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RP- HPLC Method for the Simultaneous estimation of Losartan and Spironolactone in Tablet Dosage Form

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Abstract-

A RP- HPLC method has been developed and validated for the simultaneous estimation of Losartan and Spironolactone in tablet dosage form. The simultaneous equation method allows rapid, simple and direct estimation of Losartan and Spironolactone in tablet dosage form commercially available without the need of previous separations and can thus be useful in case of routine analysis. This method is based on a HPLC separation of the two drugs on the Thermo, P4000 Quaternary pump, UV 6000 PDA Detector with CHROMQUEST software and a simple mobile phase containing Methanol:water (70:30) at a flow rate of 1.0 mL/min using UV detection at 240nm. The method showed linearity in a concentration range of 10–50 µg mL−1 for losartan (r = 0.9949) and 50–250 µg mL−1 for spironolactone(r = 0.9984). The method results in repeatability and precision. The percent recovery of Losartan and Spironolactone was seen to be in the range of 99.3-100.3% and 99.13-100% respectively (98.0 to 102.0%).Finally, the method was applied successfully in the simultaneous determination of losartan and spironolactone pharmaceutical formulations.

Keywords: Losartan, Spironolactone, RP- HPLC, linearity, Accuracy, Precision.

1. INTRODUCTION-

Losartan is chemically known to be **[**2-butyl-5-chloro-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] imidazol-4-yl] methanol and is an antihypertensive agents known as angiotensin II receptor blockers [1]. Losartan and its long acting active metabolite, E-3174 interferes the blood pressure and increases the effect of angiotensin II [4, 8]. Losartan inhibits competitively the binding of AT2 to AT1 in majority of the tissues which also includes vascular smooth muscle as well as the adrenal glands. The estimation of Losartan is carried out in tablets by HPLC, super-critical fluid chromatography, in urine by GC-MS and simultaneously with its active metabolite in by HPLC [1, 4].

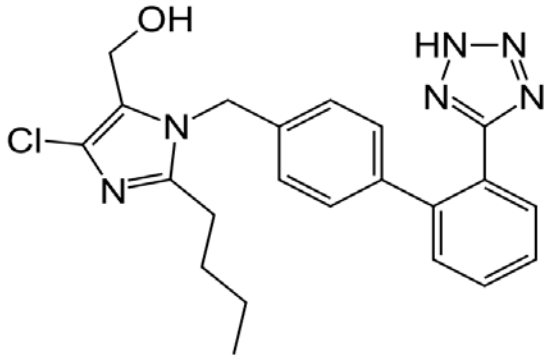

Fig. no. 1: Structure of Losartan

Spironolactone is chemically 7α-Acetylthio-17α-hydroxy-3-oxopregn-4-ene-21-carboxylic acid γ-lactone. Spironolactone is a steroid and is renal competitive aldosterone antagonist which belongs to the class called potassium-sparing diuretics [2]. Spironolactone acts initially via competitive binding of receptors at the aldosterone-dependent sodium-potassium exchange site. Thus, it leads to increase in the of sodium and water level to be excreted, and potassium is retained. Due to this mechanism Spironolactone acts as a diuretic and also as an antihypertensive drug [4, 5].

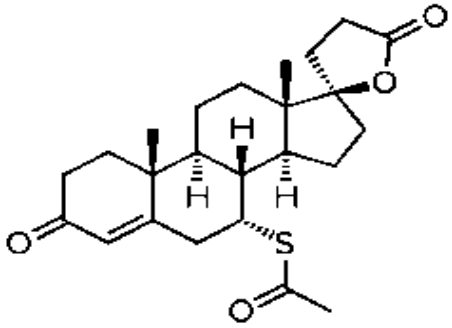

Fig. no. 2: Structure of Spironolactone

To our knowledge simple, reproducible and economical analytical method for simultaneous determination of Spironolactone and Losartan is not reported yet so far. The present research describes simple, sensitive, accurate, rapid and economic method for Simultaneous estimation of Spironolactone and Losartan in tablet form [3]. The proposed method was validated as per ICH guidelines and was found to be precise, accurate and reproducible [6].

2. MATERIALS AND METHOD-

Instrumentation:

The HPLC system which consists of a Pump (P4000 Quaternary pump) was used. The detector consisted of UV 6000 PDA Detector with CHROMQUEST software. The column used was a CHEMSIL ODS-C18 (250 mm X 4.6 mm), 5µ column.

