International Journal of Chemistry and Pharmaceutical Sciences

Journal Home Page:www.pharmaresearchlibrary.com/ijcps

R E S E A E C H A R T I C L E

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Spironolactone and Furosemide in Bulk and Pharmaceutical Dosage Form

Dr. GampaVijayKumar[1*], Dr.D.Naresh[2], N. Krishna Nayak[3]

[1]*Professor and Head, Dept. of Pharmacy, KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana, India.*
[2]*KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana,India.*
[3]*KGR Institute of Technology and Management, Rampally, Kesara, Rangareddy, Telangana,India.*

A B S T R A C T

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Spironolactone and Furosemide was done by RP-HPLC. The Phosphate buffer was p^H4.5.and the mobile phase was optimized with consists of Phosphate buffer:Methanol P^H 4.5(20:80 v/v). Kromasil C18 (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1ml min^{-1}. The linearity range of Spironolactone and Furosemide were found to be from 100-500 µg/ml of Spironolactone and 1-5µg/ml of Furosemide . Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Spironolactone and Furosemide. LOD and LOQ were found to be within limit.The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Inertsil $C_{18,}$ Spironolactone and Furosemide, RP-HPLC

A R T I C L E I N F O

Corresponding Author
Dr. Gampa Vijay Kumar
1Professor and Head, Dept. of Pharmacy,
KGR Institute of Technology and Management,
Rampally, Kesara, Rangareddy, Telangana, India.
MS-ID: IJCPS3756

PAPER-QRCODE

A R T I C L E H I S T O R Y: Received 29 September 2018, **Accepted** 31 Oct 2018, **Available Online** 27 December 2018

Citation: Dr. Gampa Vijay Kumar, *et al.RP- HPLC Method Development and Validation for the Simultaneous Estimation of Spironolactone and Furosemide In Bulk and Pharmaceutical Dosage Form*. *Int. J. Chem, Pharm, Sci.*, 2018, 6(12): 339-344.

C O N T E N T S

1. Introduction

Spironolactone, sold under the brand name Aldactone among others, is a medication that is primarily used to treat fluid build-up due to heart failure, liver scarring, or kidney disease. It is also used in the treatment of high blood pressure, low blood potassium that does not improve with supplementation, early puberty in boys, acne and excessive hair growth in women, and as a part of feminizing hormone therapy in transgender women. Spironolactone is taken by mouth. Common side effects include electrolyte abnormalities, particularly high blood potassium, nausea, vomiting, headache, rashes, and a decreased desire for sex. In those with liver or kidney problems, extra care should be taken. Spironolactone has not been well studied in pregnancy and should not be used to treat high blood pressure of pregnancy. It is a steroid that blocks the effects of the hormones aldosterone and testosterone and has some estrogen-like effects. Spironolactone belongs to a class of medications known as potassium-sparing diuretics.

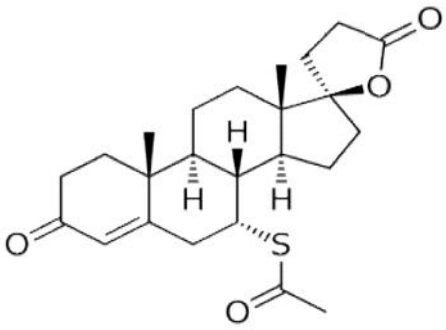

Fig 1: Structure of Spironolactone

Furosemide, sold under the brand name Lasix among others, is a medication used to treat fluid build-up due to heart failure, liver scarring, or kidney disease. It may also be used for the treatment of high blood pressure. It can be taken intravenously or by mouth. When taken by mouth, it typically begins working within an hour, while intravenously, it typically begins working within five minutes.Common side effects include low blood pressure with standing, ringing in the ears, and sensitivity to sunlight. Potentially serious side effects include electrolyte abnormalities, low blood pressure, and hearing loss. Blood tests are recommended regularly for those on treatment. Furosemide is a type of loop diuretic that works by decreasing the reabsorption of sodium by the kidneys.

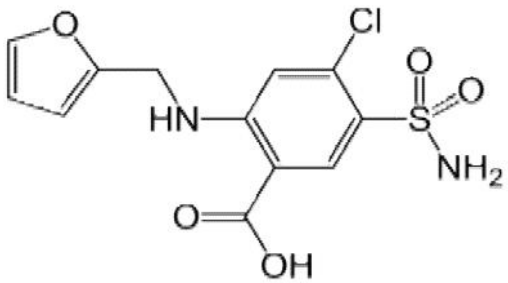

Fig 2: Structure of Furosemide

2. Materials and Methods

Instrumentation

HPLC Shimadzu, model No. SPD-20MA LC+20AD, Software- LC-20 Solution. UV/VIS spectrophotometer LABINDIA, UV 3000+pH meter, Weighing machine.

