Quantification of Spironolactone by first and second order UV Derivative Spectrophotometry in bulk and tablet dosage form

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Abstract

For determination of Spironolactone in pharmaceutical formulation, Simple, fast and reliable first and second order derivative UV spectrophotometric methods were developed. Spectrophotometrically, Spironolactone was determined by measuring the 1*D* and 2*D*-values at 226 nm and 262 nm using methanol as background solvent. The Calibration curves were shown linear within a concentration range from 5-35 µg/ml. The developed and validated method was easily applied to the analysis of the pharmaceutical tablet preparations. The percentage recoveries were found to be 100% for given methods. The excipients did not interfere in the analysis. The results showed that this method can be used for rapid determination of Spironolactone in pharmaceutical tablet with specificity and accuracy.

Keywords: Zero crossing, First order Derivative, Second order Derivative UV spectrophotometry, Spironolactone, Validation.

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Introduction

Spironolactone ($C_{24}H_{32}O_4S$), chemically 7 α-acetylthio-3-oxo-17α-pregn-4-ene 21,17β carbolactone is a Diuretic[1] used in the treatment of Hypokalemia, Hypertension, Primary hyperaldosteronism, congestive heart failure and Cirrhosis and nephrotic syndrome.[2]

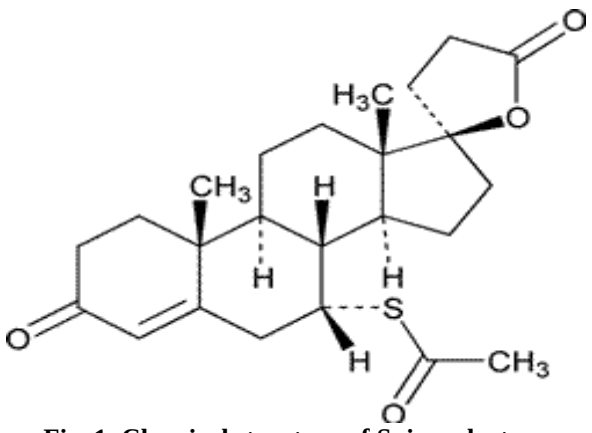

Fig. 1: **Chemical structure of Spironolactone**

Spironolactone is a specific pharmacological antagonist of aldosterone; it is acting through competitive binding of receptors at the aldosterone-dependent sodium-potassium exchange site in the distal convoluted renal tubule. Spironolactone causes increased amounts of water and sodium to be excreted, while potassium is retained. Spironolactone act as a diuretic and as an antihypertensive drug by this mechanism. It may be given alone or with other diuretic agents that act more proximally in the renal tubule.[3] Literature survey reveals that the analytical method reported for quantitative estimation of the Spironolactone are UV[4-7], HPLC[8-11], HPTLC[12-13], TLC[14], UPLC[15] individually or in combination. This revealed that no analytical method was reported for the Quantitative estimation of the Spironolactone by UV derivative spectrophotometry. Derivative UV–VIS spectrophotometry involves calculating and plotting one of the mathematical derivatives of a spectral curve, which offers an alternative approach to drug analysis. So, the development and validation of the Spironolactone by highly sensitive UV spectroscopic method for the estimation of Spironolactone in bulk and pharmaceutical dosage form.

Materials and Methods

Spironolactone [Bulk Drug] was generously gifted by Chemdyes Corporation Ahmedabad; Methanol AR grade was used throughout the analysis. Aldactone tablet containing 50mg of Spironolactone was used for the analysis.

Instruments: A double beam Agilent Cary 60 UV-visible spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Data were analyzed by Cary win software.

Preparation of Standard Stock Solution

Accurately weighed Spironolactone (10.0 mg) was transferred to 100 ml volumetric flask, dissolved in about 50 ml of methanol and volume was made up-to 100 ml with methanol to obtain stock solution of 100 µg/ml.

