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Analytical Method Development and Validation of Losartan Potassium and Hydrochlorothiazide in Combined Dosage Form by RP-HPLC

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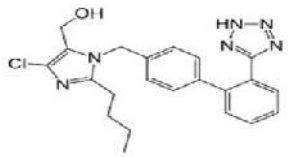
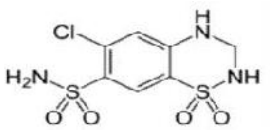
Abstract: A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Losartan potassium and Hydrochlorothiazide in combination. The separation was carried out using a mobile phase of Buffer and Acetonitrile are taken in 65:35%v/v (adjust pH 3.0 with orthophosphric acid). The column used was Reverse phase C18 column (Agilent XDB C18, 150 x 4.6 mm, 5µ) with flow rate of 1.0 ml min using PDA detection at 254 nm. The described method was linear over a concentration range of 25-75 µg/ml and 6.25-18.75 µg/ml for the assay of losartan potassium and hydrochlorothiazide respectively.The retention times of losartan potassium and hydrochlorothiazide were found to be 4.901 and 2.176 min respectively. Results of analysis were validated statistically and by recovery studies. The limit of quantification (LOQ) for losartan potassium and hydrochlorothiazide were found to be 5.393 and 1.138 µg/ml respectively and the limit of detection (LOD) values were found to be 1.779 ug/ml and 0.375 ug/ml respectively for losaratan potassium & hydrochlorthiazide.Results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of losartan potassium and hydrochlorothiazide bulk drug and in its pharmaceutical dosage form.
Keywords: Losartan potassium, Hydrochlorothiazide, Reverse phase HPLC & validation.

Introduction

Losartan potassium 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 (2-butyl-4-chloro-1-{[2'- (1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*- Imidazol-5-yl)methanol and It empirical formula is $C_{22}H_{22}ClKN_6O$ and its structural formula is-



Hydrochlorothiazide 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 believed to lower the peripheral vascular resitance. It empirical formula is $C_7H_8ClN_3O_4S_2$



Chemical stucture of Hydrochlorthiazide

Literature survey reveals the availability of several methods for estimation of both Losartan potassium and Hydrochlorothiazide includes Spectrophotometric[5], HPTLC[4,7] determination, HPLC in single dosage form or combination with other drugs[1-3,]. No method has been reported for the estimation of Losartan potassium and Hydrochlorothiazide in combined dosage form. Present work emphasizes on the quantitative estimation Losartan potassium and Hydrochlorothiazide in their combined dosage form by RP-HPLC.

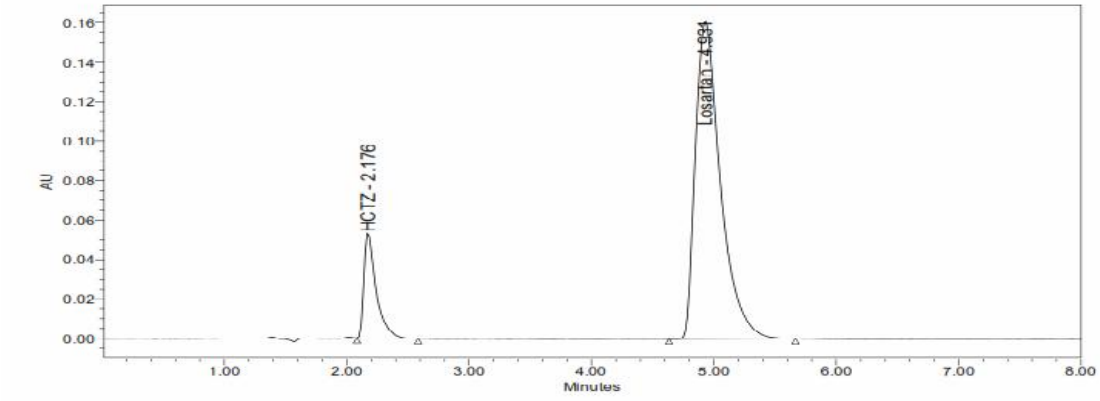


Fig 1: Standard chromatogram (Retention time versus peak height)

Preparation of Solutions:

Standard Preparation:

Acurately Weighed and transferred 50mg of Losartan potassium and 12.5mg of Hydrochlorothiazide working Standards into a 25 ml clean dry volumetric flask, add 15ml of diluent, sonicated for 5 minutes and make up to the final volume with diluent (standard stock).

Sample Preparation:

5 Tablets powder, was transferred into a 500 mL volumetric flask, 300mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1 Filtered through 0.45µ filter ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method validation[6]

The proposed method was validated as per ICH guidelines. The drug solutions were prepared as per the earlier adopted procedure given in the experiment.

