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Research Article

SIMULTANEOUS ESTIMATION OF FUROSEMIDE AND SPIRONOLACTONE IN COMBINED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A new, simple, rapid, accurate, precise and sensitive method has been developed for the simultaneous estimation of Furosemide and Spironolactone in their combined tablet dosage form. The method was carried out on a Hiber C_{18} column (250 mm×4.6mm, i.d.5 µm) with a mobile phase consisting of Acetonitrile: Water at a flow rate of 1 ml/min and the detection was carried out at 237 nm. The retention time of Furosemide and Spironolactone was 3.81 min and 7.28 min. respectively. Linearity for Furosemide and Spironolactone were found in the range of 2-10 µg/ml and 5-25 µg/ml respectively. The developed method was validated in terms of linearity, accuracy, and precision, limit of detection (LOD) and limit of quantification (LOQ). The proposed method can be used for estimation of both drugs in their combined dosage form.

Keywords: Furosemide, Spironolactone, RP- HPLC, 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.

MATERIAL AND METHOD

Chemicals and reagents

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.

Instrumentation and chromatographic condition

The LC system (YL-9100) consisted of following components: YL9160 PDA detector, YL9101 vacuum degasser and YL9110 quaternary solvent delivery pump. Chromatographic analysis was carried out on a Hiber C_{18} column (250 mm×4.6mm, i.d.5 µm) using mobile phase Acetonitrile : Water with flow rate of 1ml/min. Detection of eluent was made at 237 nm by PDA detector. The column was maintained at room temperature and injection volume of 20µl was used. The mobile phase was filtered through 0.45µm Chrom Tech Nylon-66 filter paper.

Preparation of standard solution

Standard stock solution of pure drugs were prepared separately by dissolving 10 mg of each drug with Acetonitrile in 10 ml of volumetric flask and made up to volume to get concentration of 1000 µg/ml. 1 ml from stock solution of Furosemide and 1 ml from stock solution of Spironolactone were transferred in 10 ml of volumetric flask and made up to volume with Acetonitrile separately to get a concentration of 100 µg/ml. 0.4 ml stock solution of furosemide

And 1 ml stock solution of spironolactone were mixed in 10 ml volumetric flask and made up to volume with Acetonitrile to get concentration of 4 µg/ml of Furosemide and 10 µg/ml of Spironolactone.

Preparation of sample solution

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 50 mg of Spironolactone was transferred to 50 ml volumetric flask containing 40 ml of mobile Acetonitrile, gentle shaking was carried out for 5min and ultrasonicated for 5 min. The volume was made up to the mark with Acetonitrile. The tablet sample solution was filtered through Whatman filter paper no.41. 1 ml of filtrate was further diluted to 10 ml with Acetonitrile to get 100 µg/ml concentrations. From the above solution 1 ml was further diluted to 10 ml with mobile Acetonitrile to get the final concentration 10 µg/ml. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present in tablet was estimated from their respective calibration curve (Table-1).

Table 1: Assay results of combined dosage form

Drug	Label claim (mg)	Amount found (mg)*	% Label claim
Furosemide	20	20.29	101.45
Spironolactone	50	49.55	99.1

***Each value is a mean of six observations.**

Method validation

The method was validated for linearity, accuracy, intraday and interday precision, LOD and LOQ, in accordance with ICH guidelines.

Linearity

Aliquots of 0.2,0.4,0.6,0.8 and 1 ml from100 µg/ml standard solution of Furosemide and aliquots 0.5, 1, 1.5, 2 and 2.5 ml from 100 µg/ml standard solution of Spironolactone transferred to series of 10 ml volumetric flasks and made up to volume with Acetonitrile. Each solution was injected and chromatogram was recorded. Retention time (mean ± s.d) of Furosemide and Spironolactone were found to

be and min respectively. The peak area of Furosemide and Spironolactone in each chromatogram was recorded.

s.d= standard deviation.

Accuracy

To study accuracy of the method, recovery studies were carried out by addition of standard drug sample in a tablet sample at 50%, 100% and 150%. The percentage of recovery was calculated (Table-3).

Precision

It was carried out by preparing 3 replicates of 3 different concentrations within the linearity range and then injecting each solution. The peak area of Furosemide and Spironolactone in each chromatogram was recorded in order to record any intra day variation. To record inter day variation, 3 different concentration solution within the linearity range were analyzed for 3 different days. The peak area of each drug was recorded and % RSD (% relative standard deviation) was calculated for both series of analysis.

Limit of detection (LOD) and limit of quantification (LOQ)

They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y- intercept) and S is the mean of the slope of calibration plot.

