International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.4, pp 1876-1885, April-June 2013

Simultaneous Determination And Validation Of Spironolactone And Furosemide By Second Order Derivative Method And Area Under Curve Method In Bulk Drug And Pharmaceutical Formulations

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Abstract: The proposed method involves Second order derivative spectroscopy and Area under curve (AUC) method. A novel, simple and rapid UV Spectrophotometric determination method for Simultaneous estimation of Spironolactone and Furosemide was successfully developed and validated in bulk and pharmaceutical formulation. First method is second order derivative method. It involves the measurement of absorbances at selected wavelengths. 238.0 nm and 277.0 nm were selected as working wavelength for the estimation of Spironolactone and Furosemide. Second method is Area under curve method. It involves the measurement of area at selected analytical wavelength ranges. Two analytical wavelength ranges selected were 228.0 nm to 248.0 nm (λmax of Spironolactone is 238.0 nm) and 267.0 nm to 287.0 nm (λmax of Furosemide is 277.0 nm) for the estimation of Spironolactone and Furosemide respectively in bulk drug and formulation. Both the methods showed linearity from 2–10 µg/ml and 0.8-4.0 µg/ml for Spironolactone and Furosemise respectively. Recovery studies showed that the method is accurate. Precision of the proposed methods were found to be within the acceptable limits. Thus the two proposed methods and results were validated according to ICH guidelines. So, the methods can be applied for routine analysis in bulk and pharmaceutical formulation.
Keywords: Second order derivative method, Area under curve method, Validation.

INTRODUCTION:

Spironolactone is a potassium-sparing diuretic used in the treatment of heart failure and ascites in patients with liver disease,low-renin hypertension, hypokalaemia and secondary hyper aldosteronism. Spironolactone is a specific antagonist for the mineralocorticoid receptors, thus it compete with aldosterone for its intracellular receptors, thereby reducing Na^+, water retention and K^+ secretion, resulting in increase in the volume of tubular fluid[1,2].

Spironolactone chemically is 7 α-acetyl thio-3-oxo-17 α-pregn-4-ene-21,17 β-carbolactone 4-chloro-N-furfuryl-5-sulphamoyl anthranilic acid with a molecular formula of $C_{24}H_{32}O_4S$ and molecular weight of $C_{24}H_{32}O_4S$. It is an official drug in Indian Pharmacoepia[3] and British Pharmacopoeia[4](Fig 1).

Furosemide is a loop diuretic used in the treatment of congestive heart failure and edema. Like other loop diuretics, Furosemide acts by blocking the Na^+-K^+-$2Cl^-$ symporter in the thick ascending

limb of the loop of henle, decreasing the sodium reabsorption from the tubular fluid resulting in increased water secretion into the tubule and hence reducing the blood pressure[5,6].

Furosemide chemically is 4-chloro-N-furfuryl-5-sulphamoyl anthranilic acid with a molecular formula of $C_{12}H_{11}ClN_2O_5S$ and molecular weight of 330.75g/mol. It is an official drug in Indian Pharmacopoeia and British Pharmacopoeia (Fig.2).

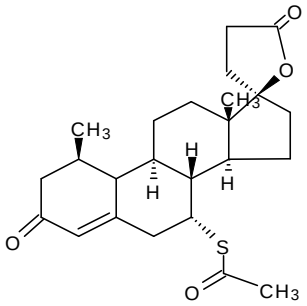

Fig.1.Chemical structure of Spironolactone.

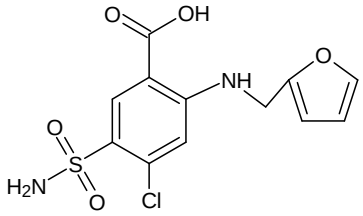

Fig.2.Chemical structure of Furosemide.

The combination of Furosemide and Spironolactone is very useful in the treatment of heart failure. Spironolactone prevents hypokalaemia due to Furosemide in their combined dosage forms. The combination is not only safe, but has low side effects[7,8].

