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SIMULTANEOUS ESTIMATION OF LOSARTAN AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

The aim of this study was to develop and validate an assay method for simultaneous determination of Losartan and Hydrochlorothiazide in pharmaceutical formulations. A RP-HPLC procedure was developed, using Aglilent C-18 column (150 mm × 4.6 mm, 4 µm). The mobile phase was composed of 0.1% OPA [pH 3.5]: ACN: Methanol (65:30:5 % v/v), with a flow rate of 0.8 mL min−1. Both the drugs were separated in less than 5 min. The method was validated according to International Conference on Harmonization (ICH). The method showed linearity in a concentration range of 10–50 µg/mL for Losartan (r = 0.9991), 5–25.00 µg/mL for Hydrochlorothiazide (r = 0.9996. The method also showed repeatability and precision. The % RSD for average intra-day precisions were 0.086 and 0.564 for losartan and Hydrochlorothiazide. Similarly, the inter-day precisions were 0.082799for losartan, and 0.454275for Hydrochlorothiazide. The method accuracy was also tested and validated — in this case, the average recovery values were 100.55 for losartan, and 99.98for Hydrochlorothiazide. Finally, the method was successfully applied in the simultaneous determination of losartan and hydrochlorothiazide commercial pharmaceutical formulations.

Keywords: HPLC–UV, antihypertensive, simultaneous determination, quantitative and validation.

INTRODUCTION

Hypertension remains one of the most important risk factors for cardiovascular diseases and if neglected can lead to cardiac stroke, coronary thrombosis and kidney failure[1]. Over 1.4 billion people worldwide have high blood pressure, but just 14% have it under control [WHO, 2021] and treatment of hypertension remains challenging. WHO initiated with two drugs for effective results as combined-dose formulations increased antihypertensive efficacy, act by different mechanisms, and reduction of adverse effects due to the use of lower doses, lower cost, and improvement of treatment adherence[2,3].

Losartan (LOS) (Fig. 1) is an angiotensin II receptor antagonist and has a smooth-muscle relaxing action. It has a satisfactory hypotensive effect and is indicated mainly for young person's suffering with hypertension, diabetics, or patients with heart failure[4,5]

Hydrochlorothiazide (HCZ) (Fig. 2), is the potent diuretic, effectively reduces blood pressure, by decreasing the amount of fluid flowing through veins and arteries[6].

However, several methods have been described for the determination of losartan potassium and Hydrochlorothiazide drug substance in tablets. Various methods developed on RP- HPLC[7-16].

Though several methods are available the present study was focused to develop and validate simple, precise and cost effective RP-HPLC method.

CHEMICALS AND REAGENTS

API's, losartan from Atom Laboratories, Gujarath, India. HCZ from Hetero Labs Hyderabad, India. Potassium dihydrogen phosphate, Orthophosphoric acid AR grade purchased fromS. D. Fine chemicals (Mumbai). HPLC solvents like, Acetonitrile, and Methanol from Merck, Mumbai, Water from Millipore. The pharmaceutical dosage form containing 12.5 mg HCZand 25mg LOS, Losanorm H (Ipca Laboratories Ltd.) purchased from a local drug store.

INSTRUMENTATION

The development and validation of the assay was performed on a HPLC (Infinity II 1260, Agilent, US) - 1260 VWD UV–VIS detectorprovided with high speed auto sampler, column oven, degasser and UV detector to provide a compact and convenient for LC with , EZ Chrome software (version 4.07) for chromatographic analysis using Agilent C18(4.6 X 150mm,4µ)column. The flow rate was 0.8 mL/ minand the injection volume 10 µl, UV detection was performed at 229 nm. Peak identity was confirmed by both retention time comparison and comparison spectra obtained from the UV detector.

METHODOLOGY

Preparation of standard solutions:

10 mg of LOS and 5mg of HCZ were weighed accurately and transferred in to a 10ml volumetric flask. The drug was dissolved in sufficient quantity of mobile phase (diluent) and the volume was made up to the mark with the same solvent. The stock solution was suitably diluted to get a concentration of 100 µg/ mL and 50 µg/ mL of LOS and HCZ.