RESULTS AND DISCUSSION

For RP-HPLC method, several different mobile phases were tried and finally mobile phase containing Acetonitrile: water was found to be optimized and well defined. Resolved peaks of Furosemide and Spironolactone with retention time (mean ± s.d.)3.81 min and 7.28 min were obtained for Furosemide and Spironolactone respectively. The representative chromatogram of sample solution of Furosemide (4 µg/ml) and Spironolactone (10 µg/ml) is shown in Fig 1. The calibration curve for each drug was obtained separately by plotting as peak area → concentration over the range of 2-10 µg/ml for Furosemide and 5 -25 µg/ml for Spironolactone. From, calibration curve of Furosemide (Fig 2), it was found to linear with r^2= 0.995 and from calibration curve of Spironolactone (Fig 3) it was found to linear with r^2= 0.999. The % recoveries for Furosemide and Spironolactone were found to be 99.25%-101.45% and 99.1%-100.26% respectively, which were satisfactory (Table-3). The precision is usually expressed as % RSD. The intraday precision for Furosemide and Spironolactone were found to be 0.09-1.181 and 0.863-1.585 respectively. The inter day precision for Furosemide and Spironolactone were found to be 0.096-1.623 and 0.044-1.22 respectively. The limit of detection (LOD) for Furosemide and Spironolactone were 0.0025µg/ml and 0.00099 µg/ml respectively. The limit of quantification (LOQ) for Furosemide and Spironolactone were 0.00033 µg/ml and 0.00302 µg/ml respectively.

Table 2: System suitability parameters for RP-HPLC

Sr no.	Parameter	Furosemide	Spironolactone
1	Asymmetry	1.327	1.043
2	Resolution	8.350	8.350
3	Tailing	1.184	0.994

Table-3: 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
RP-HPLC	0%	4	10	0	0	4.058	9.91	101.45	99.1
	50%	4	10	2	5	6.082	15.04	101.33	100.26
	100%	4	10	4	10	7.946	19.93	99.25	99.65
	150%	4	10	6	15	9.917	25.04	99.17	100.19

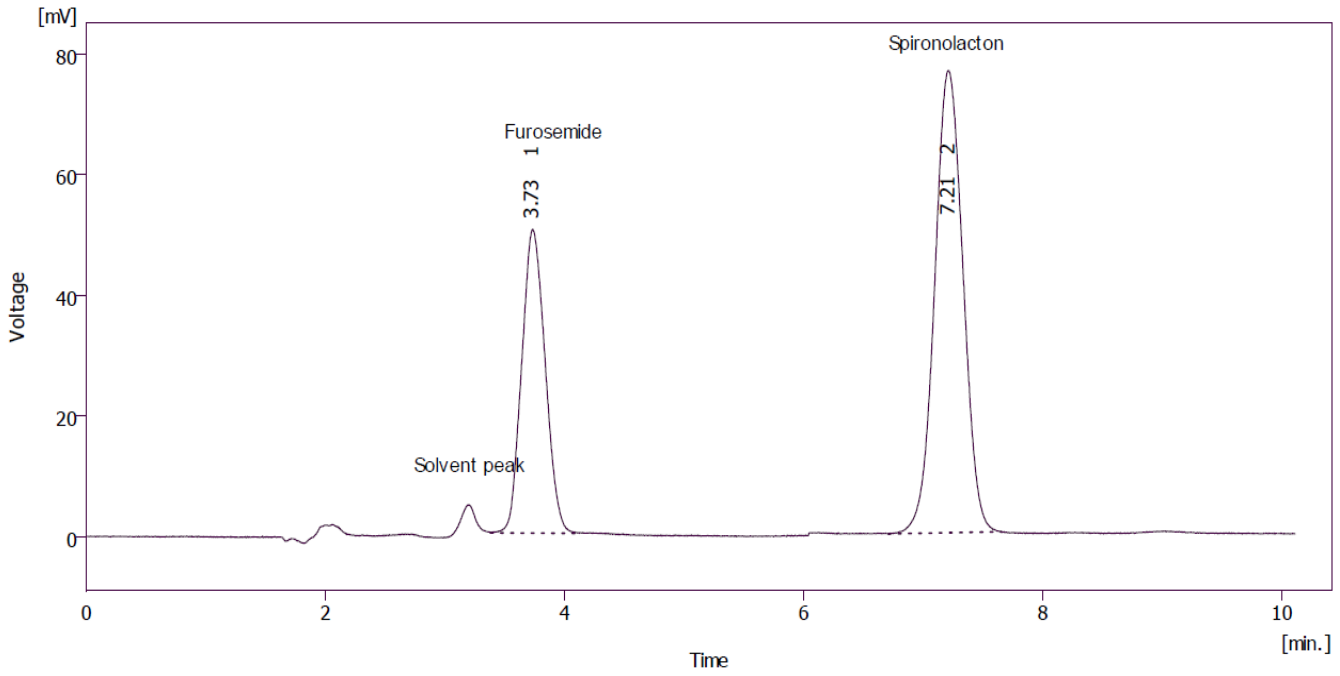

Fig 1: Representative chromatogram obtained from Sample solution.

