Analytical Method Development And Validation Of Spironolactone By Rp-Hplc Method

Uniket Gosavi[1], Onkar Doke[2], Chaitali Dhale[3*], Vaibhavkumar Bhagwat ,Pranita S. Kavitake[5]

[1]*Assistant Professor, Vidya Niketan College of Pharmacy, Lakhewadi
[2]Associate Professor, Vidya Niketan College of Pharmacy, Lakhewadi
[3]Associate Professor, Vidya Niketan College of Pharmacy, Lakhewadi
[4]Assistant Professor, Vidya Niketan College of Pharmacy, Lakhewadi
[5]Assistant Professor, Vidya Niketan College of Pharmacy, Lakhewadi

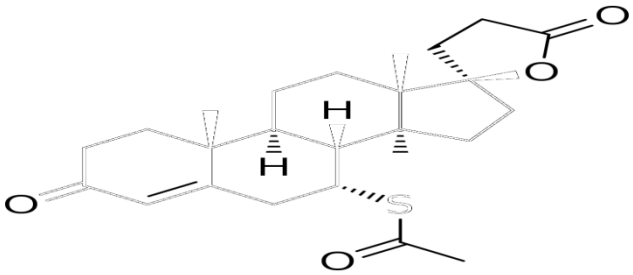
***Corresponding author:**Chaitali Dhale
*Associate Professor JBVP's Vidya Niketan College of Pharmacy, Lakhewadi
Email- chaitalidhalevncop@gmail.com

Abstract
A reversed-phase liquid chromatographic method has been developed and validated for estimation of Spironolactone in Pharmaceutical Dosage Form. RP-HPLC method, Column used was 150 x 4.6mm 5µ C18, Hypersil BDS with mobile phase containing 10mM Potassium dihydrogen Phosphate + 1% TEA (pH 4.5 Adjusted using Ortho phosphoric acid): Acetonitrile (50:50). The flow rate (1.0 ml/min) and wavelength (226 nm). The retention time of Spironolactone was found to be 4.509 min, respectively. Correlation co-efficient for Spironolactone was found to be 0.999. Assay resultof marketed formulation was found to be in 99.7% for Spironolactone
Olamine. The proposed method was validated with respect to linearity, accuracy, precision and robustness. Percentage recovery for Spironolactone was found to be 99.7 –100.0%. Analysis proves that the developed method was successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

Keywords: Analytical method development, validation, Spironolactone , HPLC, ICH guidelines.

INTRODUCTION

The IUPAC name of Spironolactone is 7α-Acetylthiospirolactone; 7α-Acetylthio-17α-hydroxy-3-oxopregn-4-ene-21-carboxylic acid γ-lactone. With molecular formula and molecularweight C29H36N6O6 and 564.64 g/mol respectively.



Spironolactone is used primarily to treat heart failure, edematous conditions such as nephrotic syndrome or ascites in people with liver disease, essential hypertension, low blood levels of potassium, secondary hyperaldosteronism.

MATERIALS AND METHODS

1. Chemicals

The Spironolactone reference standard (RS) was gifted by Hetero drugs Ltd Hyderabad. The Revolade (Spironolactone 50mg) tablet marketed drug GlaxoSmith Kline Ltd, purchased from Pune. All solvents (HPLC grade) were provided by our college.

2. RP-HPLC Instrumentation

Jasco PU-2080Plus HPLC system were used. SPD-10 detector (SPD- M20A, Japan). A Zodiac C (18) column (50 mm x 4.6 mm, 5 µm) with pore size 95Å. The column temperature was maintained at 27ºC and the flow rate was 1ml/min. The injection volume was 20 µl, 226 nm was set as a wavelength and the HPLC run time was set for 10 minutes.

3. METHOD

- **Chromatographic method.**

Methods Working Standard preparation

Solution Preparation of Spironolactone : (25 µg/ml)

About 2.5mg of Spironolactone was dissolved in 100mL of water with 15minutes sonication toachieve 25µg/mL concentration of Spironolactone .

- **Sample Preparation for marketed formulation**

Transferred 1 tablet in to 100mL volumetric flask and added 70-75 mL of diluent, sonicated for 45 minutes and then it was shaked for 30 minutes by mechanical means, tablet was checked visually if it got dispersed and then volume was made up to mark with diluent and mixed well. The solution was filtered through 0.45 µ PVDF filter. Further 5mL of filtrate wastransferred to 50mL of volumetric flask and volume was made up to mark with diluent.