Preparation of working standards

From the above prepared stock solutions 10-50 µg/mL concentrations was prepared for LOS and 5-25 µg/mL concentrations for HCZ. The working standards were prepared by using mobile phase.

Preparation of sample solution

Losanorm H tablets [25 mg/12.5 mg]

Twenty tablets were weighed and crushed to a fine powder; a known quantity of placebo powder was added. A quantity equivalent to 25 mg of LOS and 12.5mg of HCZ were weighed and transferred to 100ml standard flask. The powder was dissolved by sonication using sufficient amount of selected diluent and then made up to the mark with the same. The solution was filtered using 0.22µ disposable membrane filter and the stock solution was suitably diluted.

VALIDATION OF THE METHOD[17-18]

The developed method was validated for as per ICH Q2 (R1) guidelines[21] for various parameters such as accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ).

Specificity

The specificity of the method was evaluated by assessing whether excipients present in the pharmaceutical formulations interfered with the analysis. The results obtained with the pure drug were compared to those obtained with the sample solutions. The excipients used in the tablet did not interfere with the method.

Linearity and range

The five series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

Accuracy

The accuracy of the method was determined by recovery experiments using the standard addition method. Each solution was injected in triplicate and percentage recovery was calculated.

Precision

Themethod was assessed by studying intra-day and inter-day variation. In the intra-day studies, standard and sample solutions were analyzed for six times on the same day and percentage RSD was calculated. In the inter-day studies, sample solutions were analysed in triplicate on six consecutive days and percentage RSD were calculated.

LOD and LOQ

Accordance with ICH recommendations, the approach based on the standard deviation of the response and the slope of the calibration plots was used to determine detection and quantification limits. LOD and LOQ values were estimated as [(standard deviation of repeatability)/(Slope of the regression equation)] by multiplying with 3.3 and 10 respectively.

Robustness

For demonstrating the robustness of the method, slight variations in the optimized conditions were done and the standard solution was injected. The variations made were ±2% in the ratio of acetonitrile in the

mobile phase, ±0.2 unit in the pH of the buffer, ±0.1 mL/min in the flow rateand ±1 nm in the wavelength. The peak area from the observed results, mean, SD and %RSD were calculated.

System suitability parameters

The standard solution containing 30 and 15 mcg/mL (LOS and HCZ) was injected six times and analysed. The number of theoretical plates, peak area, and tailing were observed. From the observed results, mean, SD and %RSD were computed.

RESULTS AND DISCUSSION

Optimization of method

Selection of the HPLC method depends on the nature of the sample like its molecular weight, and solubility and pka. The drugs selected for the current study are polar in nature; hence, Reversedphase -HPLC method was selected for its separation.

Optimization of the chromatographic condition

Method optimization for the simultaneous estimation of the combination of LOS and HCZ was carried out by performing several experiments with different columns, varying organic phase ratio in mobile phase, pH and detector wavelength. It was found that the peak shape, retention time, tailing factor, and column efficiency were good with AgilentC18 column (150 mm, 4.6 mm, 4 mm), mobile phase 0.1% OPA [pH 3.5]: ACN: Methanol (65:30:5 % v/v), with a flow rate of 0.8 mL/min at 229nm as detection wavelength using UV detector at 25^{O}C and 10µl injection volume.

The specificity of the method is illustrated in (Fig. 3 & 4), which indicates separation of the compounds was complete. Average retention times ± standard deviation for LOS and HCZ were 2.156±0.001 and4.272 ±0.002 respectively, for six replicate analyses. In determination of accuracy and precision, recovery was 100 ± 2%, which indicates the method is accurate, and intra-day and inter-day variation, as RSD, were no more than 1.0%, indicating the method is precise. In determination of the robustness of the method, slight variation of mobile phase pH, amount of buffer, flow rate, and detector wavelength had no significant effect on chromatographic resolution.