Preparation of calibration curve for Spironolactone

Method A: Zero order spectroscopic method: From the standard stock solution, 0.5 ml, 1.0 ml, 1.5 ml,2.0 ml, 2.5 ml, 3.0 ml and 3.5 ml were pipette out into 10 ml volumetric flasks and volume was made up to the mark with methanol to produce the concentrations ranging from 5-35µg/ml respectively. The analytical wavelength was selected by scanning 10 µg/ml in the wavelength range of 400-200 nm using methanol as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 237.02 nm and the corresponding UV spectrum was shown in the figure 2 and the overlay spectrum was shown in figure 3. Then, the calibration curve was plotted in the concentration range of 5-35 µg/ml at 237 nm by taking concentration on X-axis and absorbance on Y-axis. The correlation coefficient (r^2) was found to be 0.9994. The calibration curve of Spironolactone was shown in Fig. 4.

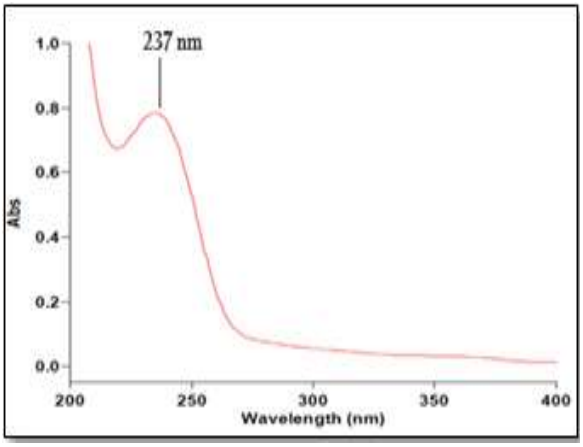

Fig. 2: Absorbance maxima of Spironolactone

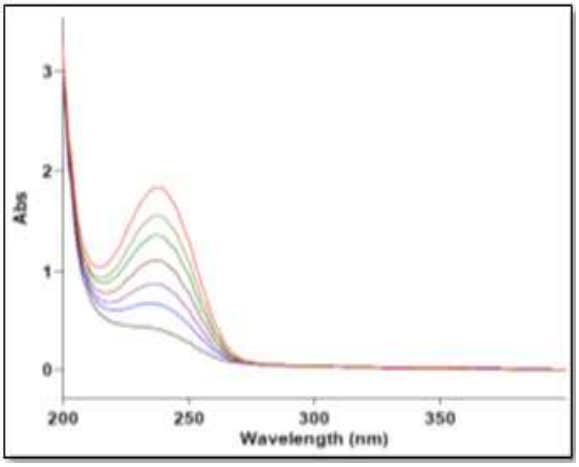

Fig. 3: Overlay spectrum of Spironolactone (Zero order)

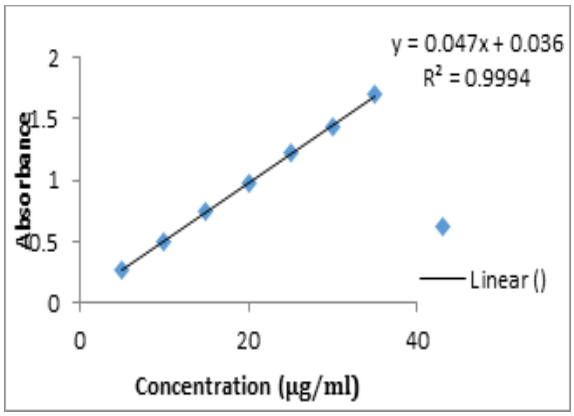

Fig. 4: Calibration curve of Spironolactone (zero order)

Method B: First order derivative spectroscopic method

For the selection of analytical wavelength solution of 10 µg/ml was scanned in the spectrum mode in the wavelength of 200-400nm and the absorption spectra thus obtained was derivatized in the first order. First order derivative spectrum showed one sharp positive peak at λ max of 226 nm and one sharp negative peak at 253 nm, the corresponding spectrum was given in the Fig. 5. The amplitude of absorbance was measured for all solutions in the concentration range of 5-35 µg/ml at 226 nm and was plotted against concentration for getting the calibration curve and the regression equation was calculated. The calibration curve for the first order derivative spectra was shown in the Fig. 6.

