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Research Article

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Development and Validation of High Performance Liquid Chromatography Assay Method of Spironolactone

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ABSTRACT

*A new, precise and accurate reversed-phase high performance liquid chromatograph a method was developed for determination of spironolactone. High performance liquid chromatograph was carried out by isocratic technique on a reverse-phase using C_{18} inertstil (250 *4.6 mm), 5µm column with a mobile phase consisting of phosphate buffer solution PH = 4 and acetonitrile in (1:1) ratio. The flow rate was adjusted at 1.5 ml/min, detection wavelength at 240 nm, temperature at 40 °C and retention time was found to be 4.5 min. Beer's low was obeyed in concentration range 20-30 ppm. The assay percentage of spironolactone in tablet dosage form was found to be (98.25±0.59) %. The % of recovery was found to be (99.4-101.99) %. The limit of detection (LOD) and limit of quantization (LOQ) were found to be 0.553 ppm and 1.677 ppm respectively. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of accuracy of developed method which was carried out according to ICH guidelines [3].*

Keywords: Spironolactone; HPLC; Method development and Validation

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INTRODUCTION

Spironolactone (aldactone) is potassium -aspiring diuretic agent and has half-life of about 16 hour. Spironolactone one diuretics agents which increase renal excretion of water and solutes (mainly sodium salt). It is used mainly in the treatment of refractory edema in patients with congestive heart failure nephrotic syndrome, or hepatic cirrhosis.On its own, spironolactone is only a weak diuretic, but it can be combined with other diuretics. [1][4]
Spironolactone is 7α-acetyle thio-3-oxo-17α-pregn-4-ene-21,17β- carbolactone. Its molecular formula is C_{24} $H_{32}O_4S$ having a molecular weight 416.58 gm/mole. It has following structure:

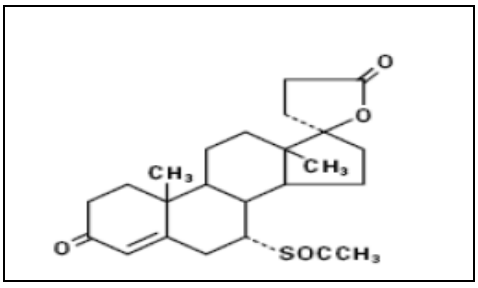

Figure 1: 7α-acetyle thio-3-oxo-17α-pregn-4-ene-21,17β- carbolactone

The literature reviews regarding spironolactone suggest that various analytical methods were reported for its determination as drugs, in pharmaceutical formulation and in various biological fluids such as artificial neural network and near-infrared spectrometry [6], colorimetric [5], micellar enhanced spectrofluorimetry [2] and RP-HPLC [4] methods.
In this method compered to method ananlysis of spironolactone in USP in tablet dosage form it simpler which used the mobile phase as diluent and time of preparation of sample is less than USP method.

EXPERIMENTAL SECTION

The instruments used for this work; UV-visible double beam spectrophotometer; model UV-2201, SHIMADZU. A pair of matched quartz cell (10mm) was used for the measurement, HPLC SHIMADZU CORP 03137 serious number LC202544 (Model of pumb LC-20Ab, Model of detector: SPD-20A, Model of oven: CTO – 20 AC) with LC- Solution Software: LC- Solution, PH/Temperature Bench Meter Mi 150 (Milwaukee) and Analytical Balance (keranabs).

Chemicals and reagents

Acetonitrile HPLC GRADE reagent, potassium dihydrogen phosphate anhydrous A.R, ortho phosphoric acid, Spironolactone working standard (purity 99.4%) gift from Shanghai- Sudan Pharmaceutical CO-LTD and spironolactone tablet 25 mg dosage form from the local market (yesalactone).

Method development

Selection of solvent

After study the solubility of spironolactone in different solvents and different buffer solutions acetonitrle and buffer solution PH 4 of potassium dihydrogen phosphate in 1:1 ratio were chosen for developing of the method.

Mobile phase preparation

6.8 g of potassium dihydrogen phosphate were weighted and transferred to 1000 ml volumetric flask and dissolved by purified water then completed to the mark by purified water. The solution was adjust to PH = 4 with orthophosphoric acid. Acetonitrile and buffer solution were mixed by (1:1) ratio.

Preparation of diluents

The same mobile phase

Preparation of standard stock solution

20.6 mg of spironolactone working standard was dissolved in 100 ml volumetric flask by mobile phase and sonication for 30 second then completed to the mark by mobile phase. Solution was diluted by pipetting 5ml of solution to 100ml volumetric flask. The solution was scanned and found to have maximum absorption wavelength at 240 nm using mobile phase as blank.

Preparation of the sample for assay

Equivalent to 25 mg of spironlactone from crushed 20 tablet of spironlaction were weighted and dissolved in 100 ml volumetric flask by mobile phase and sonication for 20 minutes then completed to the mark by mobile phase. 2.5 ml of solution were transferred to 25 ml volumetric flask then completed to the mark by mobile phase.