Method validation

Specificity

The specificity of the RP-HPLC method was determined by the complete separation of HCZ, and LOS as show in (Fig. 3 & 4) with parameters like peak area. The peaks obtained for HCZ, and LOS were sharp and have a clear baseline separation.

Linearity

The calibration curves were prepared by plotting the peak areas of the drug to IS which were linear in the range of 10–50 and 5–25 µg/mL LOS and HCZ. Peak area ratios and concentrations were subjected to least square linear regression analysis to calculate the calibration equations and correlation coefficients. The mean regression equations were found as $y = 91628x + 17243$($R^2 = 0.9991$, $n = 6$), and $y = 100499x + 47178$ ($R^2 = 0.9996$) for LOS and HCT, respectively. The result shows that there is an excellent correlation coefficient (Table 1, Fig 5&6).

LOD and LOQ

The LOD was 0.0022 µg mL^{-1} for HCZ, 0.0011 µg/mL for LOS at a signal to noise ratio of 3.3:1. The limit of quantification was determined as 0.0067 HCZ and µg/mL for 0.0032 LOS at a signal to noise ratio of 10:1.

Precision

Intra-day precision was performed by relative standard deviation of six repeated assays of samples. Inter-day precision was determined by analyzingthe same set of samples of six different days. The % RSD values were found to be 0.0828 and 0.454 for LOS and HCZ for inter day , and 0.086 and 0.564 % for LOS and HCZ for interday precision respectively, indicating good precision (Table 2).

Recovery

To examine the accuracy of the method, recovery studies were carried out by standard addition method. The percent recovery of the added standard to the assay samples was calculated from:

Recovery%=[(C1−Cu)/Ca]×100

Were C_1 is the total concentration of analyte found; C_u is the concentration analyte present in the formulation; and C_a is the concentration added to the formulation. The average percent recoveries obtained as 100.55 and 99.95% (LOS and HCZ) indicate good accuracy of the method (Table 3)

Robustness

To ensure the robustness of the HPLC method deliberate changes were made like slight variation of mobile phase pH, flow rate, and detector wavelength had no significant effect on chromatographic conditions and the % RSD was found to be not more than 1%.

minor changes in the experimental conditions it is important to demonstrate robustness of the method. None of the modifications caused a significant change in the resolution between the drugs and IS, peak area RSD, USP tailing factor, theoretical plates (Table 5).

System suitability

The RSD values of peak area and retention time and theoretical plates for drugs are within 2% indicating the suitability of the system (Table 4).

CONCLUSION

A simple, rapid, and reliable RP-HPLC method has been established for simultaneous determination of HCTZ and LOSin bulk and dosage form. The method has several advantages, including rapid analysis, simple mobile phase, less Rtand improved sensitivity in contrast with previous methods. This makes the method suitable for routine analysis in quality-control laboratories.

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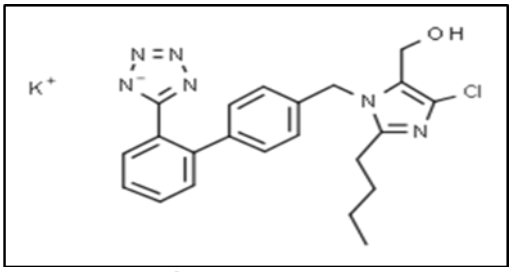