On literature survey, it was found that only RP-HPLC and Ratio spectra derivative methods have been reported for the simultaneous estimation of Spironolactone and Furosemide in combined dosage forms and no method is available in the pharmacopoeia. In the view of the need in the industry for routine analysis of Spironolactone and Furosemide in formulation, attempts are being made to develop simple and accurate analytical methods for simultaneous estimation of Spironolactone and Furosemide and extend it for their determination in formulation.

EXPERIMENTAL

Instrument used:

For both the methods, Shimadzu model 1700 double beam UV-VIS spectrophotometer with spectral bandwidth of 1.8cm, wavelength accuracy of 2nm and a pair of 5 ml matched quartz cells of 10 mm optical path length was used as an instrument for spectral measurements. Analytically pure samples of Spironolactone and Furosemide were procured as gift samples from Lupin Ltd, Mumbai, India. Tablet formulation FRUSELAC containing Spironolactone (50 mg) and Furosemide (20 mg) was purchased from local market.

Solvent Used:

Methanol AR grade and distilled water was used as solvent.

Preparation of standard stock solution:

50 mg each of Spironolactone and Furosemide were weighed separately and transferred in two different 50 ml volumetric flasks. Both the drugs were dissolved in 25 ml of methanol by ultrasonication and then volume was made up to the mark with methanol in water(70:30) to obtain final concentration of 1000 µg/ml of each component (stock A and A' solution).

From the above stock A and A' solution 10 ml of aliquot was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with methanol in water (70:30) to obtain the final concentration of 100 µg/ml of each component (stock B and B' solution).

Preparation of sample stock solution using formulation:

From the powder of twenty tablets, a quantity equivalent to 100 mg of Spironolactone was weighed accurately and transferred to a flask containing 25 ml of methanol , ultrasonicated for 15mins, solution was filtered through whatmann filter paper no.41 into a 100ml volumetric flask, volume was made up to mark with distilled water to

get 1000 µg/ml (stock I). Aliquots were further prepared by diluting stock II (100 µg/ml) in solvvent to get a concentration of 2-10µg/ml.

Methodology:

Calibration curve

Method A: Second order derivative method[9-12]

Using appropriate dilutions of the standard stock solution, the solution was scanned in the wavelength region of 400 – 200 nm. The absorbance spectrum, thus obtained was derivatized to remove the interference of absorbing species. From the examination of the second derivative spectra of Spironolactone and Furosemide, 238 nm (λ_1) and 277 nm (λ_2) were selected as working wavelengths for the second order derivative spectroscopy (Fig.3 and 4). The calibration curve was plotted (Fig.5 and 6). Similarly absorbances of samples solution were measured and amount of Spironolactone and Furosemide was determined from standard calibration curve (Fig.7 and 8).

Method A: Area under curve method[13]

Area under curve method involves the measurement of area at selected analytical wavelength ranges. Using appropriate dilutions of the standard stock solution, the solution was scanned in the range from 400-200 nm and the analytical wavelength ranges selected were 228.0 nm to 248.0 and 267.0 nm to 287.0 nm for the estimation of Spironolactone and Furosemide respectively (Fig.9 and 10). Beer's law is obeyed in the concentration range of 2-10 µg/ml and 0.8-4.0 µg/ml for Spironolactone and Furosemide respectively and the calibration curve was plotted. (Fig.11 and 12). Similarly absorbances of sample solutions were measured and amount of Spironolactone and Furosemide was determined from AUC formula. (Fig.13 and 14).

