STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE DRUGS BY RP-HPLC

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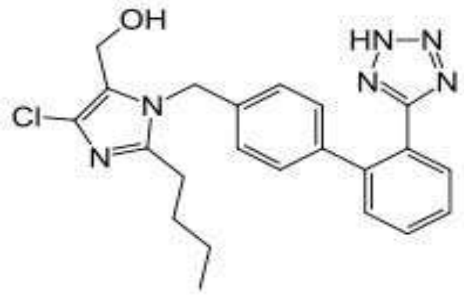
ABSTRACT

A simple Reverse phase liquid chromatographic stability indicating method for assay 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 45:55%v/v. The column used was reverse phase C18 column (3.9-mm × 15-cm; 5-µm packing L7) 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 12.0 and 7.7 min respectively. Results of analysis were validated statistically and by recovery studies. Results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is stability indicating of losartan potassium and hydrochlorothiazide drugs in solid dosage form.

Keywords: *Losartan potassium, Hydrochlorothiazide, stability indicating.*

1. INTRODUCTION

Losartan is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). It was the first angiotensin II antagonist to be marketed. As with all angiotensin II type 1 receptor (AT1) antagonists, losartan is indicated for the treatment of hypertension. It may also delay progression of diabetic nephropathy and is associated with a positive clinical outcome in that regard. It is a suitable pharmacological agent for the reduction of renal disease progression in patients with type 2 diabetes, hypertension, and micro-albuminuria (>30 mg/24 hours) or proteinuria (>900 mg/24 hours). It is designated chemically (2-butyl-4-chloro-1-{[2'- (1H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1H- Imidazol-5- yl)methanol and It empirical formula is C22H22ClKN6O and its structural



formula is -

Hydrochlorothiazide (abbreviated HCTZ, HCT, or HZT), is a diuretic medication often used to treat high blood pressure and swelling due to fluid build up. Other uses include diabetes insipidus, renal tubular acidosis, and to decrease the risk of kidney stones in those with high calcium level in the urine. For high blood pressure it is often recommended as a first line treatment. Hydrochlorothiazide is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis. It is also used for the prevention of kidney stones in those who have high levels of calcium in their urine. The empirical formula of Hydrochlorothiazide is C7H8ClN3O4S2

and its structural formula is –

Preparation of Solutions: Standard Preparation: Accurately 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 up to 10ml with diluent.

Instrument: HPLC equipped with Injector, Pump, UV detector, and Recorder.

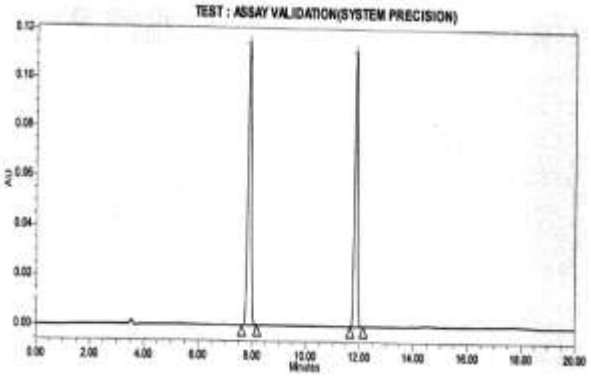
Column: 3.9-mm × 15-cm; 5-µm packing L7
Flow Rate: 1 ml/min
Wavelength: 270 nm
Injection Vol: 20 µl
Run Time: 20 minute
Auto samp. temp: 15°C
Diluent Water: Acetonitrile (45:55)
Mobile phase Buffer: Acetonitrile Buffer 0.5% Ammonium acetate in water.