1. System precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Mixed standard solutions of losartan potassium (50µg/ml) and hydrochlorothiazide (12.5µg/ml) were prepared as per test method and injected for 6 times. Results are shown in Table 1.

Table 1: System precision study

Injection	Area of HCT	Area of LOS
1	462535	2858879
2	462220	2932002
3	463198	2857237
4	475141	2860680
5	464407	2869028
6	463980	2869497
Mean±SD.	465247	2874554
Std.dev	4917.5	28616.3
% RSD	1.1	1.0

2. Method precision

Six samples were prepared and analyzed as per the test method and calculated the % RSD for Assay of six preparations. Results are shown in Table 2.

Table 2: Method precision study

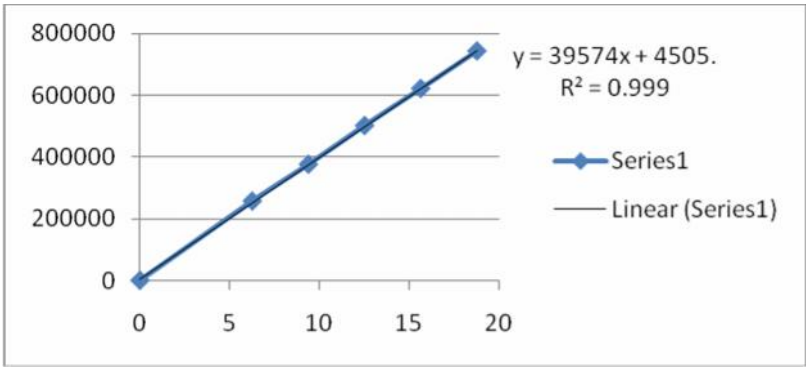
Sample no.	Area		% Assay	
	HCT	LOS	HCT	LOS
1	465373	2835455	99.74	99.22
2	462290	2846656	99.74	99.61
3	463007	2831896	100.09	99.09
4	464226	2859022	99.71	100.04
5	463956	2849123	100.08	99.69
6	464640	2843289	100.23	99.49
Mean±S.D.	463415 965.3	2844240 9784.1	99.96 ±0.2082	99.52 ±0.3424
% RSD	0.2	0.3	0.21	0.34

3. Linearity study

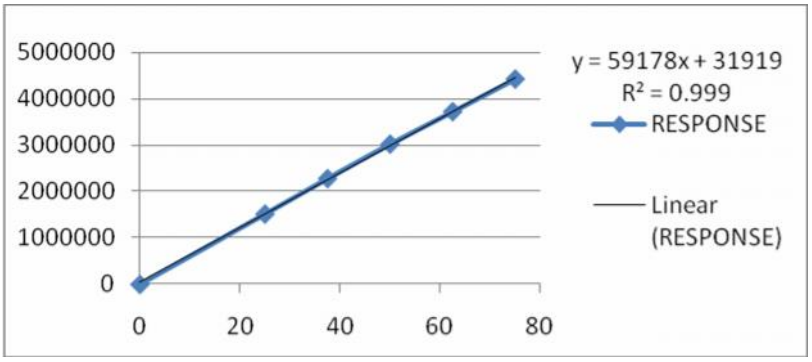
From stock solution aliquots of 0.125, 0.187. 0.25 0.31, 0.375 were taken in 100ml volumetric flasks and diluted upto the mark with mobile phase such that the final concentration of Losartan potassium in the range of 25 to 75 µg/ml and hydrochlorothiazide in the range of 6.25 to 18.75 µg/ml. Volume of 8 µl of each sample was injected in for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration. Results are shown in Table 3.

Table 3: Linearity study

Spike level %	Concentration(ug/ml)		Area	
	HCT	LOS	HCT	LOS
50	6.25	25	256901	1517965
75	9.375	37.5	376327	2283106
100	12.5	50	501859	3027038
125	15.625	62.5	621859	3727038
150	18.75	75	743470	4430758
Slope			39574	59178
Intercept			4505	31919
Correlation coefficient			0.999	0.999



HPLC Calibration curve for hydrochlorothiazide



HPLC Calibration curve for Losartan potassium

4. Ruggedness

It was done by different analyst by using different column, different system on different day. The system suitability criteriawas evaluated and the overall % RSD for % assay of experiment results and method precision results was also calculated.

5. Accuracy as Recovery[10]

It was done by recovery study. Sample solutions were prepared by spiking at about 50 %,100% and 150 % of specification limit to Placebo and analyzed by the proposed HPLC method. Results are shown in Table 4 and 5.