Fig. 1: Losartan

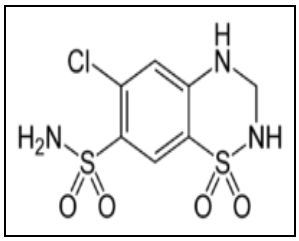

Fig. 2: Hydrochlorothiazide

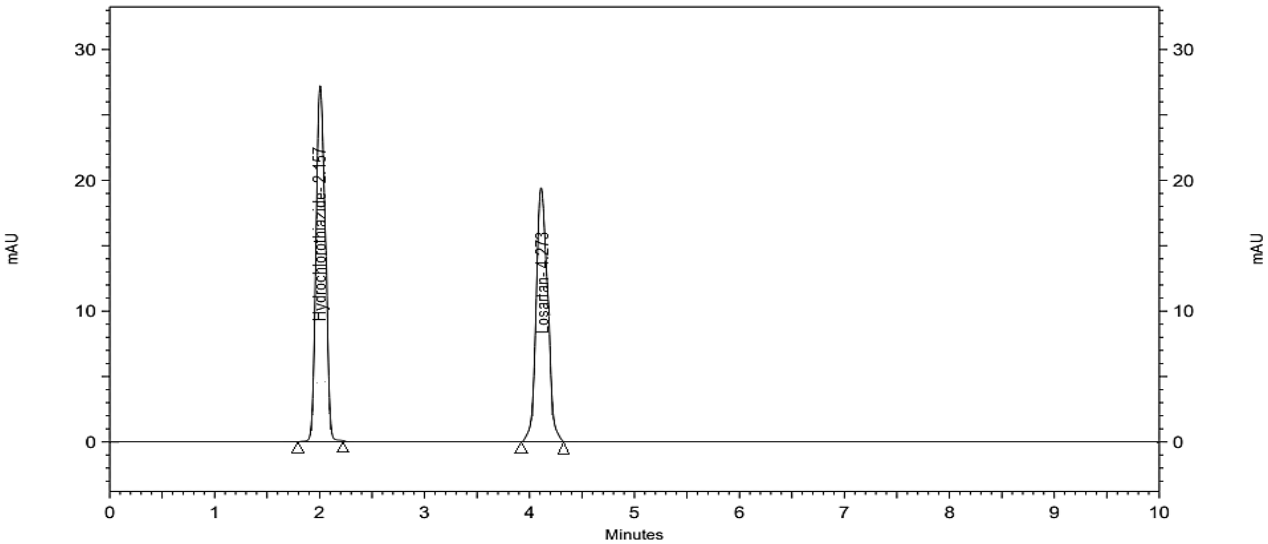

Fig. 3: HPLC Chromatogram of Pure drug

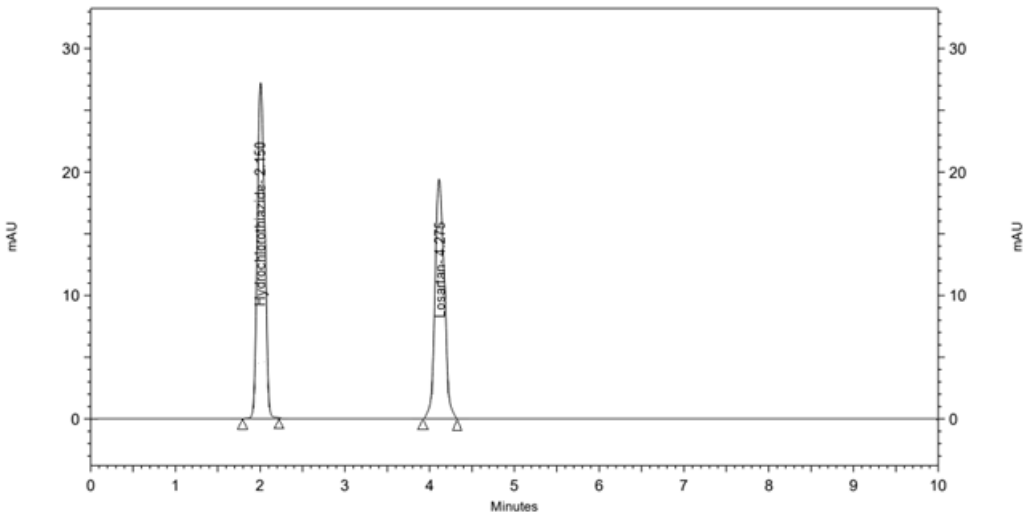

Fig. 4: HPLC Chromatogram of tablet formulation

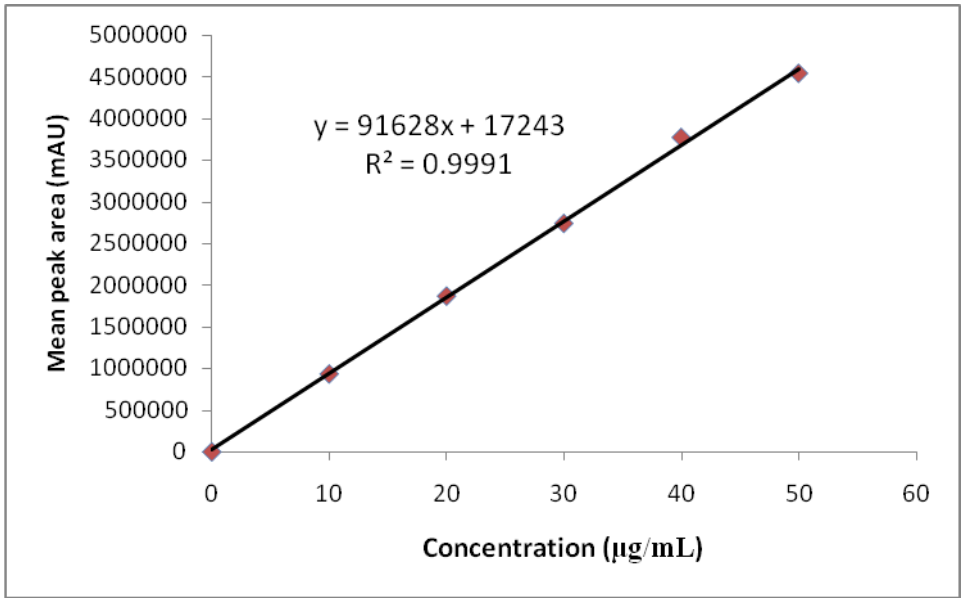

Fig. 5: Linearity plot of Losartan

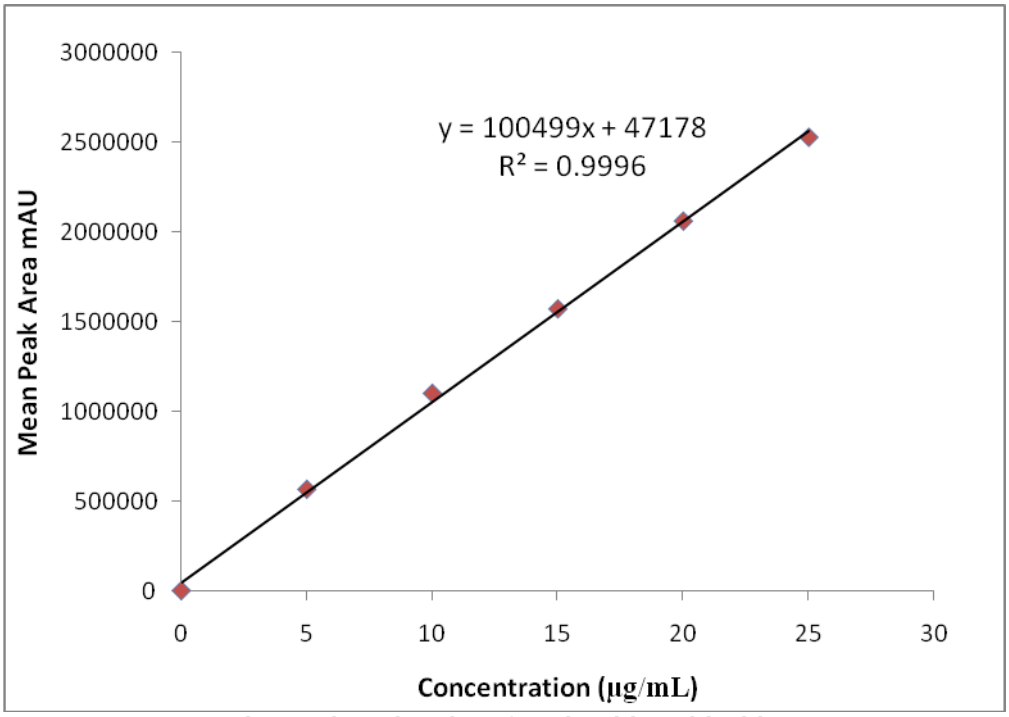

Fig. 6: Linearity plot of Hydrochlorothiazide

Table 1: Linearity data of LOS and HCZ

Losartan		Hydrochlorothiazide	
Concentration (µg/mL)	**Mean Peak area *($\bar{x}$±sd)**	**Concentration (µg/mL)**	**Mean Peak area *($\bar{x}$±sd)**
0	0	**0**	0
10	933739 ± 111.85	**5**	564833 ±19.35
20	1867548± 95.68	**10**	1099585±264.35
30	2738414± 169.02	**15**	1570241±84.79
40	3769843± 102.80	**20**	2059392±123.41
50	4538136± 39.71	**25**	2526577±73.85
Slope (B)	91628± 1.51	**Slope (B)**	100498± 5.66
Intercept (A)	17242.81± 29.56	**Intercept (A)**	47208.21± 67.55
Regression coefficient	**0.999**	**Regression coefficient**	**0.999**

