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SIMULTANEOUS ESTIMATION OF LOSARTAN POTASSIUM AND HYDROCHLOTHIAZIDE PRESENT IN TABLET FORMULATION BY RP-HPLC

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Abstract

A simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method was developed for Simultaneous estimation of Losartan potassium and Hydrochlorothiazide (HTZ) in tablet dosage form on RP C-18 Column (BDS Hypersil 250*4.6 mm) using Acetonitrile: Water(50:50) as mobile phase. The flow rate was 1.0 ml/min and effluent was monitored at 232nm. The retention time for Losartan potassium and HTZ was found to be as 1.98 and 3.04 respectively. Proposed method was validated for Precision, Accuracy, Linearity range, Robustness and Ruggedness.

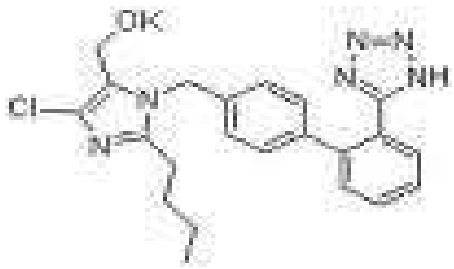
Key Words; Losartan potassium, Hydrochlorothiazide, Reverse Phase High Performance Liquid Chromatography.

Introduction:

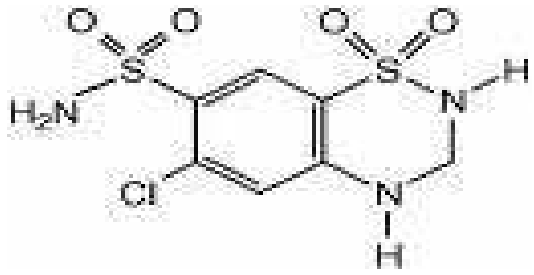
Losartan Potassium is chemically 2-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5-yl) biphenyl-4-yl] methyl}-1*H*-imidazol-5-yl) methanol and with the Molecular weight of 422.91 grams.Losartan Potassium white crystaline powder freely soluble in water and slightly soluble in alcohol[(1,2).]Losartan potassium is an orally active, nonpeptide angiotensin II (AII) receptor antagonist. It is the first of a new class of drugs to be introduced for clinical use in hypertension. This novel agent binds competitively and selectively to the AII subtypes 1 (AT_1) receptor, thereby blocking AII-induced physiological effects[(3).]

Hydrochlorothiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 7 -sulfonamide and with the molecular weight of 297.74 g. Hydrochlorothiazide is white crystalline powder and is very slightly soluble in water and soluble in alcohol[4] . Hydrochlorothiazide belongs to the thiazide class of diuretics, acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na+-Cl- co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport [5,6] . Losartan potassium and Hydrochlorothiazide combined formulation available as Hyzaar, Cozaar and Losart.H.

Literature survey reveals that various analytical techniques to analyze the formulation by spectroscopic methods[7.8,9],High performance low pressure method [9] ,high pressure methods[10,11] and Bioanalytical techniques[12] were available. The developed RP-HPLC method very economic, specific, accurate and précised method for analysis combination formulation of Losartan potassium and Hydrochlorothiazide



Hydrochlorothiazide



Losartan potassium

Materials and Methods

Instrument: High Performance Liquid Chromatographic system (Shimadzu) equipped with two LC 20AT liquid pumps, Rheodyne Injector (2E 7725, 20 µl loop), SPD 20A UV/Vis detector and Spinchrome software, Glass Van Hypodermic injecting syringe, an BDS Hypersil C-18 RP column (250 cm* 4.6 mm ID).

Reagents: Acetonitrile of HPLC grade (Merck), Double Distilled Water.

Drugs: Losartan Potassium, Hydrochlorothiazide.

Chromatographic condition: The mobile phase containing Acetonitrile: Water (50:50) was found to resolve Losartan potassium and HTZ. The mobile phase was filtered through 0.45-µ-membrane filter and the ultrasonicated for 30 min. the flow rate was set at 1.0 ml/min. All three drugs showed good absorbance at 232 nm, which was selected as wavelength for further analysis all determinations were performed at ambient column temperature**.**

Standard Stock Preparation: Weigh and transfer accurately about 50.0 mg of Losartan potassium and 12.5mg of hydrochlorothiazide into a 100ml clean dry volumetric flask, and make up to volume 100 ml of with diluent, sonicate for 5 minutes.

