JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Furosemide and Spironolactone in their Combined Tablet Dosage Form

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ABSTRACT:

The simple, sensitive, accurate, precise, rapid and economical reverse phase high pressure liquid chromatographic method was developed for the simultaneous estimation of Furosemide and Spironolactone in combined tablet dosage form. The method was developed using an Inertsil C_{18} (250 x 4.6mm), 5µ column with a mobile phase consisting of Methanol:Water (70:30 v/v),pH 3.20 ±0.05, which was adjusted by o-phosphoric acid at a flow rate of 1.0 ml/min and detection was carried out at 236 nm. Retention times were found to be 3.64 min and 6.69 min for Furosemide and Spironolactone respectively. The linearity was found to be in the concentration ranges of 10-60 µg/ml & 25-150 µg/ml for Furosemide and Spironolactone respectively. The proposed method can be used for the estimation of these drugs in combined tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS: Spironolactone, Furosemide, RP-HPLC, Tablet, Validation

Article history:
Received 15 April 2012
Accepted 04 May, 2012
Available online 13 Jun 2012

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INTRODUCTION:

Furosemide is chemically 4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid. Furosemide is primarily used for the treatment of two conditions: Hypertension and Edema. It is the first line agent in most people with edema due to congestive heart failure. Spironolactone is chemically 7α-Acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone. Spironolactone is used primarily to treat low-renin hypertension, hypokalemia, and Conn's syndrome. Both drugs are official in IP, BP and USP. Literature survey reveals that the methods like UV-Spectrophotmetry and HPLC were reported for the estimation of Furosemide and Spironolactone individually and in combination with other drugs. Whereas no RP-HPLC method has been reported for their simultaneous estimation. Hence, it is necessary to develop a rapid, accurate and validated RP-HPLC method for the determination of Furosemide and Spironolactone from combined dosage form. The method proved to be simple model since it does not contain a buffer system. This paper describes the development and validation of reliable, simple reversed phase HPLC method, using UV detection, for the simultaneous estimation of Furosemide and Spironolactone in combined tablet dosage forms. The developed method was validated according to ICH guidelines.

MATERIALS AND METHODS

Apparatus

A double beam UV/Visible spectrophotometer (Heλios Alpha) was used to measure detection wavelength. An analytical balance (CONTECH), HPLC instrument (LC-100 binary CYBERLAB Instruments) were used in the study.

Reagents and Materials

Spironolactone and Furosemide were obtained as gift sample from RPG Life Sciences Ltd., Maharashtra, India. Methanol and Water of HPLC grade were procured from RFCL limited. Marketed formulation used was spiromide tab (RPG Life Sciences Ltd.) having Spironolactone 50 mg and Furosemide 20 mg per tablet.

Chromatographic Conditions

Inertsil C_{18} (250mm x 4.6mm, 5µ) column was used as the stationary phase. A mixture of Methanol and Water in the ratio of (70:30 v/v) was used as as mobile phase and pH 3.20±0.05 adjusted with ortho-phosphoric acid. It was filtered through 0.45 µ membrane filter. The flow rate used was 1 ml/min. The detection was carried out at 236 nm. The injected volume was 20 µl. Run time used was 10 min.

Preparation of Standard Solutions

A stock solution containing Furosemide 10-60 µg/ml and Spironolactone 25-250 µg/ml was prepared by dissolving Furosemide and Spironolactone in methanol. The solutions were covered with aluminium foil to prevent from light.

Preparation of Mobile Phase

A mixture of methanol and water in the ratio of (70:30 v/v) was used as as mobile phase and pH 3.20±0.05 adjusted with ortho-phosphoric acid. It was filtered through 0.45 µ membrane filter.

Selection of Detection wavelength

The detection wavelength was selected as 236 nm where both drugs show significant absorbance and hence this λ_{max} was selected for further studies.

Preparation of Calibration Curves

In a series of 10 ml volumetric flask several dilutions of Furosemide (10-60 µg/ml) and Spironolactone (25-150 µg/ml) were prepared in methanol. All these solutions were injected into HPLC system and the chromatograms were recorded. The calibration graph was plotted by using peak area against concentration.