Double beam UV –Visible spectrophotometer, Model LabindiaUV 3200.

Analytical balance, Model Shimadzu.

Reagents and Chemicals:

Methanol, Acetonitrile, per chloric acid, Trimethyl amine,Potassium dihydrogen Phosphate and Ortho phosphoric acid were supplied by Thermocil fine Chem Ltd. Pune. Methanol, Acetonitrile, Water were of HPLC grade while remaining solvents were of AR grade.

A) Determination by UV spectroscopy:
Drugs found to be freely soluble in Methanol, water and Acetonitrile. In the present study the mobile phase was 70% methanol and 30% water (pH 3 was adjusted by phosphate buffer). UV spectrophotometric method involves the estimation of Losartan and Spironolactone bulk and pharmaceutical formulation as mentioned in Fig. 3.

Preparation of standard stock solution:
Standard stock solution was prepared when 100 mg of Losartan and Spironolactone was dissolved in mobile phase and the volume was made up to 100 ml with mobile phase .(Stock solution-I, 1000 mcg / ml). 10 ml of stock solution-I was diluted to 100 ml with mobile phase (Stock solution-II, 100 mcg / ml). 1 ml of this stock solution-II was withdrawn and placed in 10 ml standard flask diluted so as to reach 10 ml with mobile phase to get the concentration 10 mcg/ml. The resulting solution absorbance was checked and measured against respective blank solution in the UV region of 200-400 nm. The maximum absorbance of Losartan and Spironolactonewas at 237 and 242 nm respectively.

Preparation of standard curve:
Aliquots from standard stock solutions were transferred to 10 ml capacity of volumetric flasks. With mobile phase the volume was adjusted to get concentrations of 10-50 mcg / ml for Losartan and 50-300mcg / ml for Spironolactone. The obtained absorbance values were plotted against the concentrationto get the calibration graph. The regression equation and correlation coefficient was determined. Validation was carried out for Losartan and Spironolactone by calculating range, linearity, precision, ruggedness, accuracy, robustness, LOD and LOQ as per ICH guidelines.

B) Analytical method development by RP-HPLC:
Chromatographic Condition:
The mobile phase selected was Methanol: water (70:30%v/v). The mobile phase was allowed to filter through 0.45 µ filter under vacuum filtration and then ultra-sonicated for 5 min. The flow rate was set to 1.0 ml/min and 20 µL was injection volume. Wavelength selected was 240 nm with Run time of 15min. All determinations were performed atambient temperature.

Preparation of standard solution (Mixed standard) :
10 mg of Losartan and 10 mg of Spironolactone working standards were weighed accurately and added in a 100 ml volumetric flask and approx. 70 ml of diluent was added and was allowed to sonicate in order to dissolve it completely. Volume was made to the mark with the similar solvent (Stock solution).

Preparation of sample solution:
The powder (of 10 tablets of Losartan and Spironolactone) equivalent to the amount of active ingredient present in 10 tablets was transferred in a 100 ml volumetric flask and 70 ml of diluent was added to it and it was sonicated for 30 minutes and was shaken for five minutes and then was diluted to the mark. 0.6 ml of upper solution was shifted to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 mcg/ml filter prior injecting in HPLC system.
Test Procedure:
20 µl of the sample, blank, standard, and placebo preparations in duplicate were injected into HPLC system and the peak responses for Losartan and Spironolactone were observed. The quantities in mg of Losartan as well as Spironolactone were calculated according to each tablet taken. The RP-HPLC method developed for the simultaneous estimation of Losartan and Spironolactone was carried out. Fig.No. 6

METHOD VALIDATION:
The proposed HPLC method was validated as mentioned in the ICH guidelines.

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

Linearity:
Appropriate volume from stock was diluted to achieve final concentration of 10, 20, 30, 40, 50 µg/mL for Losartan and 50, 100, 150, 200and 250 µg/mL for Spironolactone. Then the chromatogram was recorded. For each concentration, plot the graph concentration versus area fig no.7 and 8.

Accuracy:
Assay was performed in triplicate for various concentrations of Losartan and Spironolactone equivalent to 50, 100, and 150 % of the standard was injected in the HPLC system per the test procedure. Table no.2 and 3.