*Mean of three replicates

Table 2:Intra-day and inter-day precision data of LOS and HCZ

Chromatogram Runs	Intra day Precision (30/15µg/mL)		Inter day Precision (30/15µg/mL)	
	Sample concentration of LOS	**Sample concentration of HCZ**	**Sample concentration of LOS**	**Sample concentration of HCZ**
1	30.01	14.95	29.98	14.85
2	29.98	15.02	29.99	14.91
3	30.02	14.97	29.97	14.92
4	29.97	14.99	29.98	14.88
5	29.99	14.78	30.04	15.03
6	29.95	14.95	29.99	14.99
Mean	29.98667	14.94333	29.99167	14.93
S.D	0.02582	0.084301	0.024833	0.067823
% R.S.D	**0.086105**	**0.564138**	**0.082799**	**0.454275**

Table 3: Results of recovery studies by standard addition method

Drug	Claimed amount (≅µg/ml)	Level of addition (%)	Amount of standard added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average % Recovery
LOS	30	50	15	45.48	101.06	100.5578
	30	100	30	59.99	99.98	
	30	150	45	75.53	100.70	
HCZ	15	50	7.5	22.48	99.91	99.98593
	15	100	15	29.99	99.96	
	15	150	22.5	37.53	100.08	

Table 4: Results of system suitability study

