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REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF SPIRONOLACTONE AND HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

The present research work describes a novel, simple, accurate, sensitive, rapid reversed-phase liquid chromatographic method for simultaneous estimation of Spironolactone & Hydrochlorothiazide in bulk & pharmaceutical formulations. The chromatographic separation was achieved on WATERS HPLC with PDA detector and column Symmetry, phosphate buffer and methanol as mobile phase at a flow rate of 1.0ml/min. The detection was carried out at 250 nm. The retention time of Spironolactone & Hydrochlorothiazide was found to be 3.388and 1.688. %Recoveries obtained for Spironolactone and Hydrochlorothiazide were 99.87% & 100.3%.The %RSD below 2.0 shows the high precision of proposed method. The method was validated for precision, Recovery, Specify Detection & Quantification limits in accordance with ICH guidelines.

Keywords: *Spironolactone, Hydrochlorothiazide RP-HPLC, Validation.*

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INTRODUCTION:

Spironolactone is indicated for the treatment of Class III-IV heart failure, management of edema in cirrhotic adults not responsive to fluid and sodium restrictions, primary hyperaldosteronism short-term preoperatively, primary hyperaldosteronism long-term in patients with aldosterone producing adrenal adenomas that are not candidates for surgery or patients with bilarteral micro/macronodular adrenal hyperplasia, as an add-on therapy in hypertension, and in nephrotic syndrome when treatment of the disease as well as fluid and sodium restriction with other diuretics is inadequate. Spironolactone has antiandrogenic activity which leads to many of its off label uses. Spironolactone is used off label in the treatment of hirsutism, female pattern hair loss, and adult acne vulgaris. Spironolactone is also frequently used for its antiandrogenic effects in transgender female patients due to its low cost and reducing male-pattern hair growth.

Hydrochlorothiazide is indicated alone or in combination for the management of edema associated with congestive heart failure, hepatic cirrhosis, nephrotic syndrome, acute glomerulonephritis, chronic renal failure, and corticosteroid and estrogen therapy. Hydrochlorothiazide is also indicated alone or in combination for the management of hypertension. Hydrochlorothiazide is transported from the circulation into epithelial cells of the distal convoluted tubule by the organic anion transporters .From these cells, hydrochlorothiazide is transported to the lumen of the tubule by multidrug resistance associated protein (MRP4). 4 Normally, sodium is reabsorbed into epithelial cells of the distal convoluted tubule and pumped into the basolateral interstitial by a sodium potassium ATPase, creating a concentration gradient between the epithelial cell and the distal convoluted tubule that promotes the reabsorption of water.

MATERIALS AND METHODS:

Chemicals and Reagents: Spironolactone and Hydrochlorothiazide were obtained as a gift sample fromA.R .Life science, Methanol and Acetonitrile from Merck, anhydrous di hydrogen phosphate and citric acid from finar chemicals.

Equipment and Chromatographic Conditions:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector Analysis was carried out at 250 nm with column Phosphate Buffer (pH-4.8): Methanol (55:45% v/v), dimensions at 35^0C temperature. The optimized mobile phase consists of. Flow rate was maintained at 1 ml/min and run time for 8 min.

Preparation of solutions:

Preparation of mobile phase:

Accurately measured 700 ml (70%) of HPLC Methanol and 300 ml of Acetonitrile (30%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Preparation of Standard Solution:

Weigh 10 mg of Spiranolactone and 10 mg of Hydrochlorothiazide then transfer them into a 10 mL & 10 ml volumetric flask. Add roughly 7 mL & 7 ml of diluents, sonicate to dissolve them fully.

Further, pipette 0.3 ml of the above stock solutions for hydrochlorothiazide & spiranolactone into a 10 ml volumetric flask, then dilute with diluents to the appropriate level.

Preparation of Sample Solution:

Ten combination tablets should be weighed. & pestle, then transfer the equivalent of 10 mg of the commercially available formulation of Spiranolactone and Hydrochlorothiazide into a 10 mL volumetric flask. Add around 7 mL of diluents.

Further, pipette 0.3 ml of the stock solution of Spiranolactone & Hydrochlorothiazide into a 10-ml volumetric flask, then dilute with diluent to the appropriate level.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters:

To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 8 minutes to equilibrate the column at ambient temperature.

Assay of pharmaceutical formulation:

The proposed validated method was successfully applied to determine Spironolactone and Hydrochlorothiazide in their pharmaceutical dosage form. The result obtained for Spironolactone and Hydrochlorothiazide was comparable with the corresponding labeled amounts and they were shown in Table-2.