Estimation of Furosemide and Spironolactone in marketed tablet

Twenty tablets were accurately weighed and powdered. Tablet powder equivalent to 20mg of Furosemide and 50mg of Spironolactone was transferred in 50ml volumetric flask. Volume was made upto mark. 1ml of this was taken and transferred into 25 ml volumetric flask. Volume was made up upto mark. These gave concentration of 16 µg/ml of Furosemide and 40 µg/ml of Spironolactone. The solution was filtered through 0.45nm membrane filter. The solution was injected 20 µl. The areas of resulting peak were measured at 236 nm.

METHOD VALIDATION

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation.

Linearity and Range

The linearity of the method was determined at six concentration levels ranging from 25-150 µg/ml for Spironolactone and 10-60 µg/ml for Furosemide.

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery study was carried out by the standard addition method at three levels of 80, 100 and 120%. Each solution was injected in triplicate and the percentage recovery was calculated.

Precision

Repeatability

Standard solutions containing Spironolactone 75,100 and 125 µg/ml and Furosemide 30,40 and 50 µg/ml were injected three times and area of peaks were measured and %R.S.D. was calculated.

Intraday Precision

Standard solutions containing 50-150 µg/ml of Spironolactone and 10-60 µg/ml of Furosemide were analyzed three times on the same day and % R.S.D was calculated.

Interday Precision

Standard solutions containing 50-150 µg/ml of Spironolactone and 10-60 µg/ml of Furosemide were analyzed three times on different day and % R.S.D was calculated.

This shows that values are not more than 2%, indicates that the developed method is precise.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula:

LOD = (3.3 x standard deviation)/ Slope of calibration curve

The LOD for Spironolactone and Furosemide were found to be 1.24 µg/ml and 0.46 µg/ml, respectively.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula:

LOQ = (10 x standard deviation) / Slope of calibration curve

The LOQ was 3.76 µg/ml and 1.39 µg/ml for Spironolactone and Furosemide respectively.

RESULTS AND DISCUSSION

For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for Spironolactone and Furosemide.

Different mobile phases containing water, methanol and acetonitrile were examined and the mobile phase containing Methanol:Water (70:30 v/v,pH 3.20 ±0.05, which was adjusted by o-phosphoric acid) was selected as optimal for obtaining well resolved peaks with acceptable system suitability parameters (theoretical plates, resolution factor and asymmetry). The optimum wavelength for detection and quantitation was 236 nm, at which the best detector response was obtained for both the substances.

The method was found to be linear in the concentration range of 25-150 µg/ml for Spironolactone and 10-60 µg/ml for Furosemide. It was also found to be accurate, precise with acceptable values of LOD and LOQ.

Table-1: Linearity studies of Spironolactone and Furosemide

Parameter	Spironolactone	Furosemide
Linearity range	25-150 µg/ml	10-60 µg/ml
Coefficient of correlation (r^2)	0.997	0.996
Regression equation	y = 4210.x + 10095	y = 11679x + 47218
Intercept	10095	47218
Slope	4210	11679

Table-2: System suitability and validation parameters

Parameter	Spironolactone	Furosemide
Retention Time	6.69 min	3.64 min
Theoretical plates	2005.85	682.93
Tailing Factor	1.57	1.11
Resolution	00	3.05
LOD (µg/ml)	1.24 µg/ml	0.46 µg/ml
LOQ (µg/ml)	3.76 µg/ml	1.39 µg/ml

Table-4: Repeatability studies of Spironolactone and Furosemide

Drug	Concentration (µg/ml)	% RSD
Spironolactone	75	0.17
	100	0.33
	125	0.5
Furosemide	30	0.81
	40	0.66
	50	0.36

Table-5: Intraday and Interday studies of Spironolactone and Furosemide

Drug	Concentration (µg/ml)	% RSD	
		Intraday	Interday
Spironolactone	25	0.88	1.29
	50	0.19	0.33
	75	1.03	0.82
	100	0.76	0.68
	125	0.51	0.61
	150	0.60	0.76
Furosemide	10	0.99	1.42
	20	0.92	0.82
	30	0.67	0.80
	40	0.45	0.70
	50	0.24	0.47
	60	0.41	0.51

Table-6: Estimation of Furosemide and Spironolactone in marketed tablet

Brand	Label claim (mg/tab)	% Mean recovery ± S.D.	Label claim (mg/tab)
	Furosemide: Spironolactone	Furosemide	Spironolactone
Spiromide Tablet	20:50	99.78± 0.7429	98.45± 0.9487