METHOD VALIDATION

Chromatographic conditions and System Suitability ParametersChromatographic parameters:

Use suitable High Performance Liquid Chromatography equipped with following:

Column	:	Zorbax SB C8, 5µ 4.6 X 150 mm
Flow rate	:	1.5 mL/min
Wavelength	:	230 nm
Injection volume	:	20 µL
Column oven temperature	:	30ºC
Sample compartment temperature	:	25ºC
Run time	:	10 minutes

System Suitability Parameters

Table 1: System Suitability Test Parameters for Spironolactone .

Sr. No.	System SuitabilityParameters	Spironolactone
1	Retention Time(min)	5.3
2	Theoretical plate number (N)	23568
3	Tailing factor ()	**1**

Precision Repeatability

Five replicate of 25µg/ml concentration of Spironolactone were prepared andchromatographic were recorded at the optimized condition. SD and RSD were calculated.

Accuracy (% Recovery)

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 5 tablet powder were weighed and analysis was carried out as per assay. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

RESULT

Validation ParameterLinearity and Range

Linear correlation was obtained between peak area and concentration of Spironolactone in the range of 13.5-38.5 µg/ml. The linearity of the calibration curves was validatedby the value of correlation coefficients of the regression (r).

Table 2: Linearity data for Spironolactone .

% LinearityLevel	Concentration (µg/ml)	Mean area	Correlation Coefficient
50	13.5	3784448	
80	21.0	6064026	
100	26.0	7583549	0.999
120	31.0	9098754	
150	38.5	11424543	

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at threelevel of standard addition. Percentage recovery for Spironolactone was found to be 99.8 – 100

Table 3: Recovery Data of Spironolactone .

Accuracy Level %	Set No.	Amount Added (mg)	Amount recovery (mg)	% Recovery	Mean	% RSD
80	1	20	19.84	99.3	99.9	0.8
	2	20.24	20.30	100.2		
	3	19.80	19.75	99.6		
100	1	25.30	25.43	100.7	100.6	0.6
	2	25.24	30.03	101.5		
	3	24.75	24.72	99.9		
120	1	30.13	30.10	99.9	100	0.7
	2	30.27	30.05	99.5		
	3	29.98	30.18	100.9		

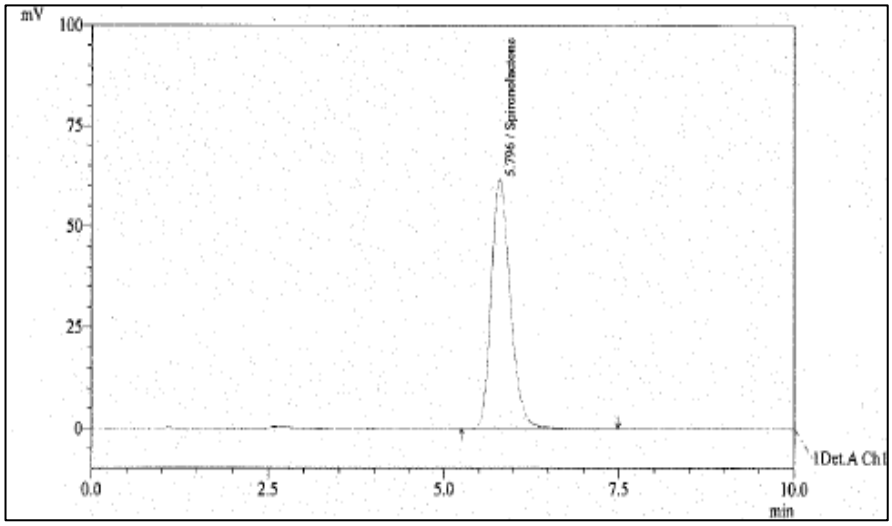


Figure 2: Overlay chromatogram of different concentration of Spironolactone .

PRECISION

Repeatability (Method precision, n=6)

Table 4: Repeatability of Spironolactone .

Sr. No.	Area	Mean	SD	%RSD
1	7571264	7553265	7742.2	0.1
2	7548865			
3	7548923			
4	7568923			
5	7581282			

Limit of Detection and Limit of Quantification

Table 5: The Limit of detection (LOD) and Limit of quantitation (LOQ) of Spironolactone as mention below.

Drug	Spironolactone
LOD	0.15
LOQ	0.50

Assay preparation Of Marketed formulation

Label claim: Spironolactone -25mg.

Sample preparation of marketed formulation

Transferred 1 tablet in to 100mL volumetric flask and added 70-75 mL of diluent, sonicated for 45 minutes and then it was shaked for 30 minutes by mechanical means, tablet was checked visually if it got dispersed and then volume was made up to mark with diluent and mixed well. The solution was filtered through 0.45 µ PVDF filter. Further 5mL of filtrate wastransferred to 50mL of volumetric flask and volume was made up to mark with diluent and it was injected.