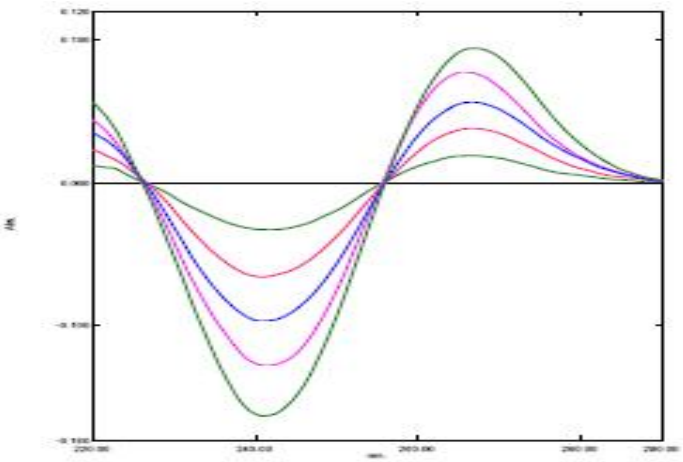

Fig.3. Second order derivative overlain spectra of SPR at 238.0 nm

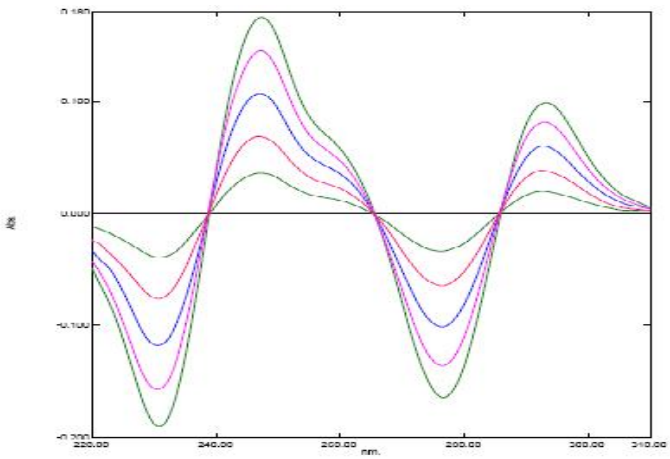

Fig.4. Second order derivative overlain spectra of FRS at 277.0 nm.

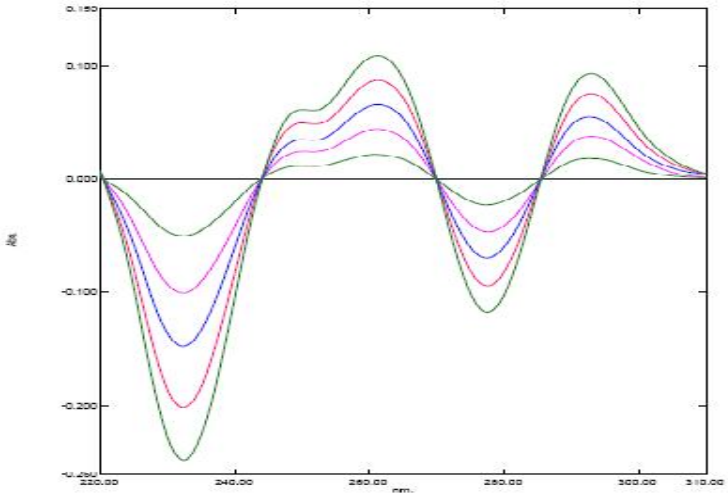

Fig.5. Second order derivative overlay spectra of formulation at 238.0 and 277.0 nm

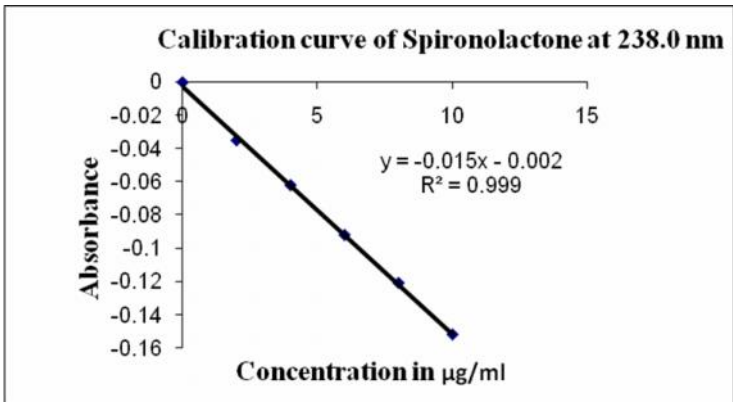

Fig.6. Calibration curve for Spironolactone at 238.0 nm by Second Order Derivative Method.