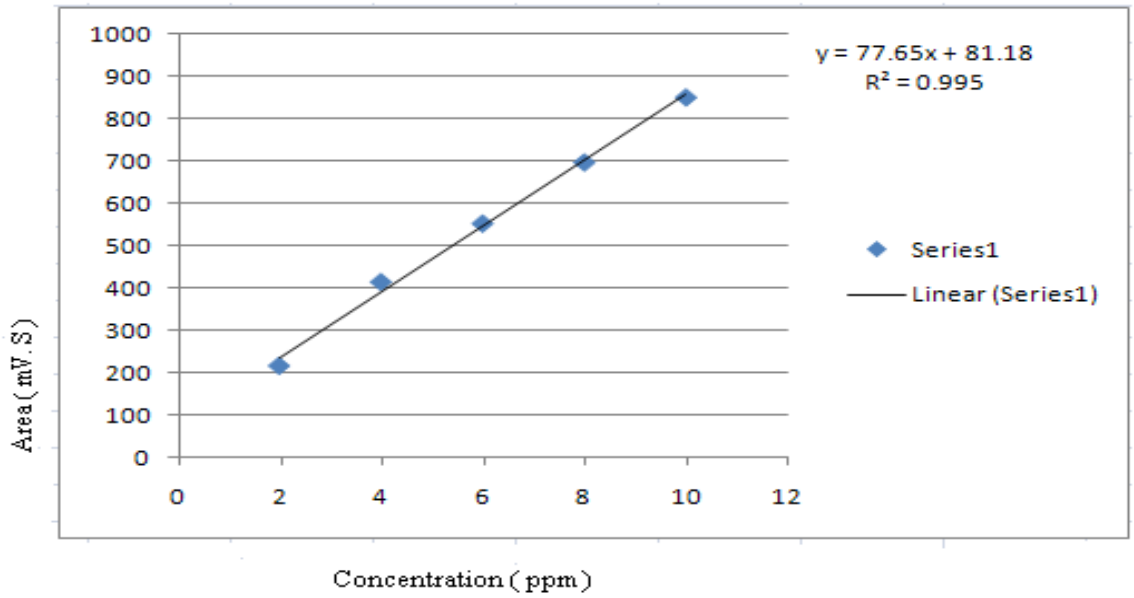

Fig 2: Calibration curve of Furosemide

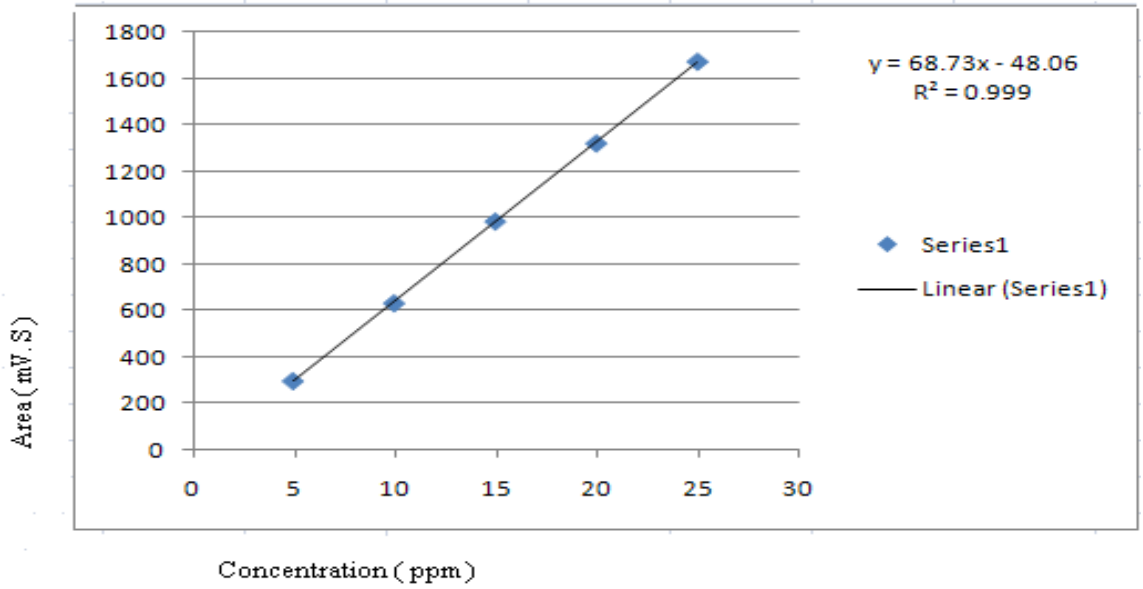

Fig 3: Calibration curve of Spironolactone

Table 4: Summary of validation parameters of proposed RP-HPLC

Parameters	Furosemide	Spironolactone
Beer's law range	2-10 µg/ml	5-25 µg/ml
Wavelength (nm)	237	237
Correlation Coefficient	0.996	0.999
Slope	0.075	68.73
Intercept	-0.017	-48.06
LOD (µg/ml)	0.0025	0.00099
LOQ (µg/ml)	0.0076	0.00302
% RSD		
Intraday precision	0.09-1.181	0.863-1.585
Interday precision	0.096-1.623	0.044-1.22

LOD= limit of detection; LOQ=limit of quantification; (%RSD)= % relative standard deviation, n = number of observations.

CONCLUSION

The validated RP-HPLC method employed here is simple, rapid, accurate, precise, sensitive and cost effective which can be used for routine analysis of Furosemide and Spironolactone in combined pharmaceutical dosage form.

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