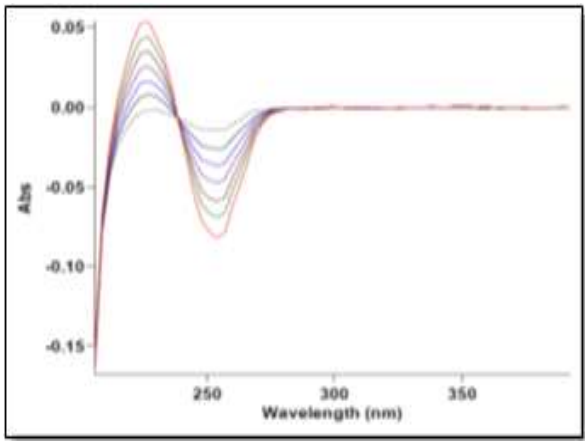

Fig. 5: First order derivative spectrum of Spironolactone

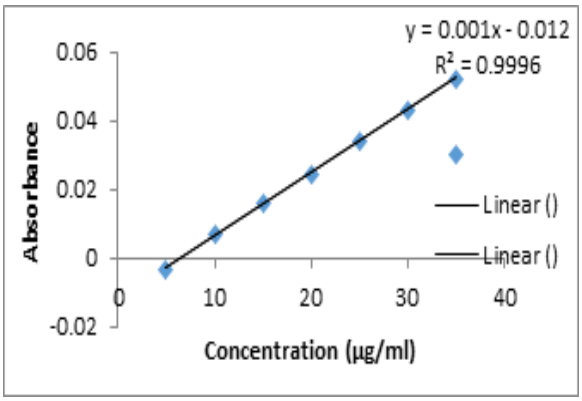

Fig. 6: Calibration curve of Spironolactone (first order)

Method C: Second order derivative spectroscopic method

For the selection of analytical wavelength solution of 10 µg/ml was scanned in the spectrum mode in the wavelength of 200-400nm and the absorption spectra thus obtained was derivatized in the first order. Second order derivative spectrum showed two sharp positive peak at λ max 217 and 262 nm and one sharp negative peak at 240 nm, the corresponding spectrum was given in the figure 7 but at 217 nm the peak obtain was not so sharp and linear so the amplitude of absorbance was measured for all solutions in the concentration range of 5-35 µg/ml at 262 nm and was plotted against concentration for getting the calibration curve and the regression equation was calculated. The calibration curve for the first order derivative spectra was shown in the Fig. 8.