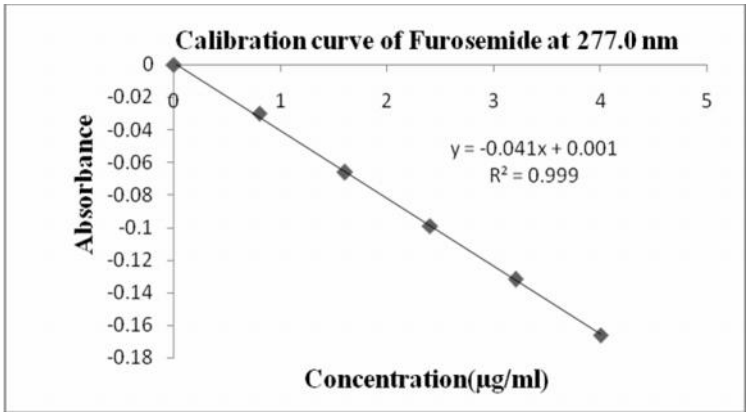

Fig.7. Calibration curve for Furosemide at 277.0 nm by Second Order Derivative Method.

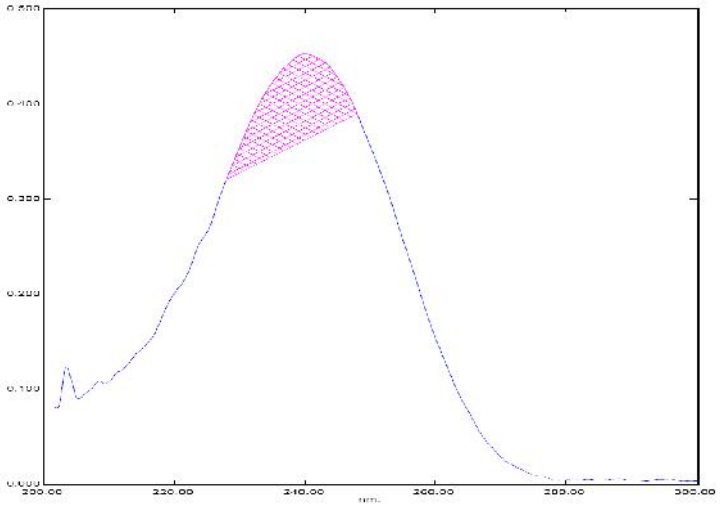

Fig.8. Area Under Curve for Spironolactone between the wavelength range of 228.0-248.0 nm.

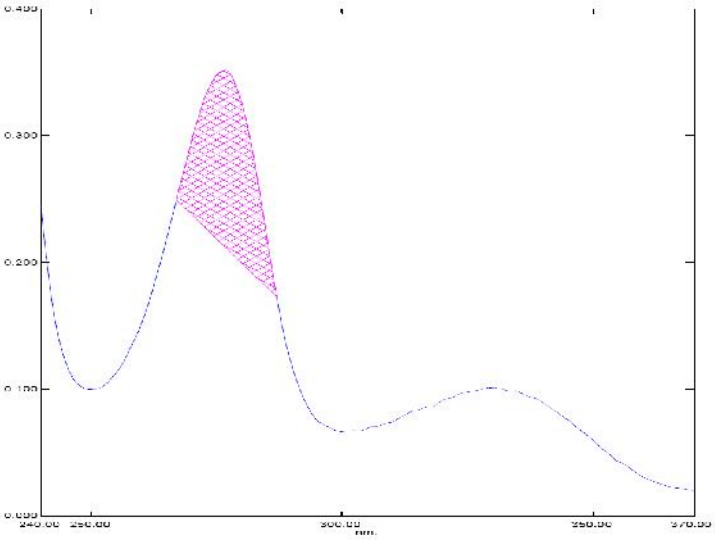

Fig.9. Area Under Curve for Furosemide between the wavelength range of 267.0-287.0 nm.