Precision:
The precision was evaluated with respect to intra-day precision (repeatability) and inter-day precision (intermediate precision). The standard solution was injected five times and area was checked for all five injections. The %RSD of five replicate injections for the area was seen to be in the specified limits. Results are tabulated in Table No. 4-9.

Limit of Detection (LOD) and Limit of Quantification (LOQ):
The LOD and LOQ were determined by analysing low concentration of the standard solution via the developed methods. The LOD is the concentration of the analyte that gives a measurable response (signal to noise ratio 3.3). The LOQ is the lowest concentration of the analyte, which gives a response that can be accurately quantified (signal to noise ratio of 10).
Results are tabulated in Table no. 10.

Robustness:
The robustness of the given method was estimated by analysis of aliquots from homogenous batches by various physical parameters like flow rate and mobile phase composition, temperature variations which might differ but the responses were within the specified limits of the assay.

System Suitability:
Sample solution of Losartan and Spironolactone were injected three times in HPLC system as per test procedure. The system suitability parameters were checked from standard chromatograms obtained, after calculating the % RSD of retention times, theoretical plates, tailing factor, and peak areas from three replicate injections.

Assay:
20 tablets were weighed and powdered, tablets powder equal to 500mg of Losartan and 12.5mg of Spironolactone was transferred into a 50 ml volumetric flask, sufficient amount of mobile phase was added and dissolved by 20 minutes ultra-sonication. Then the volume was made to the mark using the mobile phase and was filtered with 0.45 µ filter paper. Pipette out 2 ml from the above solution and diluted to 50ml with the mobile phase. The amount of Losartan as well as Spironolactone present in each tablet was calculated.Results are tabulated in Table no.12.

3. RESULT AND DISCUSSION:

The wavelength of Spironolactone and Losartan were observed to be 242 nm and 237 nm respectively. 100µg/mL solution of Losartan and 100µg/mL solution Spironolactone was prepared using methanol as solvent. The above given solutions were scanned separately from 190 to 400 nm in UV-Visible spectrophotometer. The response for the overlain spectrum in case of Losartan and Spironolactone was obtained at 240 nm. Hence the complete method was processed at the wavelength of 240 nm. Spectrums are shown in Fig.No.3-5.