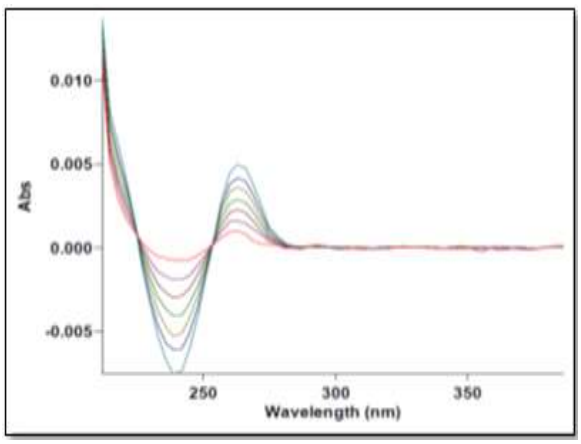

Fig. 7: Second order derivative spectrum of Spironolactone

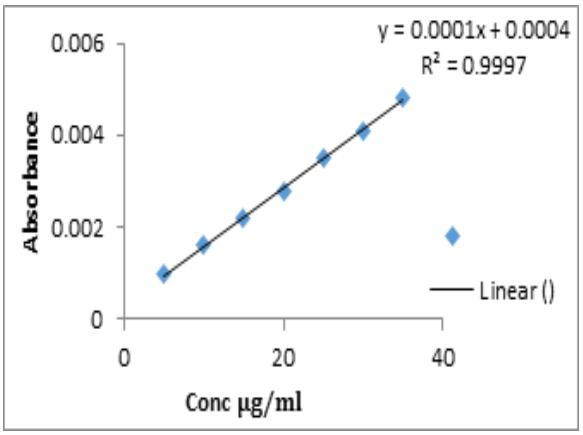

Fig .8: Calibration curve of Spironolactone (second order)

Estimation of Spironolactone in tablet formulation: For estimation Spironolactone in tablet formulation, 20 tablets of marketed brand of Aldactone containing 50 mg of Spironolactone was weighed and triturated to fine powder. Amount of powder equivalent to 10 mg drug was taken and dissolved in 50 ml of methanol and made up to the mark with methanol in 100 ml volumetric flask (100 µg/ml). From that stock solution further dilution was made with methanol to get required concentration. It was filtered through whatmann filter paper no. 41. The concentration of Spironolactone was determined by measuring the absorbance of sample solution at 237 nm. The assay procedure was repeated three times (n=3). The result of marketed formulation was given in Table 1.

Table 1: Assay of the Marketed Formulation

Conc. (µg/ml)	Mean of Absorbance	Label claim (mg)	Amount obtained ± SD	% Assay ± SD
10	0.5075	50	49.93	99.86

Validation of the proposed method: Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its

predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision and robustness.

Linearity and range: The linearity of the proposed UV spectroscopic methods was evaluated by plotting absorbance against concentrations of the analytes. Beers law was obeyed for all the given two methods in the concentration range of 5-35 µg/ml. The correlation coefficient values were found to be 0.9994, 0.9996, 0.9997 for zero, first and second order spectroscopy respectively. All the results were given in the Fig. 4, 8 and 6 respectively. The limit of detection (LOD) is the lowest concentration of an analyte in a sample that can be detected and the limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be quantitated. Both LOD and LOQ were experimentally verified and calculated using the following equation. Results were reported in table 2.
LOD = 3.3 (SD/Slope)
LOQ = 10 (SD/Slope)

Table 2: Linearity studies of Spironolactone by proposed methods

Sr. No.	Parameter	Method A	Method B	Method C
1	Linearity and range	5 – 35 µg/ml	5 – 35 µg/ml	5 – 35 µg/ml
2	Y – intercept	0.036	0.012	0.0004
3	slope	0.047	0.001	0.0001
4	Regression coefficient (R^2)	0.9994	0.9996	0.9997

Accuracy: The accuracy of the method was assessed, based on recovery study. The technique of standard addition was used to assess accuracy of the method. For this purpose a concentration of 5.0 µg/ml was selected to prepare the sample matrix of the sample drug. Again 0.5 ml of sample was taken in three, 10 ml volumetric flasks. To these three flasks 1.0 ml, 2.0 ml and 3.0 ml of standard stock solution of API mixture of Spironolactone was added and volume was made up to 10 ml. The absorbance of the sample matrix and after standard addition was measured in triplicate. The results are reported in terms of %recovery in the Table 3.

Table 3: Recovery studies of Aceclofenac by proposed methods

Spiked Level (%)	Concentration Taken (µg/ml)	Amount Added (mg)	Total amount (mg)	Amount found (mg)		%Recovery	
				Method B	Method C	Method B	Method C
50	5	10	15	15.11	15.01	100.74	100.12
100	5	20	25	24.92	25.00	99.70	100.03
150	5	30	35	34.99	35.02	99.98	100.08

Precision: For intraday and interday precisions of the method, solutions of Spironolactone were prepared at three concentration levels 10, 20, 30 (µg/ml) each in triplicate. These solutions were analyzed respectively three times within one day and three consecutive days and the results are reported in terms of relative standard deviation (RSD) in the table no. 4 and 5.