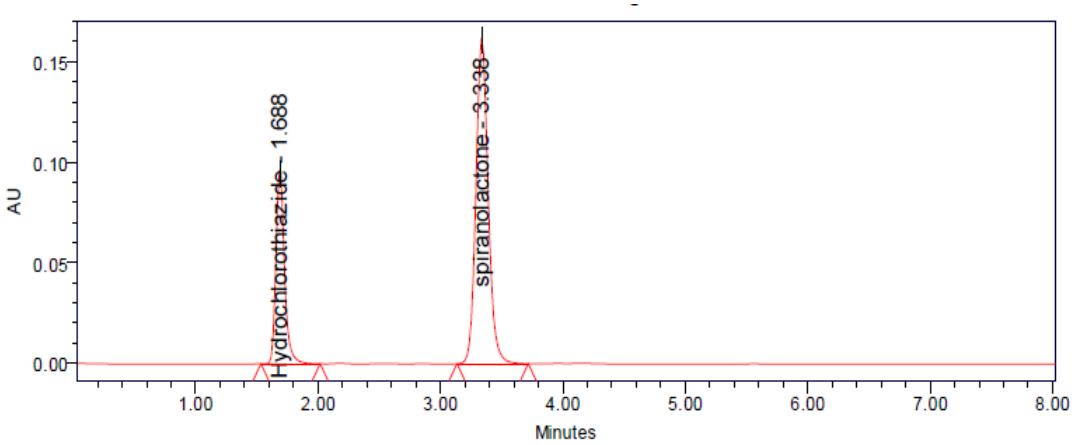

Figure 1: Standard chromatogram

Table no-1: Optimized chromatogram peak for results

S.No	Peak	R_t	Area	Height	Resolution	Tailing	plate count
I	Hydrochlorothiazide	1.688	467354	94876		1.28	2842
II	Spiranolactone	3.338	1121479	161751	10.62	1.13	5525

More plates, less tailing, and proper resolution in both peaks are displayed in this trial. Meet all requirements for system suitability. Thus, the chromatogram is optimized.

SYSTEM SUITABILITY:

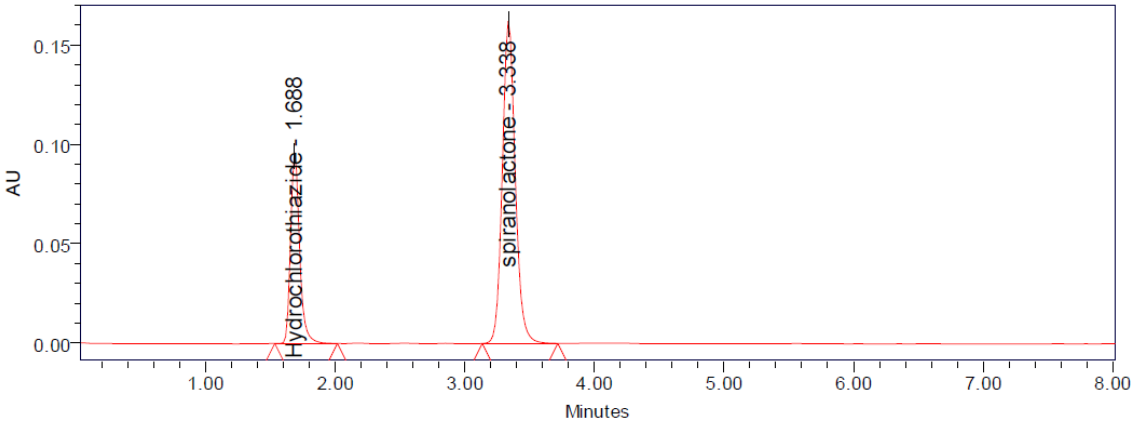

Figure no-2: system suitability

Table no-2: system suitability parameters for Hydrochlorothiazide& Spiranolactone results

S.No	Name	Retention time	Area	Height	resolution	tailing	plate count
I	Hydrochlorothiazide	1.688	462654	89941		1.29	2762
II	Spiranolactone	3.388	1119787	157009	10.13	1.15	5277

ACCURACY:

The percentage recovery was computed after sample solutions were made at various concentrations (50%, 100% & 150%).