Diluted Standard: Pipette out 1 ml of standard stock solution dilute to 10ml with diluent.

Sample preparation: Weigh equivalent to 120.4 mg of sample and transfer into a 100ml volumetric flask. Add about diluent and shake the volumetric flask on a rotary shaker for 20min, Filter the solution through 0.45µ Nylon membrane filter to obtain clear solution.

Evaluation of System Suitability:

Inject 10µl of the diluted standard solution in five replicate injections, into the chromatograph and record the chromatograms.

The column efficiency as determined from hydrochlorothiazide & losartan potassium peaks is not less than 3000 USP plate count and the tailing factor for hydrochlorothiazide & losartan potassium peaks is not more than 2.0.

The relative standard deviation for the peak areas of the five replicate injections is not more than 2.0%.

Procedure: Separately inject 10µl of the blank, Standard (five injections) and samples solution in duplicate into the liquid chromatography, record the chromatographs and measure the peak areas.

Calibration Curve;

Calibration curves were prepared by taking appropriate aliquots of Losartan Potassium and HTZ stock solution 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 and 120 mcg/ml of Losartan Potassium and 5, 10, 15, 20, 25 and 30 mcg/ml of HTZ. These solutions (n=6) were injected and chromatogram were taken. Flow rate was maintained at 1.0 ml/min. temperature of column kept ambient and the column effluents were monitored at 232 nm. Calibration curve was constructed by plotting peak area Vs concentration and regression equation was computed. R^2 values of Losartan potassium, HTZ were found to be as .999 and 0.999

Results and Discussions

To develop a simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method for simultaneous estimation of Losartan Potassium and HTZ, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. System suitability tests were carried as per ICH guidelines.

Method Validation: The proposed RP-HPLC method was validated as per ICH guidelines.

Specificity

The peak purity of Losartan potassium and HTZ were assessed by comparing the retention time of standard Losartan Potassium and HTZ and sample good correlation was obtained between the retention time of standard and sample. Placebo and blank were injected and there were no peaks. There are no interferences hence method is specific.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for Losartan Potassium and HTZ were found to be as 20-120mcg/ml, and 5-30 mcg/ml respectively. The regression equation for Losartan Potassium and HTZ were found to be as y = 3.4514X-0.2261, and y = 12.813x+ 7.7736 with correlation coefficient (R^2) 0.9991 and 0.9991 respectively.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample preparation was carried out in same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is precise.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100% and 120% concentration levels. Known amounts of standard Losartan Potassium and HTZ were added to the pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in table.

Robustness:

The Robustness of method as carried out by changing the Chromatographic conditions such as Flow rate and Temperature variations. With the change of Flow rate of 0.8 ml, 1.0 ml and 1.2ml, change of Column temperature with of 24, 25 and 26^{o}Cmore and their tailing factor, plate count obtained within the limit

Ruggedness:

The ruggedness of method carried out by using the different HPLC system and Intraday Precision .percentage RSD are calculated for both parameters

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The lowest amount of analyte in sample that can be detected, but not necessary quantified, Losartan potassium & hydrochlorothiazide Standard with those of blank.

The lowest amount of analyte in the sample that can be determined with acceptable precision and accuracy was determined by the comparison of measured signal with 22.74 µg/ml and 2.93µg/ml of Losartan potassium &hydrochlorothiazide.

Assay

Sample preparation

Weight aacurately 20 tablet(1.20mg) and from it equivalent weight of 120mg was taken and dissolve in acetonitrile make upto 100ml. from it take 1ml and makeup to 100ml he final conc to 10µg/ml

Standard Preparation

Accurately Weighed and transferred Losartan potassium &hydrochlothiazide 8mg&2mg of each into a 10 ml

clean dry volumetric flask, and diluents was added, sonicated for 5 minutes, and diluted to the mark

Calculation: Calculate the amount of each drug by using the following formula

$$\% \text{ of Assay} = \frac{A \times C \times E \times G \times (100 - WATER) \times 0.99 \times P}{(Mg/tablet)\ B \times D \times F \times L.A \times 100}$$

Where,

A = Average peak area of sample

B = Average peak area of standard.

C = Dilution factor of standard.

D = Dilution factor of sample

E = weight of standard.

F = weight of sample.

G = Average weight of sample.

P = Potency of standard.

L.A = Labeled amount.