Table 4: Intra-day and Inter-day Precision data of Spironolactone (First order)

Level (%)	Intraday (% RSD)		Interday (% RSD)	
	Day 1	Day 1	Day 2	Day 3
50	0.2532	0.1998	0.0654	0.0428
100	0.1286	0.0210	0.0690	0.0208
150	0.0351	0.0139	0.0140	0.0139

Table 5: Intra-day and Inter-day Precision data of Spironolactone (Second order)

Level (%)	Intraday (% RSD)		Interday (% RSD)	
	Day 1	Day 1	Day 2	Day 3
50	0.2226	0.1941	0.1888	0.1432
100	0.3564	0.3449	0.2245	0.0166
150	0.2226	0.0617	0.1120	0.0722

Robustness: The robustness of the proposed method was evaluated by varying method parameters such as different analyst (Analyst 1, Analyst 2), wavelengths (224, 228) for first order and wavelengths (260, 264) for second order derivative spectroscopy and using different instruments (Agilent UV, Helious α). One parameter was changed at a

time and each varied parameter was evaluated. The robustness was assessed by analyzing standard solutions of 20 µg/ml (n=6) and sample solution of Spironolactone and %RSD and %assay were calculated which were shown in Table 6 and 7.

Table 6: Robustness of Spironolactone (First order)

Parameter		% RSD	Content	Assay (%)
Analyst	Analyst 1	0.0642	19.97	99.87
	Analyst 2	0.0583	19.97	99.87
Wavelength	224 nm	0.0591	19.97	99.87
	228 nm	0.0760	19.96	99.84
Instrument	Cary win UV	0.0642	19.97	99.87
	Helious α	0.0589	19.96	99.84

Table 7: Robustness of Spironolactone (Second order)

Parameter		% RSD	Content	Assay (%)
Analyst	Analyst 1	0.2180	19.94	99.62
	Analyst 2	0.2169	19.92	99.62
Wavelength	260 nm	0.1033	19.96	99.82
	264 nm	0.0645	19.97	99.87
Instrument	Cary win UV	0.2180	19.94	99.62
	Helious α	0.1866	19.91	99.59

Results and discussion

Derivative spectrophotometry is based on a mathematical transformation of the spectra zero-order curve into the derivative spectra which allows a fast and precise resolution of a multi component mixture and overcomes the problem of overlapping of a multi component system. The methods developed in the present work provided a convenient and accurate way for the analysis of Spironolactone in bulk and in pharmaceutical dosage form. The absorbance maxima of Spironolactone was found to be 237.02 nm for the method A, the absorption maxima of first order derivative spectra was found to be 226 nm for method B and for method C the 262 nm was selected for the analysis. Linearity for all the three methods was observed in the concentration range of 5-35 µg/ml as shown in the Table 2. The assay of the three methods was found to be within the range of 98-102% as shown in the Table 1. The developed method was validated in terms of linearity, accuracy, precision and robustness in accordance with the ICH guidelines. The %recovery values vary from 98- 102% as shown in the Table 3. In given two methods both the intra-day and inter-day precision study the %RSD were found to be less than 2.0 indicating the good precision as shown in the Tables 4,5 respectively. Based on results obtained, it was found that the proposed methods were found to be specific, accurate, precise, robust and reproducible and can be employed for routine quality control analysis of Spironolactone in tablet dosage form.

Conclusion

First and second both derivative spectrophotometric methods were shown to be efficient and simple for determination of Spironolactone in Tablet dosage form. The use of polluting reagents was not required and requires relatively inexpensive equipment. All the validation parameters were found to be satisfactory including linearity, specificity, precision, accuracy, robustness. Hence, the method can be used successfully for routine analysis of pharmaceutical dosage form of Spironolactone.

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