Table.no-3 Assay results for Spironolactone and Hydrochlorothiazide

S.No	Name	Label claim(mg)	Amount Found(mg)	%Assay
1	Hydrochlorothiazide	50	49.97	99.94
2	Spiranolactone	50	49.89	99.78

Linearty: The linearity study was performed for the various concentration levels. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3

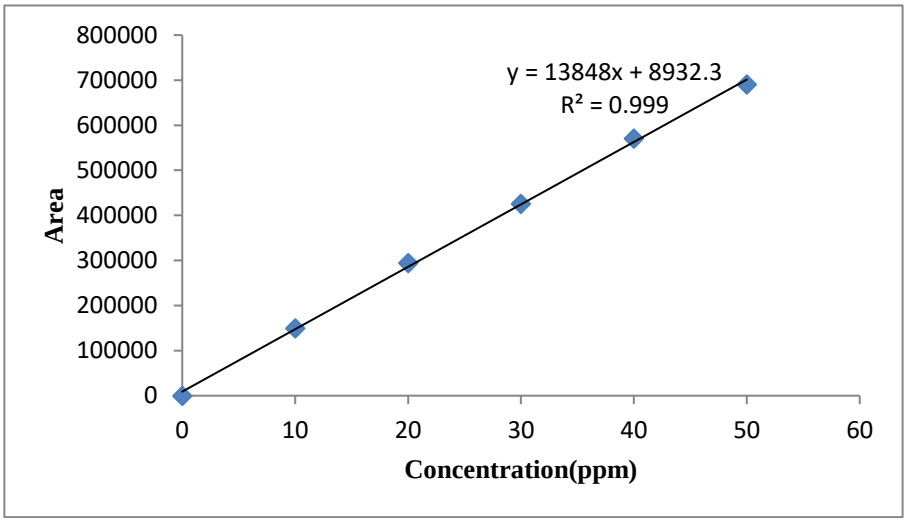

Figure no-3: calibration curve of Hydrochlorothiazide

Table no-4. Hydrochlorothiazide linearity results

S.No	Name	RT	Area	Height	Tailing	Plate Count	Injection
I	Hydrochlorothiazide	1.691	149310	83942	1.2	3126.2	1
II	Hydrochlorothiazide	1.692	294677	98443	1.3	3099.4	2
III	Hydrochlorothiazide	1.689	425907	104033	1.2	3056.3	3
IV	Hydrochlorothiazide	1.688	570529	120872	1.3	3008.2	4
V	Hydrochlorothiazide	1.689	690393	131545	1.2	2980.8	5

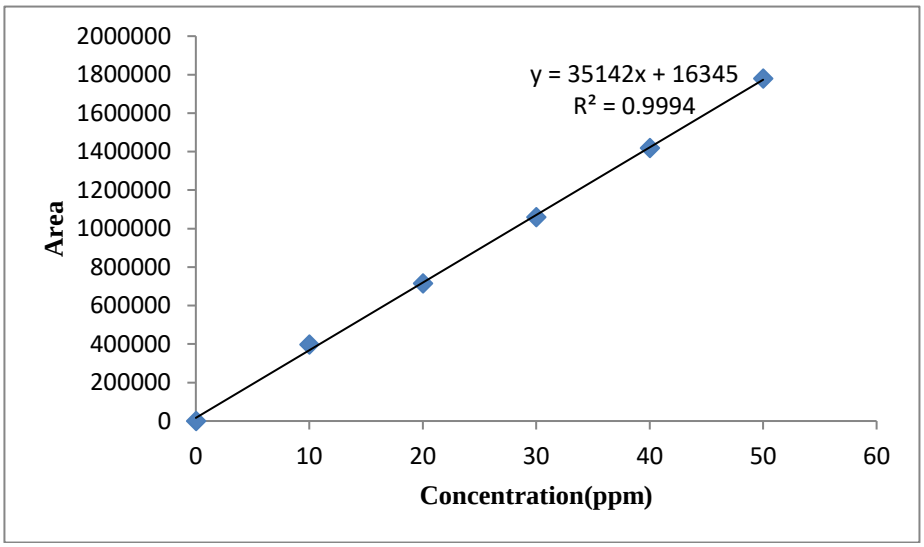

Figure 4: calibration curve of Spiranolactone

Table no-5: Spiranolactone linearity results

S.No	Name	RT	Area	Height	Tailing	Plate Count	Injection
I	Spiranolactone fumarate	3.299	397642	145809	1.1	5789.8	1
II	Spiranolactone	3.294	715547	172165	1.1	5915.2	2
III	Spiranolactone fumarate	3.290	1059209	189603	1.1	5940.4	3
IV	Spiranolactone	3.288	1417945	210333	1.1	5642.3	4
V	Spiranolactone fumarate	3.285	1779025	247790	1.1	5591.4	5

The linearity study was carried out for concentration range of 10-50 ppm of Hydrochlorothiazide and 10-50 ppm of Spiranolactone & the correlation coefficient was found to be 0.998 & 0.999.