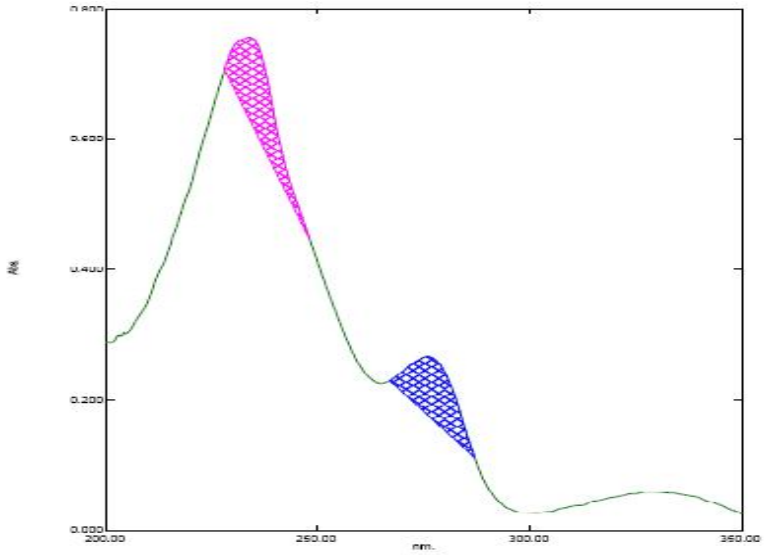

Fig.10. Area Under Curve for mixture of Spironolactone and Furosemide in formulation between the wavelength range of 228.0 to 248.0 nm and 267.0 to 287.0 nm in the Tablet dosage form.

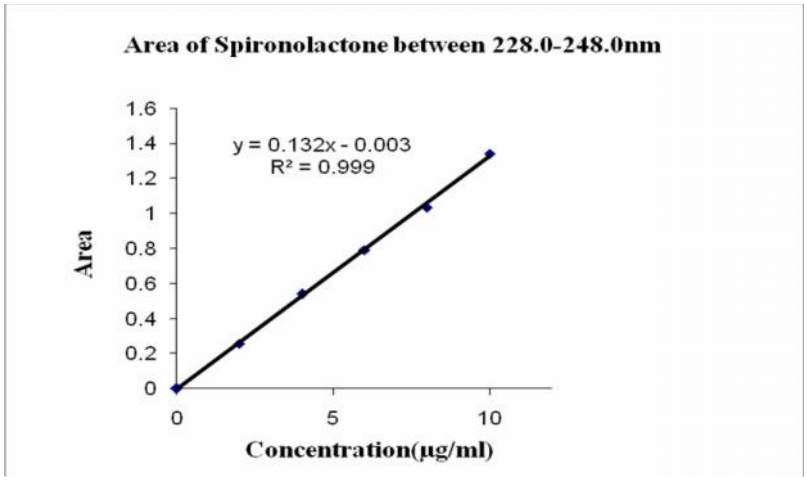

Fig.11. Calibration curve of for Spironolactone between 228.0 to 248.0 nm by Area Under Curve method.

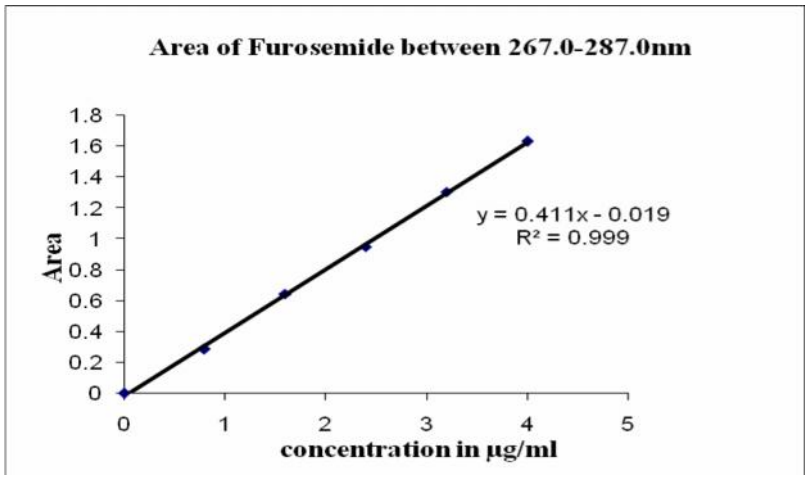

Fig.12. Calibration curve of Furosemide between 267.0 to 287.0 nm by Area Under Curve method.

Validation of methods[14,15]

The above methods were satisfactory in accordance to the ICH guidelines. Accuracy studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to previously analyzed tablet sample and the percentage recovery was calculated.