Tables and Figures:

System suitability:

Parameter	Losartan potassium	hydrochlorothiazide
Resolution	7.993	
Tailing Factor	1.500	1.400
Number of theoretical plate	3904	6787

Linearity:

Conc. in µg(Losartan potassium)	Area
20	73.888
40	131.87
60	208.035
80	273.159
100	345.208
120	416.051
Correlation coefficient	0.999

Conc in µg(HTZ)	Area
5	75.270
10	130.582
15	203.302
20	261.390
25	327.524
30	393.944
Correlation coefficient	0.9991

Accuracy:

sample	peak area				% recovery		Statistical analaysis	
	Peak area of std		Peak area of sample					
	LSD	HTZ	LSD	HTZ	LSD	HTZ	LSD	HTZ
80%	295.343	287.343	381.489	361.561	100	99.8	Avg=99.99 SD=0.005	Avg=99.53 SD=0.499
80%	292.601	281.968	381.489	361.561	99.9	98.9		

80%	295.115	282.577	381.489	361.561	100	99.9	%RSD=0.005	%RSD=0.501
100%	378.616	360.320	381.489	361.561	99.9	99.8	Avg=99.58 SD= 0.143 %RSD=0.143	Avg=99.70 SD=0.262 %RSD=0.263
100%	379.256	359.645	.381.489	361.561	99.9	99.8		
100%	380.258	359.749	381.489	361.561	99.7	99.4		
120%	453.652	428.752	381.489	361.561	100	99.5	Avg=99.99 SD=0.005 %RSD=0.005	Avg=99.25 SD=0.838 %RSD=0.90
120%	453.652	428.521	381.489	361.561	100	99.4		
120%	454.252	426.232	381.489	361.561	99.9	99.8		

Robust ness:

Flow rate variation

S.No	Flow rate (ml)		Rt		Peak Area	
	LST	HTZ	LST	HTZ	LST	HTZ
1	0.8	0.8	2.387	3.787	559.786	397.220
2	1	1	1.947	3.033	295.393	280.131
3	1.2	1.2	1.946	3.031	295.395	280.134

Temperature variation

S.No	Temp	LST		HTZ	
		Rt	Peak Area	Rt	Peak area
1	24	1.9	379.331	3.33	360.211
2	25	1.9	379.371	3.3	360.211
3	26	1.8	379.394	3.2	360.245

Ruggedness:
System-system variation
Losartan potassium

S.No	Rt	Area
1	1.973	3909
2	1.98	3879
3	1.98	3901
4	1.987	3913
5	1.983	3926
Avg	1.9806	3905.6
SD	0.00512	17.401
%RSD	0.2589	0.44554

Hydrochlorothiazide

S.No	Rt	Area
1	3.003	1262
2	3.04	1259
3	3.04	1250
4	3.033	1252
5	3.033	1262
Avg	3.029	1257
SD	0.01538	5.656
%RSD	0.50779	0.4500

Intraday Precision:

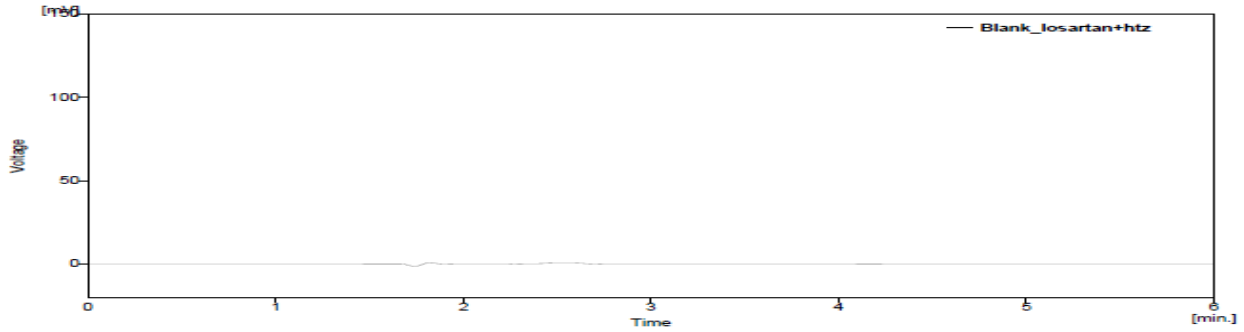
S. No.	Day	Peak area of LST	Peak area of HTZ	Statistical analysis
1	0	379.731	358.501	LST Mean =377.184 SD =4.1580 %RSD = 1.1024
2	1	372.721	360.712	
3	2	372.721	355.103	
4	3	381.489	361.561	HTZ Mean =359.144 SD = 2.5266 %RSD = 0.703
5	4	379.258	359.845	