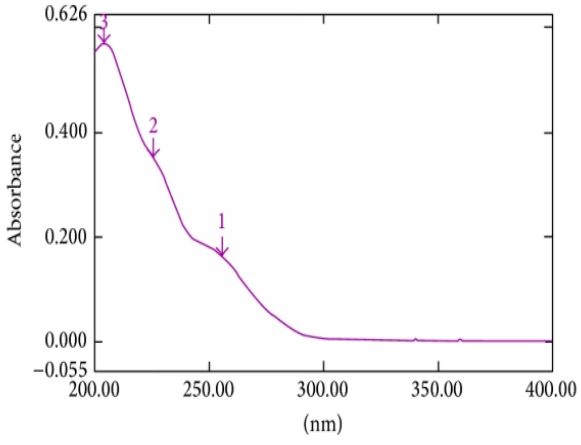

Fig no. 3:UV spectrum of Losartan

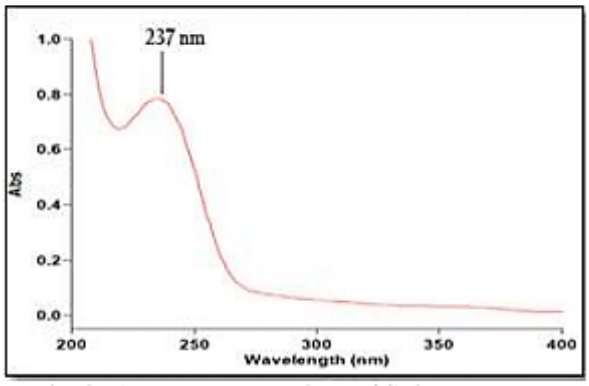

Fig. no. 4: UV spectrum of Spironolactone

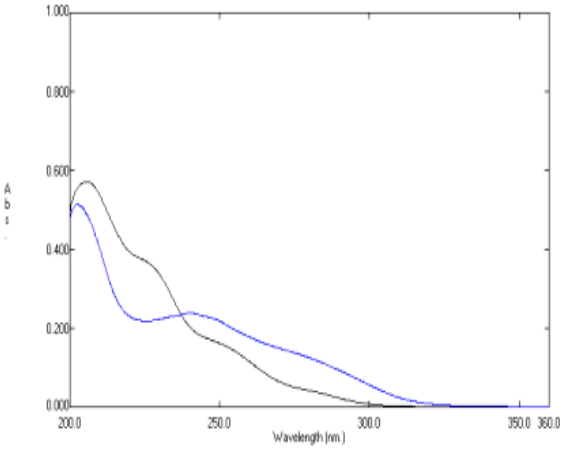

Fig. no.5: Overlay UV spectrum of Losartan and Spironolactone

Optimized HPLC method

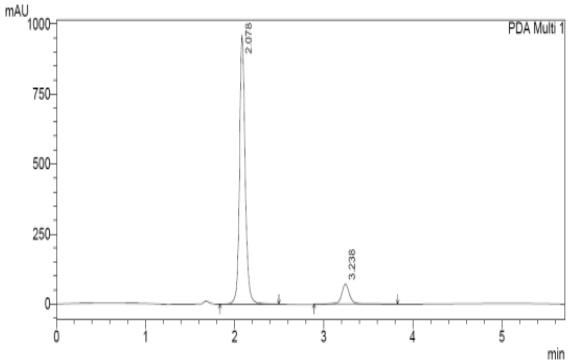

Fig no. 6: Chromatogram for optimized method

Table no. 1: Optimized method parameters

Name	Retention Time	Area	USP Tailing	USP Plate Count
LT	2.076	4207841	1.293	3567.422
SL	3.236	399852	1.133	4698.521

Losartan and Spironolactone were eluted at 2.078 and 3.238 respectively; efficiency parameters were indicating the good separation, asymmetric. So this method was selected for further analysis.

METHOD VALIDATION

SPECIFICITY

The chromatograms of standard and that of sample were identical with nearly same retention time. No changes were seen due to placebo and sample at the retention time of analyte thus confirms that the method was specific.

LINEARITY

Linearity study was performed in the range of concentration of 10-50 µg / ml for Losartan and 50-250 µg/ml for Spironolactone. Correlation co-efficient of Losartan and Spironolactone was found to be 0.9949 and 0.9984 respectively. The linearity curve is plotted and shown in Fig.No.7 and 8.

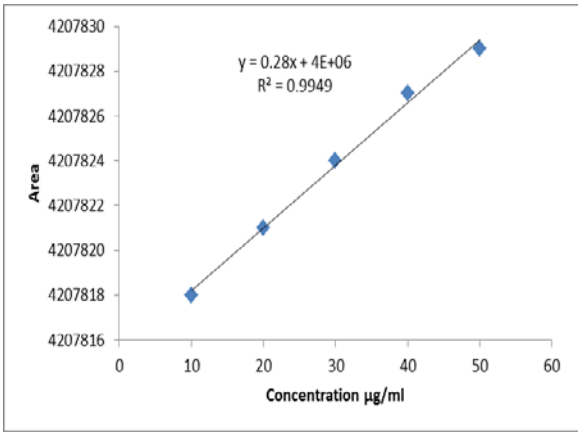

Fig. No. 7: Calibration curve of Losartan

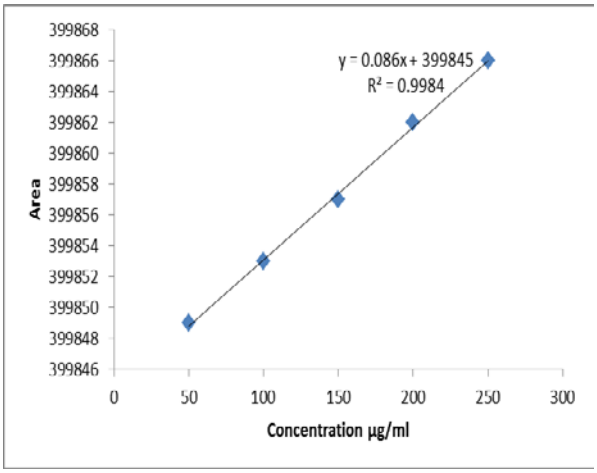

Fig No. 8: Calibration curve of Spironolactone

ACCURACY

The % recovery for 50%, 100% and 150% accuracy level of Losartan and Spironolactone was found to be within the range of 99.3-100.3% and 99.13-100% respectively (98.0 to 102.0%).

The results were tabulated in Table No.2 and 3.