Method validation: 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 and hydrochlorothiazide were prepared as per test method and injected for 5 times. Results are shown in Table 1 and chromatogram is summarized in **figure-1.**

Sr. No	Losartan Potassium 50ppm		Hydrochlorothiazide 12.5ppm	
	RT (min)	Peak Areas	RT (min)	Peak Areas
1.	11.869	826874	7.883	965066
2.	11.862	827203	7.879	966352
3.	11.860	827501	7.880	966547
4.	11.860	835453	7.887	976102
5.	11.845	827540	7.874	966344
Mean	11.8594	828914.2	7.8806	968082.2



RSD for Assay of six preparations. Results are shown in Table 2 and chromatograms are attached below figure number 2, 3,4,5,6 and 7

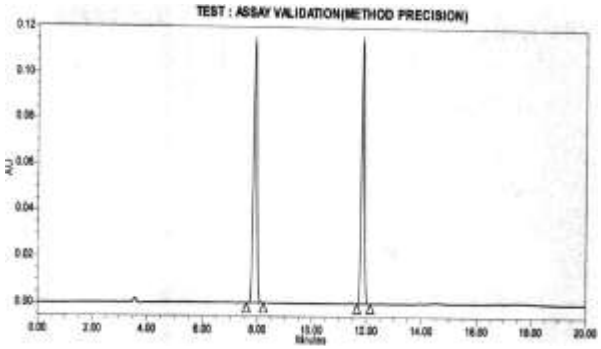
2. Method precision:

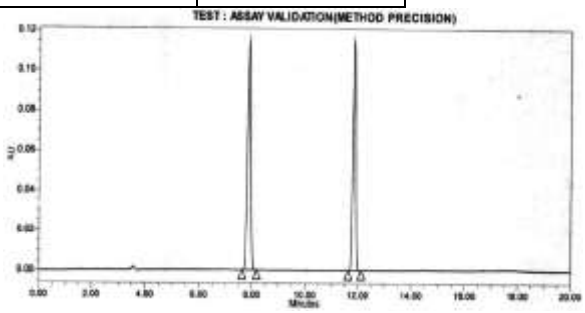
Six samples were prepared and analyzed as per the test method and calculated the %

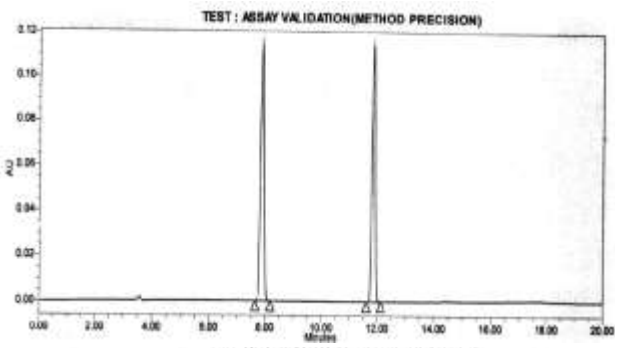
Acceptance criteria: RSD for Assay not more than 2.0.

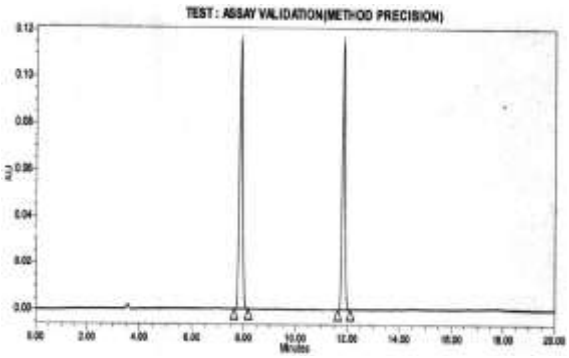
Table number 2:

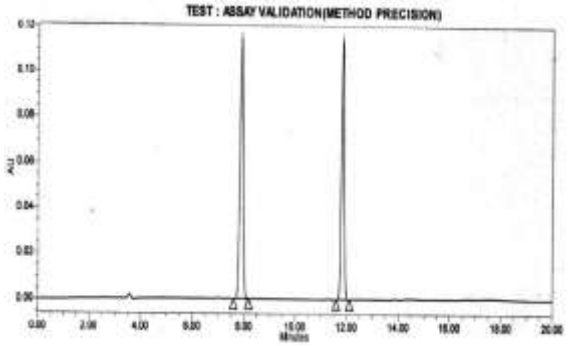
Assay No.	Mean Peak Area for Losartan	Losartan Potassium Assay (%)	Mean Peak Area for HCTZ	HCTZ Assay (%)
1	847420	102.3	966267	99.7
2	852137	102.5	973022	100.4
3	840818	101.3	960625	99.1
4	860655	103.7	984922	101.6
5	847928	102.1	969457	100.2
6	847121	102.4	968542	100.1
Mean	849346.5	102.383	970473	100.18
% RSD	0.71	0.69	0.77	0.76

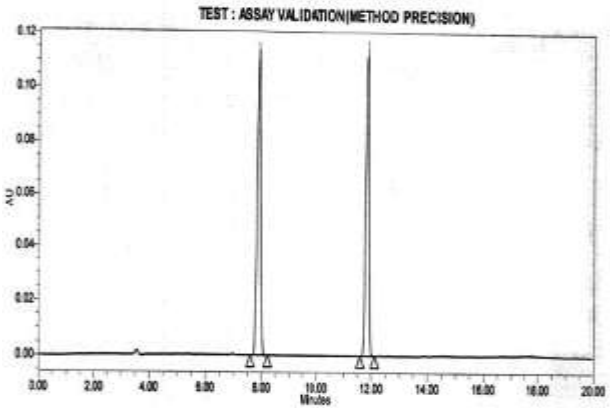












3. Linearity:

Acceptance Criteria: Correlation coefficient should not be less than 0.999.And response should be linear.

Injected 7 different concentrations of Losartan Potassium standard solution ranging from 70% to 130% of working assay concentration (35, 40, 45, 50, 55, 60, and 65 ppm). Similarly Injected 7 different concentrations of Hydrochlorothiazide standard solution ranging from 70% to 130% of working assay concentration (8.75, 10, 11.25, 12.5, 13.75, 15, and 16.25 ppm). Duplicate injections are performed at each concentration and the mean peak areas are averaged. Concentration plotted on X-axis and area on Y-axis. Calculated correlation coefficient and equation of line. Linear regression analysis will demonstrate the acceptability of the method for quantitative analysis over the full spectrum of the concentration range.

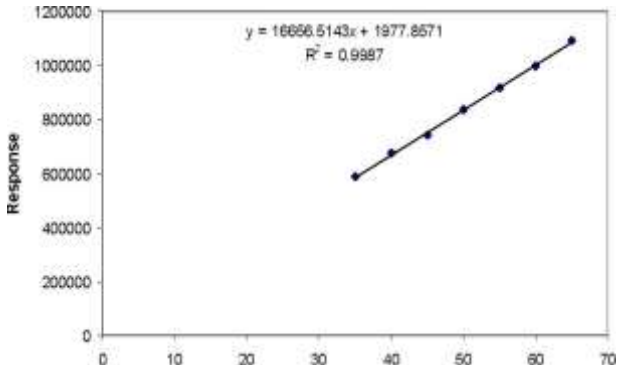
Table 3

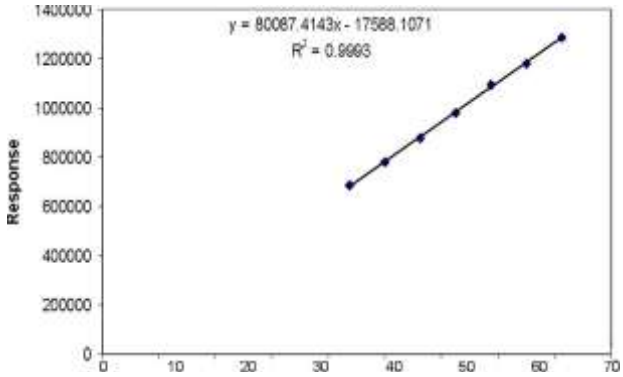
Sr. No	Conc. (in %)	Solution preparation		Conc. (in PPM)	Peak Area	Mean peak area
		Volume of stock solution (500ppm)	Total volume (ml)			
1	70	7 ml	100	35	590752 583604	587178
2	80	8 ml	100	40	678415 670844	674630
3	90	9 ml	100	45	745730 735964	740847

