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Research Article

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF FUROSEMIDE AND SPIRONOLACTONE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A simple, economical, precise and accurate method for simultaneous determination of Furosemide and Spironolactone in combined dosage form has been developed. First order Derivative spectroscopy method (Method A) and Absorbance Ratio (Q-Absorbance) method (Method B) were used. The amplitudes at 350 nm and 250.80 nm in the first order derivative spectra were selected to determine Furosemide and Spironolactone, respectively and wavelength ranges 261.21 nm (iso-absorptive point) and 276 nm (λmax of Furosemide) were selected for Absorbance ratio (Q- Absorbance) method. Beer's law is obeyed in the concentration ranges of 2-10 µg/ml and 5-25 µg/ml for Furosemide and Spironolactone for Derivative method as well as Absorbance ratio method. The % assay for commercial formulation was found to be in the range 98.25% – 102% for Furosemide and 98.8–100.9 % for Spironolactone by the proposed methods. Recovery was found in the range of 98.25–100.00 for Furosemide and 100.88–101.46 % for Spironolactone by first order derivative spectroscopic method and 99.24–102% for Furosemide and 98.8-100.55% for Spironolactone by Absorbance ratio method for both the Formulations. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed methods which were carried out according to ICH guidelines.

Keywords: Furosemide, Spironolactone, First order derivative spectroscopy, Q- Absorbance, Validation, Tablet.

INTRODUCTION

Furosemide is chemically 4-Chloro-2-(furan-2-ylmethylamino)-5-sulfa Moylbenzoic acid. Furosemide, an anthranilic acid derivative, is a potent diuretic that inhibits the active reabsorption of chloride in the diluting segment of the loop of Henle, thus preventing the reabsorption of sodium, which passively follows chloride. It is an official drug in IP & BP. Few analytical methods by RP-HPLC and spectrophotometric methods using pharmaceutical dosage forms have been reported for the estimation of Furosemide. Spironolactone is a synthetic 17-lactone drug that is a renal competitive aldosterone antagonist in a class of pharmaceuticals called potassium-sparing diuretics, used primarily to treat heart failure, ascites in patients with liver disease, low-renin hypertension, hypokalemia and Conn's syndrome. It is an official drug in IP and BP. There are very few analytical methods reported for the estimation of Spironolactone which includes HPLC, Spectrophotometry.

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. On literature survey, it was found that only Ratio Spectra Derivative Spectrophotometry method has been reported for the simultaneous estimation of Furosemide and Spironolactone in combined dosage form and no method is available in the pharmacopoeias.

MATERIALS AND METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (SHIMADZU 1800) with 10 mm matched quartz cells was used. Al weighing were done on electronic balance (Model Shimadzu AUW-220D), Ultrasonicator model 5.5L150H were used.

Reagents and chemicals

Spectroscopic grade Methanol was purchased from LOBA Chemie Pvt. Ltd., Mumbai. Tablet used for analysis was Spiromide manufactured by RPG Life sciences, Ankleshwar, India containing Furosemide 20mg and Spironolactone 50 mg per tablet. API of Furosemide was kindly supplied as a gift sample by Nucleus Heem-Deep organics, Ankleshwar, Gujarat. Spironolactone was gifted by Torrent RPG life sciences, Ankleshwar, Gujarat.

Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solution of pure drug containing 1000 µg/ml of Furosemide and 1000 µg/ml of Spironolactone were prepared separately in the methanol. These stock solutions were used to prepare series of solutions with conc. 2–10 µg/ml and 5-25 µg/ml of Furosemide and Spironolactone respectively for method A and method B and were used to prepare calibration curve.

Method A: First Order Derivative Spectroscopy

To determine derivative amplitude for Furosemide and Spironolactone solution of increasing concentrations of Furosemide and Spironolactone were prepared in combination and scanned in UV spectrum in the range 200 - 400 nm at 0.2 band width and 200 nm/min scan speed parameter. These spectrums were converted to first order derivative spectra by using instrument mode with filter size 5 and interval 1.0. After observing the derivative amplitude of first order derivative spectra, it was observed that the first derivative spectra of Furosemide and Spironolactone showed zero crossing points (Fig.1). The shape of the first derivative spectra is adequate for determining Spironolactone in the presence of Furosemide and vice versa. Spironolactone was determined by measurement of its D1 amplitude at the zero crossing point of Furosemide at 250.80 nm, While Furosemide was determined by measurement of its D1 at zero crossing point of Spironolactone at 350 nm.

Method B: Absorbance Ratio (Q – Absorbance) Method

It uses the ratio of absorbencies at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that Furosemide and Spironolactone show an isoabsorptive point at 261.21 nm (A1). The second wavelength used is 276 nm (A2), which is the λ-max of Furosemide. Five working standard solutions having concentration 2, 4, 6, 8, 10 µg/ml for furosemide and 5, 10, 15, 20, 25 µg/ml for Spironolactone were prepared in methanol and the absorbencies at 261.21 nm (isoabsorptive point) and 276 nm (λ-max of Furosemide) were measured and absorptivity coefficients were calculated using calibration curve.