Injection	Losartan (LOS)			Hydrochlorothiazide (HCZ)		
	TP	PA	Tailing	TP	PA	Tailing
Mean	4593.5	2738069.6	1.1022	3974.5	1570276	1.1700
SD	33.34	1121.97	0.00014	37.12	37.74	0.00013
%RSD	**0.726**	**0.041**	**0.0116**	**0.934**	**0.002**	**0.009**

Table 5: Results of Robustness study

Drug	W.L (nm±1nm)	Avg. (n=3)	SD	%RSD	pH (±0.1)	Avg. (n=3)	SD	%RSD	Flow rate (mL/min)	Avg.(n=3)	SD	%RSD
Losartan (30µg/mL)	235	2738217	18.14754	0.00066	3.4	2738234	13.37909	0.00049	0.7	2738227	7.76	0.00028
	235				3.4				0.7			
	235				3.4				0.7			
	239	2738197	13.52775	0.00050	3.6	2738222	10.01665	0.00036	0.9	2738238	7	0.00025
	239				3.6				0.9			
	239				3.6				0.9			
Hydrochlorothiazide (15µg/mL)	235	1570148	9.165151	0.00058	3.4	1570155	7.549834	0.00048	0.7	1570166	11.06	0.00070
	235				3.4				0.7			
	235				3.4				0.7			
	239	1570163	5.859465	0.00037	3.6	1570148	21.1266	0.00134	0.9	1570146	7.02	0.00044
	239				3.6				0.9			
	239				3.6				0.9			

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