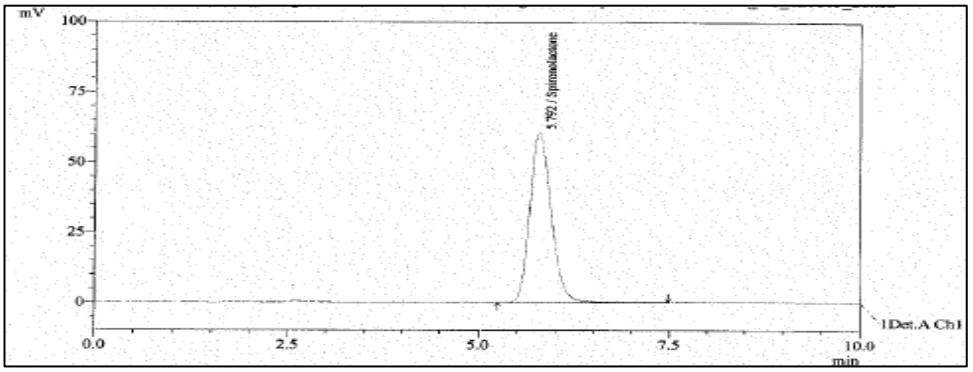


Table 6 Sample of marketed formulation.

Sr.No.	Peak	Retentiontime	Area	TailingFactor	Theoreticalplates	Resolutiontime
1	Spironolactone Olamine	4.5	7528769	1.0	12785	-

OBSERVATIONS

In formulation sample preparation, peaks are found symmetrical with good peak shape.

Table 7: % Assay Results of Formulation.

Sr. No.	Sample name	%Assay of marketed formulation
1	Revolade 25mg	98.7

DISCUSSION

A simple, accurate and precise RP-HPLC method for the estimation of Spironolactone in Pharmaceutical Dosage form has been developed and validated. 10mM Potassium dihydrogen Phosphate (pH 4.5, adjust with 1% Orthophosphoric acid): Acetonitrile (50:50% v/v) Separation of drugs was carried out using mobile phase at 10 min. run time and 226 nm. The Rt value for Spironolactone was found to be 4.509 ± 0.01 min. respectively.

The drug response with respect to peak area was linear over the concentration range 13.5- 38.5µg/ml Spironolactone. The percentage recovery of Spironolactone was found to be 98.7-100% respectively.

The %RSD values for intra-day precision study and inter-day study were ≤ 2.0%, confirming that the method was sufficiently precise.

So it is concluded that the developed method is specific. The system test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the estimation of Spironolactone in pharmaceutical dosage form.

CONCLUSION

The proposed method being reported for the assay of Spironolactone in pure form and also inits formulation is simple and inexpensive. This method was simple, rapid, accurate and precise. Thus this approach could be considered for the analysis of this drug in the quality control laboratories.

REFERENCES

1. Dr.Kasture AV., Wadodkar SG., Mahadik KR., "Introduction to Instrumental Techniques"; 12th Edn, Nirali Prakashan, Pune, 2002.
2. Syeda Kulsum, G Vidya Sagar, K. Nagalakshmi, R. Snehalatha et al, Development And Validation Of Rp-Hplc Method For Estimation Of Candesartan From Tablet Dosage Form. World Journal Of Pharmacy And Pharmaceutical Sciences, 3(4): 781-786.
3. Harshal A. Pawar and K. G. Lalitha et al, Development and Validation of a Novel RP HPLC Method for Estimation of Losartan Potassium in Dissolution Samples of Immediate and Sustained Release Tablet. Chromatography Research International, 2014; 2014.
4. ICH of technical requirements for the registration of pharmaceutical for the human use, validation of analytical procedures: Text and methodology, 2005; ICH: Q2(R1).
5. Ranjit Singh, J Pharm Educ Res., 2013; 4(1): 26-33.
6. Gunasekar Manoharan Development and Validation of a stability indicating RP-HPLC method for the estimation of same in bulk and tablet formulation, Asian Journal of Chemical Sciences, 2018; 4(1): 1-9
7. Takkis K, Aro R, Kõrgvee LT, Varendi H, Lass J, Herodes K and Kipper K: Signal Enhancement in the HPLC-ESIMS/MS analysis of spironolactone and its metabolites using HFIP and NH 4 F as eluent additives. Analytical and bioanalytical chemistry 2017; 409(12) 3145-3165