phase. (Table 8)

The results indicates that the method developed is robust.

LOQ and LOD

Limit of detection (LOD) is the minimum amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the minimum amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy. LOQ and LOD were determined using the following equation. $LOQ=10s/m$, $LOD=3.3s/m$ where s is the standard deviation of the response and m is the slope of the related calibration curve.

The limit of detection by the equation of Losartan and Chlorthalidone was found to be 1.97µg/ml and 1.67µg/ml respectively.

The limit of quantification by the equation of Losartan and Chlorthalidone was found to be 5.63µg/ml and 5.12µg/ml respectively.

Assay of market formulation

The results obtained by the assay of marketed formulation are summarized in Table 9, Figure 5.

Table 1: Linearity studies of Losartan and Chlorthalidone

Concentration (µg/ml)	Average Area *(n=5)	
	Losartan	Chlorthalidone
20	654868	533257
40	1324621	1104116
60	1817979	1484506
80	2454717	2013955
100	3129119	2601391

Table 2: Intraday precision studies of Losartan

Standard concentration (µg/ml)	Peak areas of Losartan (n=5)		
	Morning	Afternoon	Evening
20	654868	654879	654845
	654674	654746	654667
	654956	654701	654946
	654732	654849	654743
	654888	654988	654889
MEAN	654823.6	654832.6	654818
STD DEV	116.6396	113.3459	112.45
% RSD	0.0178	0.0173	0.0171

Table 3: Intraday precision studies of Chlorthalidone

Standard concentration (µg/ml)	Peak areas of Chlorthalidone (n=5)		
	Morning	Afternoon	Evening
20	508325	508355	508376
	508465	508413	508474
	508276	508289	508388
	508398	508333	508332
	508345	508466	508334
MEAN	508361.8	508371.2	508384.6
STD DEV	72.3857	69.3051	62.6522
% RSD	0.0142	0.0136	0.0123

Table 4: Interday precision studies of Losartan

Standard concentration (µg/ml)	Peak areas of Losartan (n=5)		
	Day 1	Day 2	Day 3
40mg	1245744	1245752	1245767
	1245656	1245681	1245698
	1245721	1245733	1245759
	1245693	1245696	1245701
	1245667	1245657	1245677
MEAN	1245696	1245703	1245720
STD DEV	36.67015	38.55775	40.07244
% RSD	0.0029	0.003095	0.003217

Table 5: Interday precision study of Chlorthalidone

Standard concentration (µg/ml)	Peak areas of Chlorthalidone (n=5)		
	Day 1	Day 2	Day 3
40mg	987643	987649	987667
	987635	987647	987655
	987663	987687	987698
	987655	987654	987645
	987625	987645	987654
MEAN	987644.2	987656.4	987663.8
STD DEV	15.20526	17.42985	20.65672
% RSD	0.001539	0.001764	0.00209

Table 6: Recovery studies

Drug	Amount taken	Recovery Level in Percentage	Amount of Drug Added	Amt. Of Drug µg/ml Mean (n=3)	% Recovery
LOS	20	80	36	36.15	100.41
		100	40	39.96	99.90
		120	44	43.89	99.75
CHLOR	20	80	36	35.81	99.47
		100	40	40.09	100.22
		120	44	43.94	99.86

Table 7: System suitability

Parameters	Losartan	Chlorthalidone
Retention time(min) [t _R]	1.7262	2.6422
Tailing factor	0.0579	0.0378
Theoretical plates [N]	525.32	2787.84
Calibration range(µg/ml)	20-100	20-100
Asymmetric factor	0.75	0.85

Table 8: Robustness studies of Losartan and Chlorthalidone

Parameter (n=5)	Condition	Level	Retention time		Tailing Factor	
			LOS	CHL	LOS	CHL
Flow rate(ml/min)	0.8	-1	1.77	2.70	0.063	0.045
	1	0	1.72	2.64	0.057	0.037
	1.2	1	1.68	2.57	0.069	0.052
	Mean(n=3)		1.723	2.636	0.063	0.0446
Detection wavelength(nm)	275	-1	1.67	3.71	0.056	0.046
	284	0	1.72	2.64	0.048	0.037
	295	1	1.69	3.38	0.061	0.048
	Mean(n=3)		1.693	3.243	0.055	0.043
Mobile phase composition(v/v)	6:4	-1	2.20	3.56	0.071	0.057
	8:2	0	1.72	2.64	0.057	0.037
	9:1	1	1.58	2.29	0.063	0.047
	Mean(n=3)		1.83	2.83	0.063	0.047

Table 9: Assay of market formulation

Formulation (n=5)	Labelled claim (mg)		Amount found (mg)		Assay (%)	
	Losartan	Chlorthalidone	Losartan	Chlorthalidone	Losartan	Chlorthalidone
Formulation	50	6.25	49.78	6.21	99.56	99.36

Figure 1: Chromatogram of Chlorthalidone Standard

Figure 2: Chromatogram of Losartan Standard

Figure 3: Calibration curve of Chlorthalidone

Figure 4: Calibration curve of Losartan

Figure 5: Chromatogram of Dosage form

CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of Losartan and Chlorthalidone in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than one, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of Losartan and Chlorthalidone in multicomponent pharmaceutical preparation.

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Cite this article as:

Aneesh T.P, Renju Radhakrishnan, Aravind P.M, Anuja Sasidharan, Manisha Choyal. RP-HPLC method for simultaneous determination of losartan, and chlorthalidone in pharmaceutical dosage form. *Int. Res. J. Pharm.* 2015; 6(7):453-457 <http://dx.doi.org/10.7897/2230-8407.06793>

Source of support: Nil, Conflict of interest: None Declared

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