4	100	10 ml	100	50	838772 835057	836915
5	110	11 ml	100	55	923278 909145	916212
6	120	12 ml	100	60	1002854 989535	996193
7	130	13 ml	100	65	1091955 1091348	1091652
		Slope				16656.51
		Intercept				1977.86
		Correlation coefficient (r)				0.99937

Table 4

Sr. No	**Conc. (in %)**	**Solution preparation**		**Conc. (in PPM)**	**Peak Area**	**Mean peak area**
		Volume of stock solution (500ppm)	**Total volume (ml)**			
1	70	3.5 ml	100	8.75	691525 682739	687132
2	80	4 ml	100	10.0	785181 776527	780854
3	90	4.5 ml	100	11.25	883970 873682	878826
4	100	5 ml	100	12.50	982530 978605	980568
5	110	5.5 ml	100	13.75	1102226 1085561	1093894
6	120	6 ml	100	15.00	1186628 1170734	1178681
7	130	6.5 ml	100	16.25	1284839 1284317	1284578
		Slope				80087.41
		Intercept				-17588.11
		Correlation coefficient (r)				0.99967





4. Ruggedness:

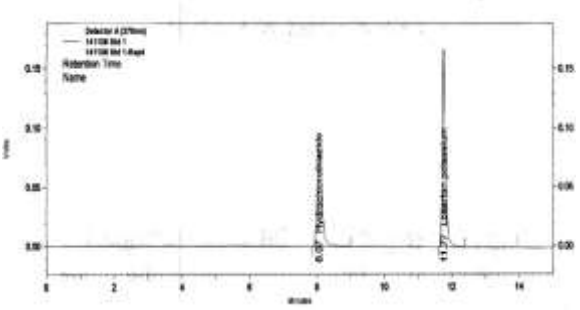
It was done by different analyst by using different column, different system on different day. The system suitability criteria were evaluated and the overall % RSD for % assay of experiment results and method precision results was also calculated.

Mean recovery found against added amount should be between 98% - 102%. %RSD of individual recoveries should be less than 2.0%. Known amount of the active ingredient at 3 levels each in triplicate, i.e. 3 x 50%, 3 x 100% and 3 x 150% of the working concentration was spiked with placebo at 100 % level of 100/25mg tablet samples were prepared in triplicate. Each sample was analyzed and calculated. The data for individual compound is summarized in Tables Below.

Amount Added

Calculation for % Recovery= ---------- x 100

Amount Recovered



5. Accuracy:

Level	Conc. (in PPM)	Amount added	Amount recovered	% recovery	% mean	% RSD
50 %	6.52	62.6	61.82	98.8	98.9	1.06
		62.4	64.24	98.1		
		62.1	64.86	98.0		
% MEAN				98.3		
% RSD				0.44		
100 %	15.5	125.1	123.05	98.4		
		125.0	122.92	98.3		
		124.9	122.92	98.4		
% MEAN				98.4		
% RSD				0.05		
		187.4	190.11	101.4		
		187.5	185.21	98.8		
		187.2	186.30	99.5		

% MEAN	99.8		
% RSD	1.34		

Range:

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

CONCLUSION:

A simple, specific, accurate, precise, stability indicating reverse phase high performance liquid chromatography method has been developed which can be used for accurately quantitative estimation losartan potassium and hydrochlorothiazide for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R1) so it can be used by pharmaceutical industries.

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