Absorptivity = Absorbance/ Concentration of that component in gm/100 ml.

The concentration of two drugs in the mixture can be calculated using following equations.

$C_{Furosemide} = [(Q_M - Q_{Spiro}) / (Q_{Furo} - Q_{Spiro})] \times A_1/aX_1$..... (1)

$C_{Spironolactone} = (A_1/aX_1) - C_{Furo}$ ..... (2)

Where, A_1 and A_2 are absorbencies of mixture at 261.21 nm and 276 nm;

aX_1 and aY_1 are absorptivities of Furosemide and Spironolactone at 261.21 nm;

aX_2 and aY_2 are absorptivities of Furosemide and Spironolactone respectively at 276 nm.

Where,

$Q_M = A_2 / A_1$,

$Q_{Furo} = aX_2 / aX_1$ and

$Q_{Spiro} = aY_2 / aY_1$.

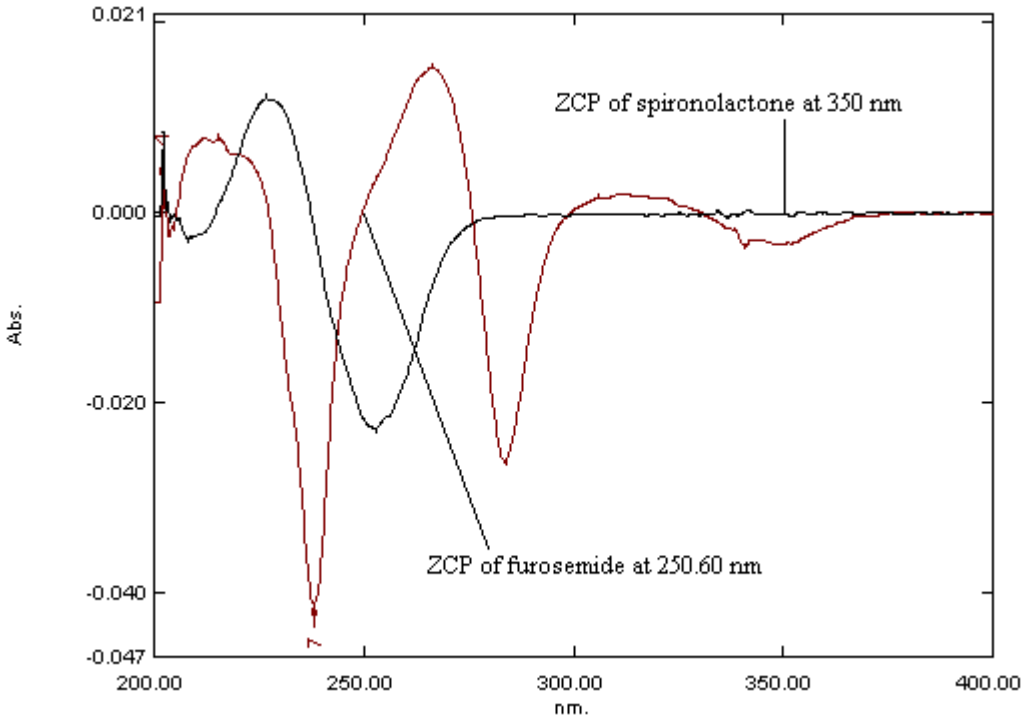

Fig. 1: Overlay derivative spectra of furosemide 4 µg/ml and spironolactone 10 µg/ml

