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Development and Validation of Ultra Violet Spectrophotometric Assay Method of Spironolactone by Hydrazine Derivatization

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ABSTRACT:

A simple, precise and accurate UV spectrophotometric method has been developed for determination of spironolactone in raw material and tablet dosage form. Ethanol was used as solvent for the method. The wavelength 251.5 nm was chosen for determination of spironolactone. Beer's low was obeyed in concentration range 20-30 ppm. The assay percentage of spironolactone in tablet dosage form was found to be (97.15±0.282) %. The % of recovery was found to be (100.20-102.65) %. The limit of detection (LOD) and limit of quantization (LOQ) were found to be 0.028 ppm and 0.0858 ppm respectively. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of developed method which was carried out according to ICH guidelines.

KEY WORDS: Spironolactone, UV spectrophotometric, Method development and Validation.

INTRODUCTION:

Spironolactone (aldactone) is potassium -aspiring diuretic agent and has half-life of about 16 hours. Diuretics agents are drugs that increase renal excretion of water and solutes (mainly sodium salt). The main functions of diuretics therapy are to decrease fluid volume of the body and to adjust the water and electrolyte balance. On its own, spironolactone is only a weak diuretic, but it can be combined with other diuretics [3].

Spironolactone is 7α-acetyl 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. Figure 1

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 [5], colorimetric [4], micellar enhanced spectrofluorimetry [1] and RP-HPLC [3] method.

In this method hydrazine agent was used to make complex with spironolactone in (1:1) ratio in ethanol which give complex that has absorption in UV region by this way the assay of spironolactone was determined and the method was validated according to ICH guidelines [2].

Experimental section

The instruments used for the study was UV-visible double beam spectrophotometer; model UV-2201, SHIMADZU. A pair of matched quartz cell (10mm) was used for the measurement. Analytical Balance (keranabs).

Chemicals and reagents:

Ethanol absolute A.R, Hydrazinehydrogensulphate A.R, Spironolactone working standard (purity 99.4%) from Shanghai- Sudan Pharmaceutical CO-LTD, and

spironolactone tablet 25 mg dosage from the local market.

Method development:

Preparation of stander solution:

25.6 mg of spironolactone working standard was dissolved in 50 ml volumetric flask by absolute ethanol. 9 ml of sodium acetate solution (1mg/ml) and 8 ml of hydrazine hydrogen sulfate solution (1mg/ml) were added respectively and shacked. The contents of volumetric flask were put in water path (88-90 ^{0}C) for 15 min then cooled at room temperature and completed to the mark by ethanol absolute. This solution was used to determine the λ_{max}.

Assay of tablet formulation:

Equivalent to 25.0 mg of spironolactone were weighted from powdered of crashed twenty tablets. Then dissolved in 50 ml volumetric flask by absolute ethanol and treated in ultrasonic bath for 10 min. 9 ml of sodium acetate solution (1mg/ml) and 8 ml of hydrazine hydrogen sulfate solution (1mg/ml) were added respectively and shacked. The contents of volumetric flask were putted in water path (88-90 ^{0}C) for 15 min then cooled at room temperature and completed to the mark by ethanol absolute. 1.25 ml of filtrated solution were transferred to 25ml volumetric flask and completed to the mark by absolute ethanol and results are given in **Table No 1**.

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 UV method was validated in the term of linearity, accuracy, precision, LOD and LOQ.

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 ethanol absolute to obtain final concentrations of spironolactone of 20, 22.5, 25, 27.5 and 30 ppm (range from 80 to 120 %) and results are given in **Table No 2**.

Five concentration of standard in range (20-30) ppm and their absorptions at wavelength at 251.5 nm. The linearity was determined by terms of correlation coefficient and results are given in **Figure No 2**.

Precision:

The precision was evaluated by intermediate precision and repeatability by using sample of 100 % and reported as RSD and results are given in **Table No 3** and **Table No 4,** respectively.

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 %).

Accuracy was carried out by adding a known amount of drug to reanalyzed sample and the percentage of recovery was recorded and results are given in **Table No 5**.

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 = 0.0283 ppm, LOQ = 0.0858 ppm.

DISCUSSION:

The proposed method was found to have R^2 =0.996 which indicate that the method have linearity in the range of concentration 20 ppm to 30ppm. Also the accuracy of the method has found to have high % recovery in the range (100.20-102.65) % 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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Table (1): Assay of spironolactone in commercial formulation for derivatization by Hydrazine:

Drug	Amount taken µg/ml	Amount found µg/ml	% recovery	Average of recovery	% RSD
	25	24.21	96.84		
Yesalactone	25	24.33	97.31	97.15	0.282
	25	24.33	97.31		09

Table (2): Calibration curve of spironolactone in derivatization by hydrazine:

Concentration ppm	Absorption at 251. 5 nm
20	0.045
22.5	0.133
25.0	0.217
27.5	0.282
30	0.356

Table (3): Intermediate precision of spironolactone in derivatization by Hydrazine

Run	ABS of 1st analyst	ABS of 2st analyst	ABS of 3st analyst
1	0.219	0.226	0.211
2	0.22	0.227	0.211
3	0.219	0.226	0.210
Mean	0.2193	0.2253	0.2107
RSD %	0.26323%	0.25509%	0.27406%

Table (4): Repeatability precision of the system of spironolactone in derivatization by Hydrazine:

Run	Absorption
1	0.221
2	0.222
3	0.22
4	0.221
5	0.221
6	0.220
Mean	0.2208
RSD%	0.3409

Table (5): Recovery study of spironolactone in dervatization by Hydrazine:

Level of the method %	Amount taken (µg/ml)	Amount added (µg/ml)	Total theoretical amount found (µg/ml)	Amount found (µg/ml)	Recovery %	Average of recovery %	Relative standard deviation %
80	25	20	45	46.08	102.41		
80	25	20	45	46.21	102.68	102.50	0.15362
80	25	20	45	46.08	102.41		
100	25	25	50	51.36	102.72		
100	25	25	50	51.31	102.61	102.65	0.06183
100	25	25	50	51.31	102.61		
120	25	30	55	55.08	100.14		
120	25	30	55	55.08	100.14	100.20	0.1052
120	25	30	55	55.18	100.32		

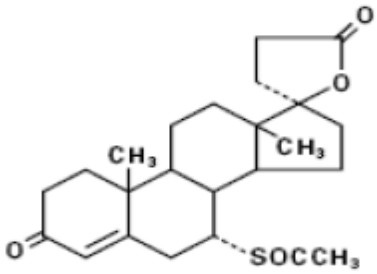

Figure (1): ***7α-acetyle thio-3-oxo-17α-pregn-4-ene-21,17β- carbolactone***

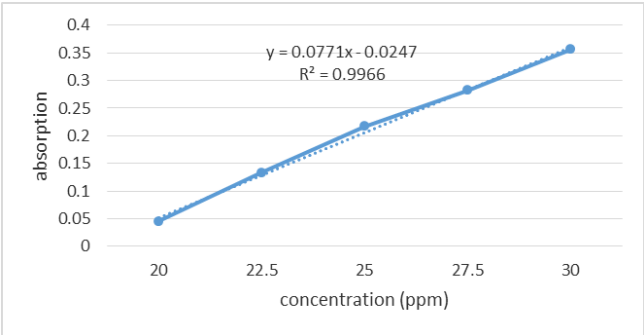

Figure (2): Calibration curve of spironolactone in derivatization by Hydrazine