Table No. 2: % Recovery results for Losartan

Sample No.	SpikeLevel	Amount added (mg)	Amount Found (mg)	Mean % Recovery
1	50%	5	4.96	100.2%
2	100%	10	9.92	99.3%
3	150%	15.3	15.2	99.2%

Table No. 3: % Recovery results for Spironolactone

Sample no.	Spike Level	Amount(µg/ml) added	Amount (µg/ml) found	Mean % Recovery
1	50%	5	4.8	100%
2	100%	10	9.87	99.12%
3	150%	14.8	14.71	99.68%

PRECISION

The RSD of % Recovery for Losartan and Spironolactone chromatograms of repeatability precision and intermediate precision is calculated. It passes repeatability and intermediate precision. The results of precision are summarized in Table No.4-9.

Repeatability

Table No.4: Sample chromatogram values for repeatability of Losartan

Injection No	Peak area	% Recovery
1	4207833	99.4%
2	4207829	100%
3	4207832	99.0%
4	4207850	99.8%
5	4207845	99.2%
Mean	4207838	99.48%
%RSD	**0.42**	

Table No.5: Sample chromatogram values for repeatability of Spironolactone

Injection No	Peak Area(mV.δ)	% Recovery
1	399841	99.2%
2	399849	99.8%
3	399851	99.2%
4	399814	99.4%
5	399801	100%
Mean	399831.2	99.52
%RSD	**0.36**	

Intermediate precision (analyst to analyst variability):

Table No.6: Intermediate precision results for Losartan (Day-1, Analyst-1)

Parameter	Peak Area	% Assay
Avg*	4207851	99.10%
% RSD*	0.41	0.38

Table No.7: Intermediate precision results for Spironolactone Day-1, Analyst-1)

Parameter	Peak Area	% Assay
Avg*	4207851	99.10%
% RSD*	0.41	0.38

Table no. 8: Intermediate precision results for Losartan (Day-2, Analyst-2)

Parameter	Peak Area	% Assay
Avg*	4207851	99.10%
% RSD*	0.41	0.38

Table no.9: Intermediate precision results for Spironolactone (Day-2, Analyst-2)

Parameter	Peak area	%Assay
Avg*	399798	99.52%
% RSD*	0.86	0.36

The % RSD for the area of five standard injections for intermediate precision of Losartan and Spironolactone was found to be 0.41 and 0.98 for day-1, analyst-1 and 0.42 and 0.36 for day-2, analyst-2 respectively (NMT 2).

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

Table No.10: Results for LOD and LOQ

	LT	SL
Peak Area	2056745	188634
	2057246	187858
	2058874	187658
SD	1113.106	515.5502
Slope	10215.91	20244.82
LOD (µg/mL)	0.359561	0.084037
LOQ (µg/mL)	1.08958	0.254658

Robustness

To estimate the robustness of the developed RP-HPLC method, minute deliberate deviations in the optimized method parameters were done. The result of change in flow rate as well as mobile phase ratio on the retention time and tailing factor were studied. The method was observed to be robust by minor changes like ± 0.1ml change in flow rate and ± 2% change in mobile phase.

System Suitability

The working standard solution was allowed to inject 3 times into the HPLC; chromatograms were recorded and measure the responses in case of major peaks. System suitability parameters like theoretical plates, retention time and asymmetric factor.

Table no. 11: System suitability results

	LT Area	SL Area	LT Theoretical Plates	SL Theoretical Plates	LT Tailing Factor	SL Tailing Factor
Avg	4207847	399798	3567.397	4698.490	1.293	1.133
SD	10397.99	1592.19	25.1889	68.2888	0.01388	0.0109
%RSD	0.247467	0.3993	0.7057	1.4230	1.0872	0.9769

Assay

Table No. 12: Results for assay

	LT	SL
Avg sample Area	4207852	399800
Amt present	500.4721	12.54347
% amount present	100.0944108	100.3477463
SD	1.195515343	0.729659142
%RSD	1.194387712	0.727130573

‘

CONCLUSION:

The given method is sensitive, simple and reproducible and could be utilized in routine for simultaneous estimation of Losartan and spironolactone in not bulk but also its formulations. Statistical analysis in case of the results also proved high accuracy and good precision. The sample recovery in the formulation was in good agreement with their label claims respectively. Hence this method can easily be adopted for the routine determination of Losartan and spironolactone depending upon the availability of chemicals and nature of other ingredients present in the sample.

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