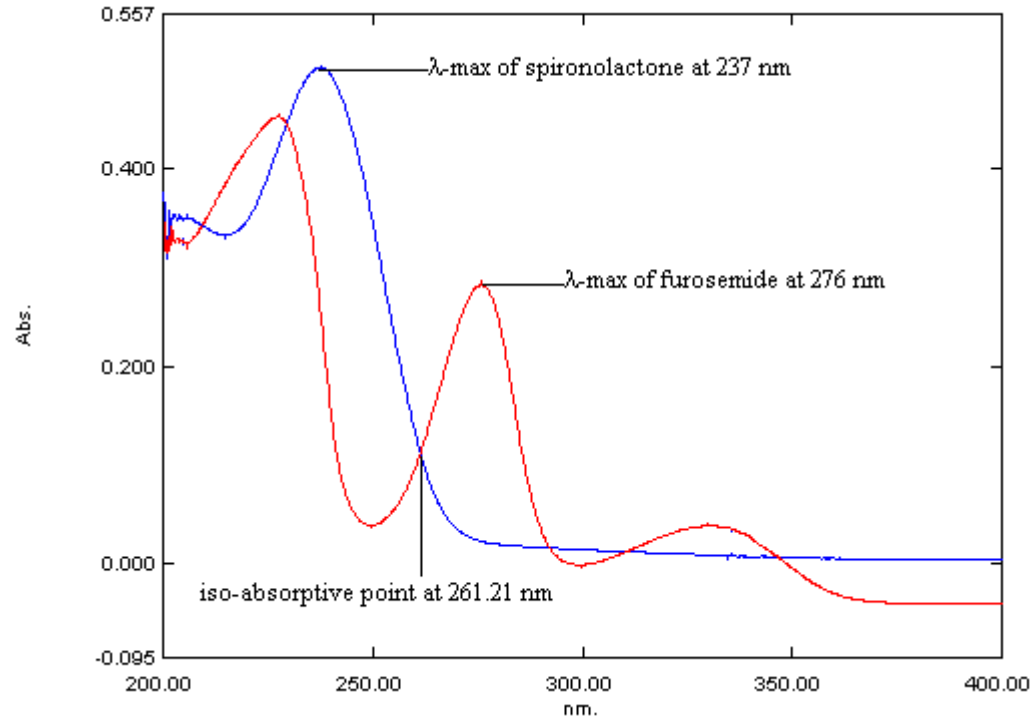

Fig. 2: Overlay spectra of furosemide 4 µg/ml and spironolactone 10 µg/ml

Preparation of Sample Stock Solution and Formulation analysis

A quantity of powder from twenty tablets / capsules equivalent to 20 mg of furosemide (50 mg of Spironolactone) was weighed and transferred to 25 ml flask containing 20 ml of methanol and ultrasonicated for 30 min and centrifuged for 10 min at 10000 RPM. Supernatant was transferred to 25 ml volumetric flask and volume was made up to mark. The solution was filtered and suitably diluted with methanol to have 4 µg/ml of Furosemide (10 µg/ml of Spironolactone) for method A and method B, respectively and samples were analyzed by the proposed methods. To determine uniformity of content ten tablets were analyzed individually by following above procedure and assay values were calculated.

Precision of the Method

To study intraday precision, method was repeated 5 times in a day and the average % RSD was calculated by method A and B, respectively. Similarly the method was repeated on five different days and average % RSD was calculated (Table 1). These values confirm the intra and inter day precision.

Recovery studies

The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs (Table 2).

Table 1: Results of Validation Parameters for First Order Derivative Method

Parameters	Furosemide	Spironolactone
Beer's law range	2-10 µg/ml	5-25 µg/ml
Wavelength (nm)	350	250.60
Correlation Coefficient	0.998	0.998
Slope	0.0008	0.0022
Intercept	0.0002	0.0005
LOD (µg /ml)	0.825	0.75
LOQ (µg /ml)	2.475	2.26
% RSD		
Intraday precision	0.29-1.37	0.28-1.35
Interday precision	0.8-1.62	0.9-1.64

LOD=limit of detection; LOQ=limit of quantification; (%RSD) = % relative standard deviation

Table 2: Results of Validation Parameters for Absorbance ratio method

Parameters	Furosemide	Spironolactone
Beer's law range	2-10 µg/ml	5-25 µg/ml
Wavelength (nm)	276	237
Correlation Coefficient	0.996	0.9938
Slope	0.075	0.062
Intercept	-0.017	0.065
LOD (µg/ml)	0.025	0.931
LOQ (µg/ml)	0.076	0.093
% RSD		
Intraday precision	0.09-1.181	0.863-1.585
Interday precision	0.096-1.623	0.044-1.22

Table 3: Results of simultaneous estimation of marketed formulation for Method A & B

Method	Label claim (mg)		Amount found (mg)*		% Label claim	
	Furo	spiro	Fur	Spiro	Furo	Spiro
A	20	50	19.65	50.45	98.25	100.9
B	20	50	20.4	49.4	102.00	98.8

*Each value is a mean of six observations.

Table 4: Results of Recovery studies of Furosemide and Spironolactone

Method	Level of recovery	Amount taken(µg/ml)		Amount added (µg/ml)		Total amount found (µg/ml)*		% recovery	
		Furo	Spiro	Furo	Spiro	Furo	Spiro	Furo	Spiro
1	0%	4	10	0	0	3.937	10.09	98.25	100.9
	50%	4	10	2	5	6.00	15.22	100	101.46
	100%	4	10	4	10	7.87	20.22	98.37	101.1
	150%	4	10	6	15	9.87	25.22	98.7	100.88
2	0%	4	10	0	0	4.08	9.88	102.00	98.8
	50%	4	10	2	5	6.0168	14.814	100.28	98.76
	100%	4	10	4	10	7.98	19.95	99.86	99.76
	150%	4	10	6	15	9.924	25.13	99.24	100.55

*Each value is a mean of three observations

CONCLUSION

These validated methods are new, rapid, accurate, precise, sensitive, and reproducible and can be employed for routine analysis for simultaneous estimation of Furosemide and Spironolactone in combined dosage form.

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