Table-3: Recovery studies of Spironolactone and Furosemide

Spike Level %	Amount of Standard Drug Added (µg/ml)		% Mean Recovery		% RSD	
	Spironolactone	Furosemide	Spironolactone	Furosemide	Spironolactone	Furosemide
80	40	16	98.88	99.06	0.48	0.5
100	50	20	98.68	99.33	0.63	0.31
120	60	24	99.61	99.31	0.83	0.76

Figure-1: Typical Chromatogram of Spironolactone and Furosemide

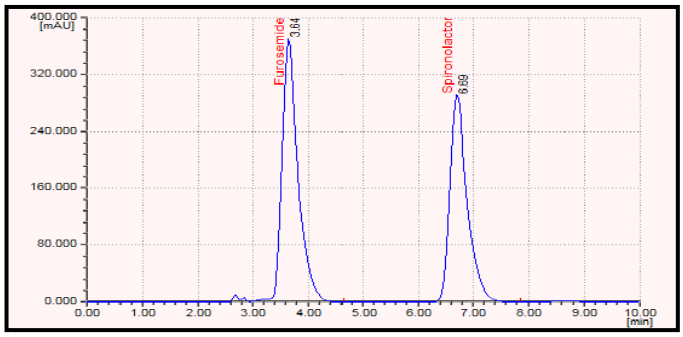


Figure-2: Overlay Chromatogram of Spironolactone and Furosemide

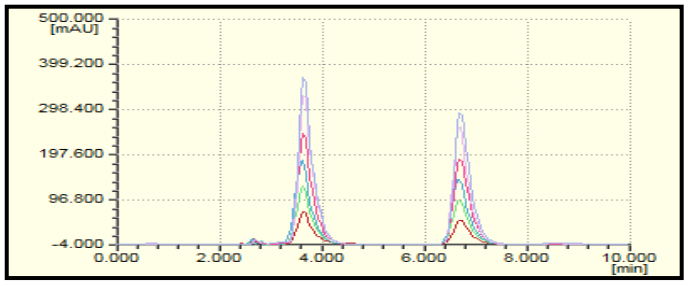


Figure-3: Calibration plot of Spironolactone (25-150 µg/ml)

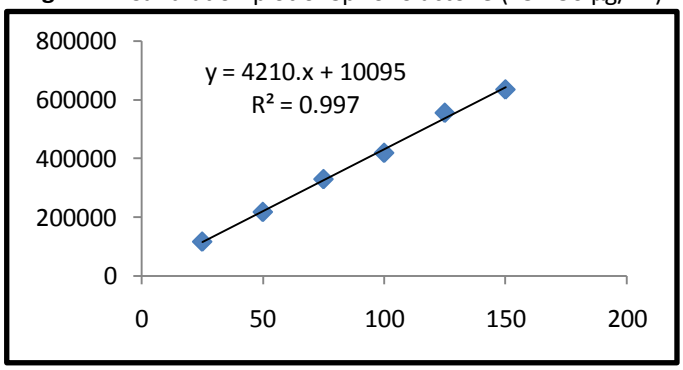
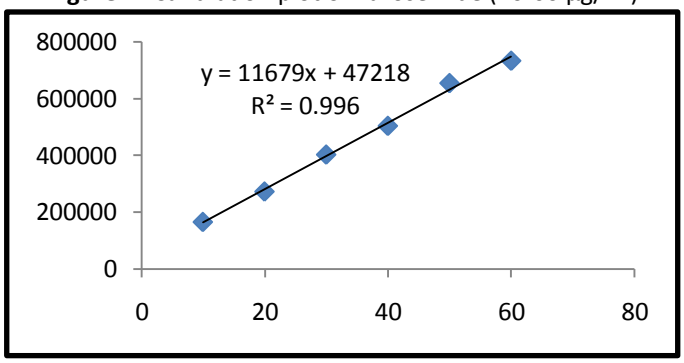


Figure-4: Calibration plot of Furosemide (10-60 µg/ml)



CONCLUSION

The method described for simultaneous estimation of Spironolactone and Furosemide are found to be simple, sensitive, accurate, precise, rapid and economical. Hence method could be successfully employed for routine analysis of Spironolactone and Furosemide in their combined tablet dosage form.

ACKNOWLEDGEMENT

The authors are thankful to RPG Life Sciences Ltd., Maharashtra, India for providing gift sample of standard Spironolactone and Furosemide for research.

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