Table no-6: variation in flow results

S.no	Drug	Flow	Area	Height	plate count	Tailing
I	Hydrochlorothiazide	Less (0.9) Actual(1) More (1.1)	518500 467354 422704	93362 94876 81335	2704 2842 2264	1.25 1.28 1.23
II	Spiranolactone	Less (0.9) Actual (1) More (1.1)	1253302 1121479 1016303	148818 161751 132846	2704 5525 3515	1.11 1.13 1.12

LIMIT OF DETECTION FOR HYDROCHLOROTHIAZIDE AND SPIRANOLACTONE

The signal to noise ratio was assessed after the sample with the lowest concentration was prepared in relation to the baseline noise.

Table no-7: LOD of results

Drug	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Hydrochlorothiazide	52	158	3.04
Spiranolactone	52	153	2.91

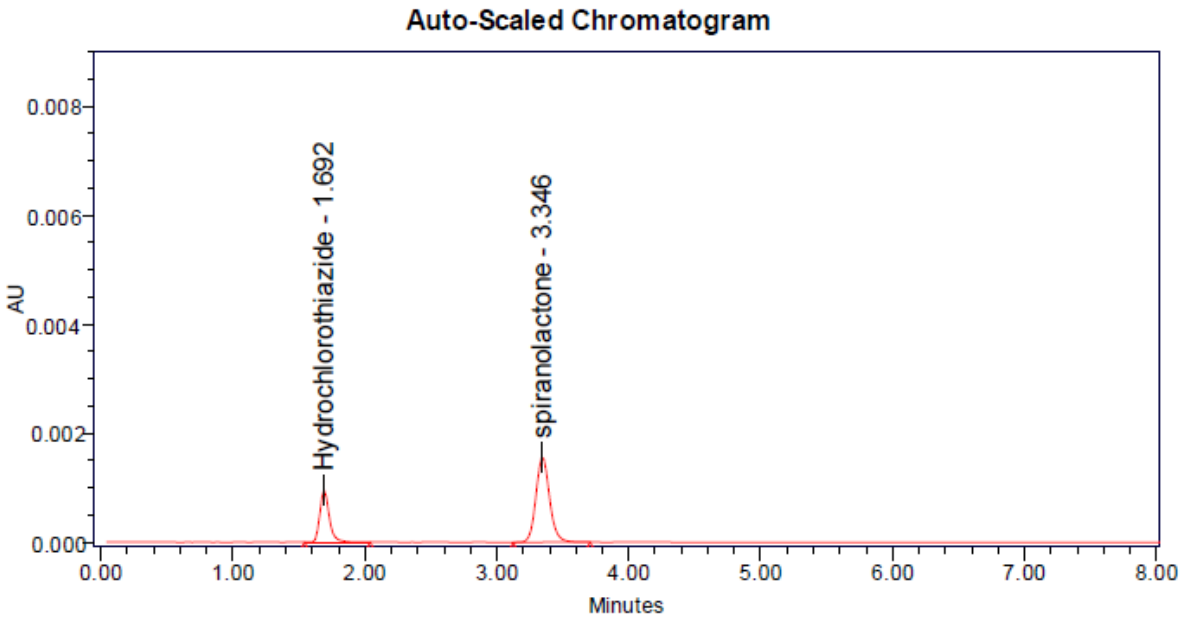

Figure no-5: Hydrochlorothiazide & Spiranolactone showing LOD

Table no-8. Mobile phase composition variation

S.no	Drug name	Organic (ml/min)	Area	Height	plate count	Tailing
1.	Hydrochlorothiazide	Less (50%) Actual More (70%)	465830 467354 473756	75693 94876 80966	2020 2842 3002	1.17 1.28 1.27
2.	Spiranolactone	Less (50%) Actual More (70%)	1158297 1121479 1093816	74259 161751 188630	3552 5525 3844	1.17 1.13 1.15

It was obtained that the percentage RSD for the mobile phase fluctuation & flow rate change was less than 1, falling within the acceptable range. Thus, the approach is reliable.

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Spironolactone and Hydrochlorothiazide in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Spironolactone and Hydrochlorothiazide in pure and its pharmaceutical dosage forms.

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