Table 1: Assay of spironolactone in commercial formulation for HPLC method

Drug	Amount taken µg/ml	Amount found µg/ml	% recovery	% RSD
Yesalactone	25	24.4	97.6	
	25	24.68	98.73	0.59
	25	24.61	98.44	

Method validation

Validation of an analytical method is the process to establish that the performance characteristics of the developed method meet the requirements of intended analytical application. The HPLC method was validated in the term of linearity, accuracy, precision, specificity, LOD and LOQ.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

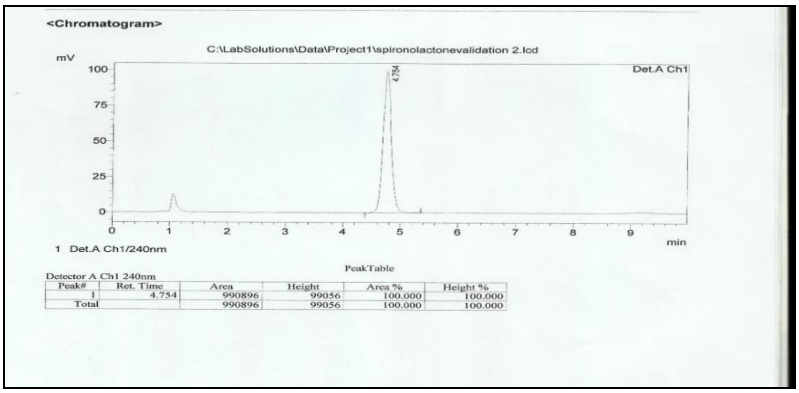

Figure 2: specificity of HPLC method

Linearity

Aliquots 2, 2.25, 2.5, 2.75 and 3 ml of spironolactone standard solution were transferred to series of 25 ml volumetric flask and made up to volume by mobile phase to obtain final concentrations of spironolactone of 20, 22.5, 25, 27.5 and 30 ppm (range from 80 to 120 %).

Table 1: calibration curve of spironolactone

Concentration ppm	Area under peak
20	799643.7
22.5	888063
25	979287
27.5	1076951
30	1168108

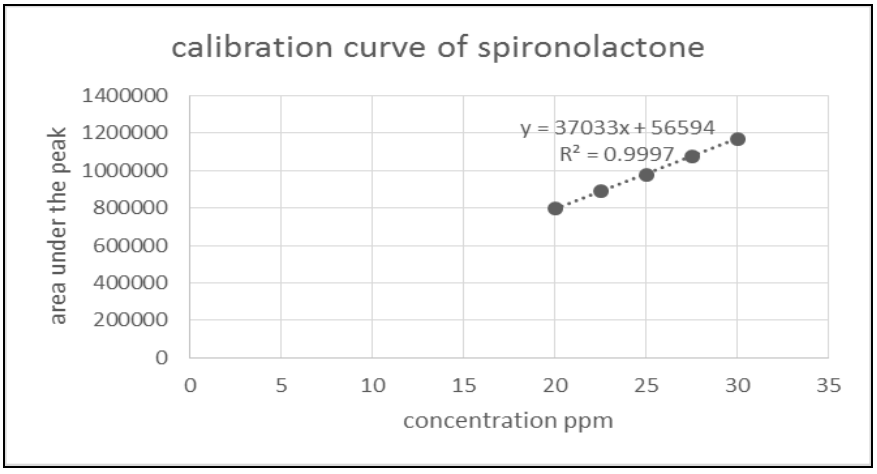

Figure 3: calibration curve of spironolactone in HPLC method

Precision

The precision was evaluated by repeatability and intermediate precision, sample % and reported as RSD. Intermediate precision of the assay was determined for 100% concentration by three analysts.

Table 4: repeatability precision of the system in HPLC method

Run	Area under the peak
1	991027
2	984376
3	984029
4	978274
5	985478
6	988032
Mean	985202.7
RSD%	0.4355

Table 5: intermediate precision of spironolactone in HPLC method

Run	Area the under peak of 1st analyst	Area the under peak of 2st analyst	Area the under peak of 3st analyst
1	1002754	1016599	1003482
2	993339	1005208	1001133
3	1000558	1007729	1006187
Mean	998883.7	1009845	1003601
RSD %	0.493126	0.59248	0.252002

Accuracy

Accuracy is closeness of test results with the true value which is express as % of recovery. The studies were performed at three levels (80,100 and 120 %).

Table 6: accuracy of HPLC method

Level of method %	Amount found %	Recovery %	Average of recovery	RSD of recovery %
80%	82.24	102.81	101.99	0.723
	81.14	101.42		
	81.4	101.75		
100%	100.22	100.22	99.4	0.761
	98.71	98.71		
	99.28	99.28		
120%	122.32	101.94	101.32	1.743
	123.22	102.68		
	119.23	99.36		

Accuracy was carried out by adding a known amount of drug to reanalyzed sample and the percentage of recovery was recorded.

Limit of detection and limit of quantization

Limit of detection (LOD) and limit of quantization (LOQ) for spironolactone was determined from the recession equation graph of spironolactone, LOD = 0.553 ppm, LOQ = 1.677 ppm.

DISCUSSION

In this work isocratic technique on a reverse-phase chromatography was used and The chromatographic condition of the method was adjust by using C_{18} inertstil (250 *4.6 mm), 5µm column with a mobile phase, flow rate at 1.5 ml/min, detection wavelength at 240 nm, temperature at 40 ^{o}C and retention time was found to be 4.5 min. Different ration of content of mobile phase were tried until get good resolution and pure peak of the method as showed at specificity. The proposed method was found to have R^2 =0.9997 which indicate that the method have linearity in the range of concentration 20 ppm to 30 ppm. Also the accuracy of the method has found to have high percentage of recovery in the range (99.40-101.99) % which indicates that the method has no interference of impurities. The precision (repeatability and intermediate precision) of the method has RSD ≤ 2% and that mean, according to ICH acceptance criteria's, the method has high precision. The method show acceptable value of LOD and LOQ. From

above information we can notice that the method is accurate and precise for determination of assay of spironolactone in raw material and tablet dosage form.

CONCLUSION

The described analytical method was validated according to ICH Q2 (R1) guideline and they meet to specific acceptance criteria. So these validated methods were found to be liner, accurate, precise, and sensitive and can be used for routine analysis for estimation of spironolactone in raw material and tablet dosage form.

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