LOD and LOQ

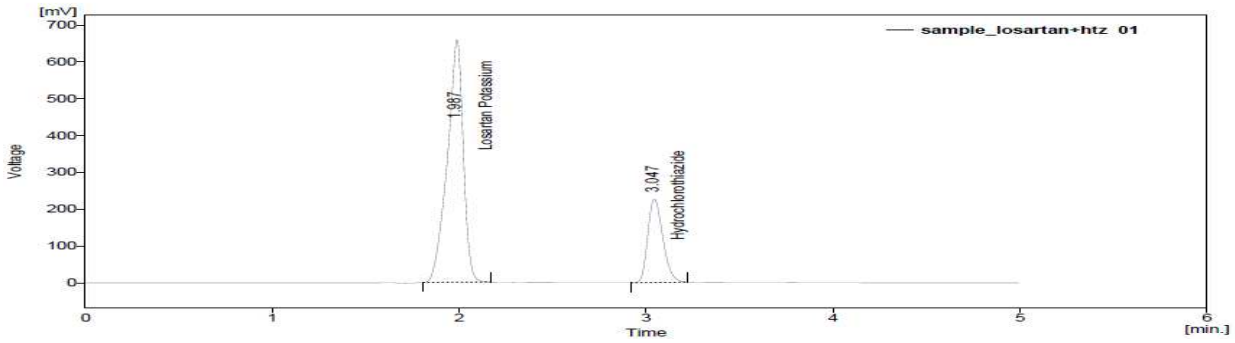
Sample	LOD	LOQ
LST	17.41 µg/ml	174µg/ml
HTZ	5.62µg/ml	57.07 µg/ml

Chromatograms:

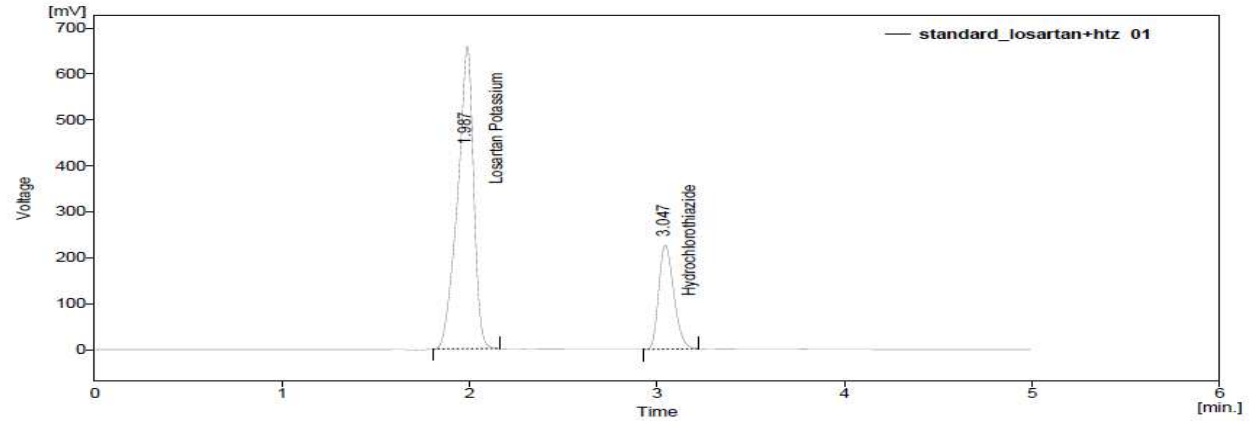
Blank



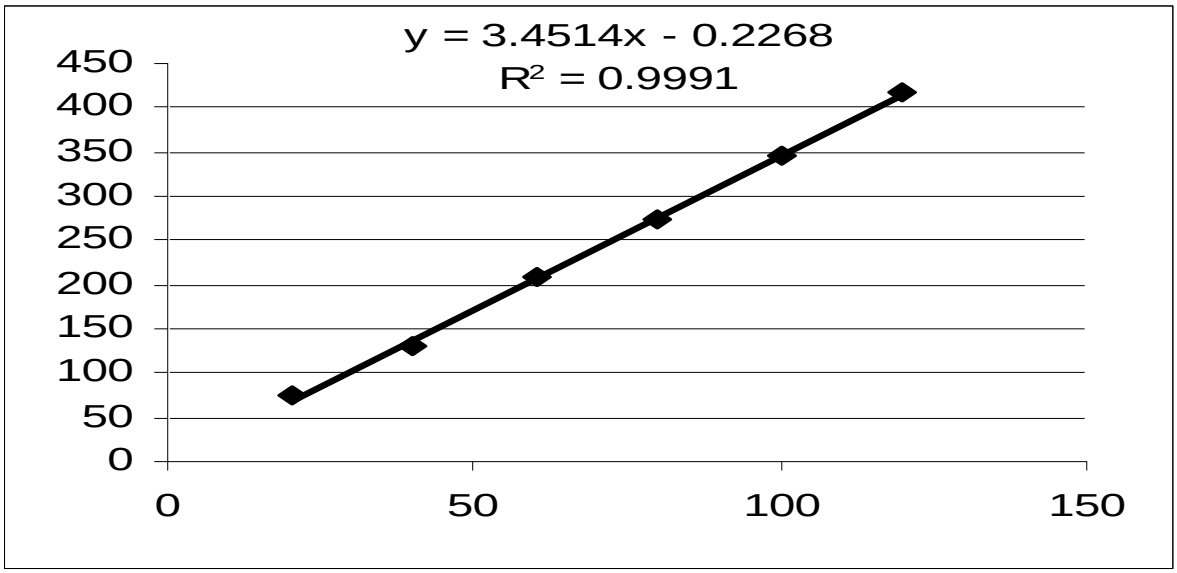
Standard



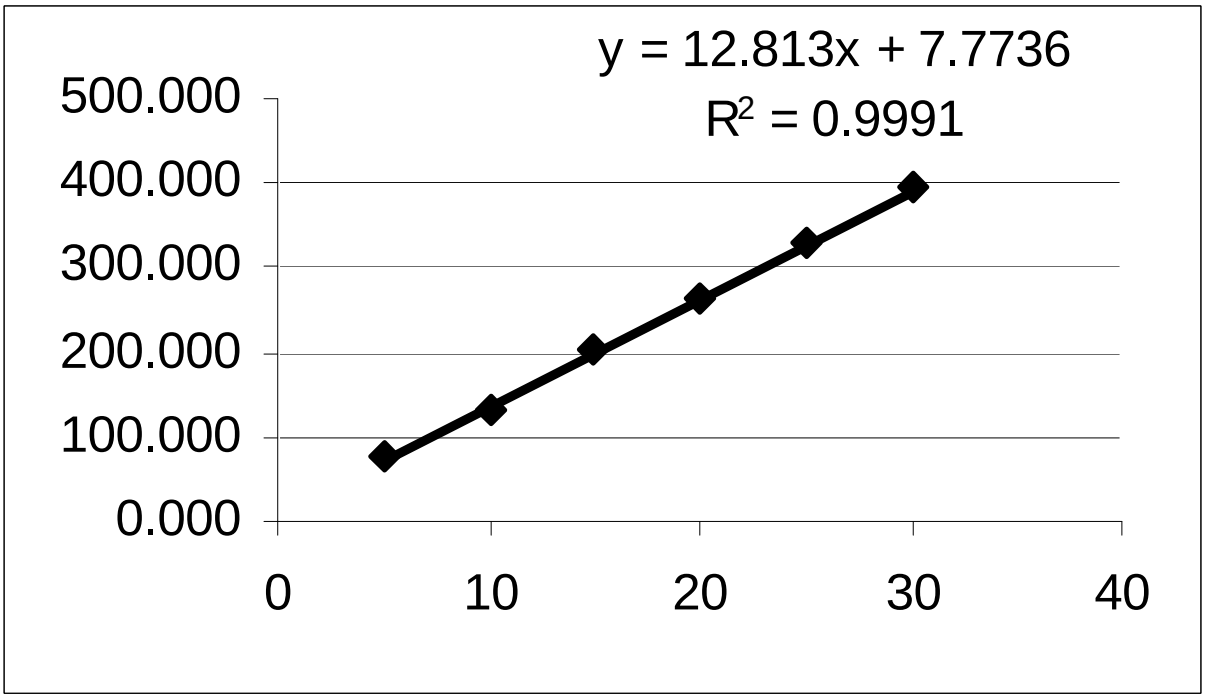
Sample



Linearity Graph for LST



Linearity Graph HTZ



Conclusion

The proposed method is simple, specific, accurate and precise and hence can be used in routine for simultaneous estimation of Losartan Potassium in tablet dosage. 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 the 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 Potassium and HTZ in tablet dosage form.

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