RESULTS AND DISCUSSION:

The absorption spectra for Spironolactone and Furosemide were recorded in the wavelength region of 200-400 nm for both method A and method B. The spectra were reported. These methods were found to be economic, simple, precise and accurate. Beer-Lambert's Law was obeyed in the concentration range of 2-10 µg/ml and 0.8-4.0 µg/ml for Spironolactone and Furosemide respectively. The accuracy was found by recovery studies at three different levels i.e.80%, 100% and 120%. The %RSD values are less than 2 for both the methods. The optical characteristics such as Beer's law limit, % relative standard deviation, limit of detection, limit of quantitation and range of errors in each method were calculated and the results were reported in Table 1,3 and Table 8,10. Also the regression characteristics like slope (m), intercept (c), and correlation coefficient (r) were calculated and are presented in same tables mentioned above. The results showed that the methods have reasonable precision and the results were reported in Table 6,7 and Table 13,14. The accuracy of the methods was confirmed by the recovery studies by adding known amount of the pure drug to the pharmaceutical formulation previously analyzed by this method and the results were reported in Table 4,5 and Table 11,12.

Table No. 1. Statistical data of Spironolactone and Furosemide at 238.0 and 277.0 nm by second order derivative method.

Parameter	SPR at 238.0 nm	FRS at 277.0 nm
Linear Range (µg/ml)	2-10	0.8-4.0
Slope	-0.015	-0.041
Intercept	-0.002	0.001
Regression co-efficient	0.999	0999
Limit of Detection (µg/ml)	0.0933	0.0355
Limit of Quantification (µg/ml)	*0.2828*	0.1076

Table No. 2: Assay Results of Tablet Formulation.

Sr. No.	Amount present in (mg/tab)		Amount obtained in (mg/tab)		% Obtained	
	SPR	FRS	SPR	FRS	SPR	FRS
1	50	20	49.50	19.86	99.00	99.30
2	50	20	49.71	19.69	99.42	98.45
3	50	20	50.30	19.78	100.60	98.90
4	50	20	49.82	20.10	99.64	100.50
5	50	20	50.10	19.89	100.20	99.45
6	50	20	49.59	19.96	99.18	99.80

Table No. 3: Statistical Validation Data for Tablet Formulation.

Components	Mean of % obtained*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
SPR	99.67	0.3080	0.6181	0.1257
FRS	99.40	0.1424	0.7163	0.0581

*n = 6

Table No. 4: Accuracy results of Spironolactone and Furosemide.

Level of % recovery	Amount of formulation (mg/tab)		Amount of standard drug added (mg)		Total amount recovered (mg)		% Recovery	
	SPR	FRS	SPR	FRS	SPR	FRS	SPR	FRS
80%	50	20	40	16	90.9	35.8	101.0	99.4
	50	20	40	16	89.6	36.2	99.5	100.5
	50	20	40	16	89.3	35.6	99.2	98.8
100%	50	20	50	20	99.76	40.2	99.76	100.5
	50	20	50	20	99.9	40.4	99.99	101.0
	50	20	50	20	99.4	40.3	99.4	100.75
120%	50	20	60	24	109.2	44.3	99.2	100.6
	50	20	60	24	109.8	43.8	99.81	99.5
	50	20	60	24	110.17	43.9	100.15	99.7

Table No. 5. Statistical Validation Data for Accuracy determination.

Level of % Recovery	Mean of % recovery*		Standard Deviation*		Co-efficient of Variation*		Standard Error*	
	SPR	FRS	SPR	FRS	SPR	FRS	SPR	FRS
80%	99.90	99.56	0.9643	0.8621	0.9653	0.8659	0.5567	0.4977
100%	99.71	100.75	0.2973	0.2500	0.2982	0.2481	0.1716	0.1443
120%	99.72	99.93	0.4813	0.5859	0.4827	0.5863	0.2779	0.3383

*n = 3

Table No. 6. Statistical Validation Data for Intra-day Precision.

Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
SPR	100.02	0.4622	0.4621	0.1887
FRS	99.84	0.8082	0.8094	0.3300

*n = 6

Table No. 7. Statistical Validation Data for Inter-day Precision.

Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
SPR	99.60	0.5125	0.5146	0.2093
FRS	99.57	0.5793	0.5817	0.2365

*n = 3

Table No. 8. Statistical data of Spironolactone and Furosemide 228.0 to 248.0 nm and 267.0 to 287.0 nm.

Parameter	SPR at 228 -248 nm	FRS at 267 - 287 nm
Linear Range (µg/ml)	2-10	0.8-4.0
Slope	0.132	0.411
Intercept	0.003	0.019
Regression co-efficient	0.9998	0.9990
Limit of Detection (µg/ml)	0.171	0.102
Limit of Quantification (µg/ml)	*0.518*	0.310

Table No. 9.: Assay Results of Tablet Formulation.

Sr. No.	Amount present in (mg/tab)		Amount obtained in (mg/tab)		% Obtained	
	SPR	FRS	SPR	FRS	SPR	FRS
1	50	20	49.50	19.86	99.30	99.60
2	50	20	49.71	19.69	99.52	98.45
3	50	20	50.30	19.78	100.60	98.90
4	50	20	49.82	20.10	99.64	100.50
5	50	20	50.10	19.89	100.20	99.45
6	50	20	49.59	19.96	99.26	99.90

Table No. 10. Statistical Validation Data for Tablet Formulation.

Components	Mean of % obtained*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
SPR	99.87	0.3060	0.6161	0.1246
FRS	99.44	0.1324	0.7363	0.0576

*n = 6

Table No. 11. Accuracy results of Spironolactone and Furosemide.

Level of % recovery	Amount of formulation (mg/tab)		Amount of standard drug added (mg)		Total amount recovered (mg)		% Recovery	
	SPR	FRS	SPR	FRS	SPR	FRS	SPR	FRS
80%	50	20	40	16	90.9	35.8	101.0	99.4
	50	20	40	16	89.6	36.2	99.5	100.5
	50	20	40	16	89.3	35.6	99.2	98.8
100%	50	20	50	20	99.76	40.2	99.76	100.5
	50	20	50	20	99.9	40.4	99.99	101.0
	50	20	50	20	99.4	40.3	99.4	100.75
120%	50	20	60	24	109.2	44.3	99.2	100.6
	50	20	60	24	109.8	43.8	99.81	99.5
	50	20	60	24	110.17	43.9	100.15	99.7

Table No. 12. Statistical Validation Data for Accuracy determination.

Level of % Recovery	Mean of % recovery*		Standard Deviation*		Co-efficient of Variation*		Standard Error*	
	SPR	FRS	SPR	FRS	SPR	FRS	SPR	FRS
80%	99.89	99.60	0.9642	0.8622	0.9653	0.8659	0.5570	0.4980
100%	99.74	100.60	0.2974	0.2501	0.2982	0.2481	0.1720	0.1439
120%	99.73	99.89	0.4814	0.5860	0.4826	0.5843	0.2769	0.3378

*n = 3

Table No. 13. Statistical Validation Data for Intra-day Precision.

Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
SPR	100.12	0.4592	0.4631	0.1890
FRS	99.79	0.8093	0.8089	0.3298

*n = 6

Table No. 14. Statistical Validation Data for Inter-day Precision.

Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
SPR	99.62	0.5128	0.5150	0.2089
FRS	99.60	0.5789	0.5820	0.2363

*n = 3

CONCLUSION

For routine analytical purpose it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. Chromatographic technique coupled with multivariate algorithms can generate large amount of quality data which serve as highly powerful and convenient analytical tool. In view of the need for a suitable method for routine analysis of Spironolactone and Furosemide in bulk and formulation, in the present work, an attempt was made to develop a newer, simple, accurate, precise and economic two spectrophotometric methods.

The methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed two spectrophotometric methods, second order derivative and area under curve method are new, simple, accurate, precise and economic and can be employed successfully for the estimation of Spironolactone and Furosemide in bulk and formulation.

ACKNOWLEDGEMENT

The author expresses their gratitude to thank Lupin Ltd, Mumbai, India for gift samples of pure drugs and for tablet formulation. Special thanks to National College of Pharmacy for providing laboratory facilities to carry out my research work.

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