Table 4: Recovery study of hydrochlorothiazide

Spike level %	%Recovery	Mean±SD	% RSD
50	100.23	100.84±0.546	0.54
50	101.29		
50	100.99		
100	99.69	100.18±0.452	0.45
100	100.58		
100	100.27		
150	99.16	99.27±0247	0.25
150	99.10		
150	99.56		

Table 5: Recovery study of Losartan potassium

Spike level %	% Recovery	Mean±SD	% RSD
50	99.23	99.38±0.1386	0.14
50	99.41		
50	99.50		
100	99.59	99.81±0.3180	0.32
100	100.17		
100	99.66		
150	99.47	99.57±0.579	0.58
150	99.60		
150	100.53		

6. Range

Range to be inferred from the data of linearity, recovery and precision experiments.

7. Specificity

The analytes should have no interference from other extraneous components and be well resolved from them.Specificity is the procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. There was no other interfering peak around the retention time of Losartan Potassium and Hydrochlorothiazide also the baseline did not show any significant noise.

8. Robustness[9]

The Robustness of the method was evaluated by changing the column oven temperature by ± 5°C, by changing the organic content of mobile phase by 2% absolute, System suitability was done for each condition. Results are shown in Table 6 and 7.

Table 6: Robustness data for HCT (for HPLC method)

S.	I	II	III	IV	V
1	99.74	104.3	103.8	103.2	99.0
2	99.74	100.08	102	104.1	99.69
3	100.1	-	-	-	-
4	99.71	-	-	-	-
5	100.18	-	-	-	-
6	99.96	-	-	-	-
Over all		102.8	102.9	99.38	99.38
Over all SD		11584.2	6013	2017.9	2017.9
Over all %RSD		2.4	1.3	0.9	0.4

Table 7: Robustness data for LOS (for HPLC method)

S.	I	II	III	IV	V
1	99.22	107.3	105.8	105.9	101.1
2	99.61	103.2	101.8	106.1	101.7
3	99.09	-	-	-	-
4	100.09	-	-	-	-
5	99.09	-	-	-	-
6	99.49	-	-	-	-
Over all		105.3	104.8	106	101.4
Over all SD		82089.0	38574.4	3959	13146.2
Over all %RSD		2.7	1.3	0.1	0.5

9 . Forced degradation[8,9]

A sample was stressed at the following conditions and the peak purity was evaluated for losartan potassium and hydrochlorothiazide peak. Degradation by 1 N hydrochloric acid, degradation by 1N sodium hydroxide, degradation by 3 % w/v solution of hydrogen peroxide, degradation by thermal energy at 105ºc for 12 hours, degradation by exposing UV light for about 7 days cycle. Results are shown in Table 8 and 9.system suitability parameter parameters are mentioned table 10.

Table 8: Forced degradation for Hydrochlorthiazide

Condition	% Assay of HCT	% Degradation	Peak Purity (Match Factor)
Acid degradation	96.45	4.14	996.51066
Base degradation	95	3.55	998.97963
Oxidative degradation	94.19	6.33	995.68541
Thermal Degradation	93.6	3.96	996.77612
Photolytic Degradation	94.29	5.2	996.36565

Table 9: Forced degradation for Losartan Potassium

Condition	%Assay of LOS	% Degradation	Peak Purity (Match Factor)
Acid degradation	96.16	2.91	995.95471
Base degradation	93.37	6.77	997.93564
Oxidative degradation	91.87	7.24	998.95027
Thermal Degradation	103.57	4.59	995.95266
Photolytic Degradation	100.45	1.43	999.95317

Table 10: System Suitability Parameters

Parameter	Hydrochlorothiazide	Losartan potassium
Theoretical plates	2774	2546
Tailing factor	1.63	1.95
LOD (µg/ml)	0.375	1.779
LOQ (µg/ml)	1.138	5.393

Results and discussion

Literature survey revealed no stability indicating HPLC was developed for the determination of losartan potassium and hydrochlorothiazide. Fig 1 shows typical chromatograms losartan potassium and hydrochlorothiazide .The retention times of losartan and hydrochlorothiazide were 4.901 and 2.176 min, respectively. The calibration curve was linear over the range 25-75 µg/ml and 6.25-18.75µg/ml for the determination of losartan potassium and hydrochlorothiazide respectively. The linearity of method was statistically confirmed. The correlation coefficients (r) for calibration curves were not less than 0.99. The relative standard deviation (R.S.D.) values of the slope were not more than 2%. The analytical recovery at three different concentrations of losartan potassium and hydrochlorothiazide was determined. Forced degradation study was also carried out. In that, Acid, Base, Peroxide, Heat, UV treatment given to losartan potassium and hydro chlorothiazide. Therefore proposed validated method was successfully applied to determine losartan potassium and hydrochlorothiazide in bulk and tablet dosage form.

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