Chemicals

Spironolactone and Furosemide,KH_2PO4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Chromatographic Conditions:

Table 1: Optimized Chromatographic Conditions

Parameter	Description
Flow rate	1.0 ml min^{-1}
Column	Kromosil C18 column (4.6×150mm)5µ
Mobile phase ratio	Phosphate buffer:Methanol PH 4.5(20:80 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 4.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of Elution	Isocratic
Detection wavelength	254 nm
Injection volume	20µl
Run time	10 min

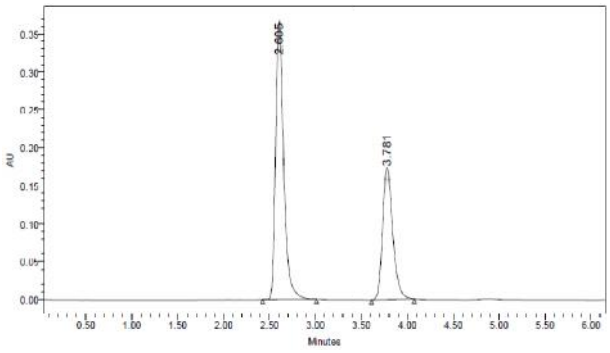

Fig 3: Optimized Chromatogram

Observation:

The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of spironolactone and Furosemide10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of spironolactone and Furosemide (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3 ml of spironolactone e and Furosemide of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

Precision:

Accurately weigh and transfer 25 mg of spironolactone and Furosemide working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Accuracy:

Accurately weigh and transfer 10 mg of Spiranolactone and Furosemide 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Linearity:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Spiranolactone and Furosemide (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection:

L**imit of Detection For Spironolactone:**Accurately weigh and transfer 10 mg of Spiranolactone working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Detection (For Furosemide):

Accurately weigh and transfer 10mg of Furosemide working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Quantification:

Limit Of Quantification (for Spironolactone): Accurately weigh and transfer 10 mg of spironolactone working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Limit of Quantification for Furosemide:

Accurately weigh and transfer 10mg of Furosemide working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

3. Results and Discussions

Linearity:

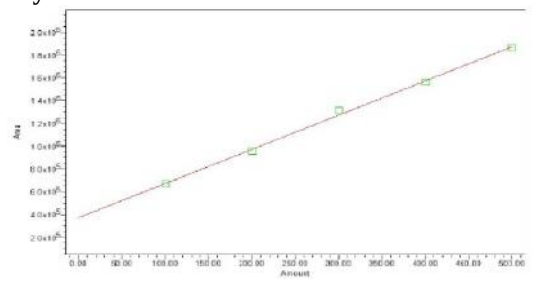

Fig 4: Calibration graph for spironolactone at 225 nm

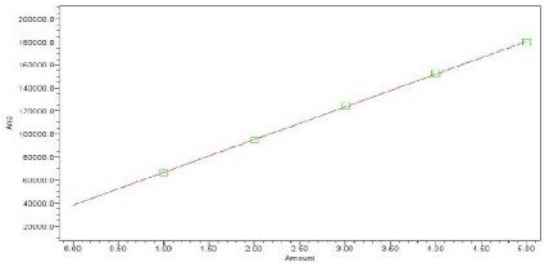

Fig 5: Calibration graph for Furosemideat 225 nm

Robustness:

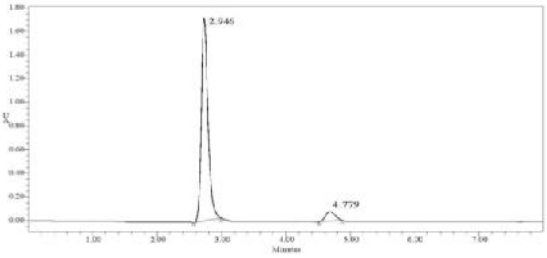

Fig 6: Chromatogram snowing less flow of 0.6ml/min

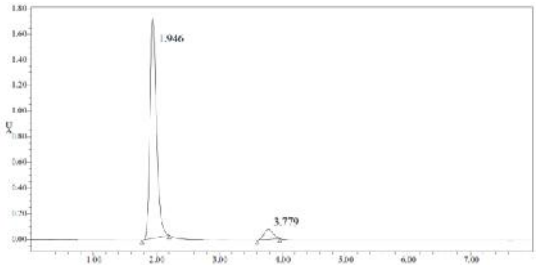

Fig 7: Chromatogram snowing more flow of 1.0ml/min

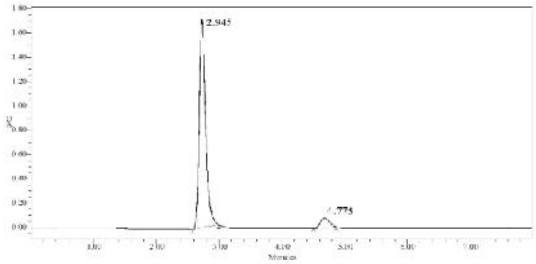

Fig 7: Chromatogram showing less organic composition

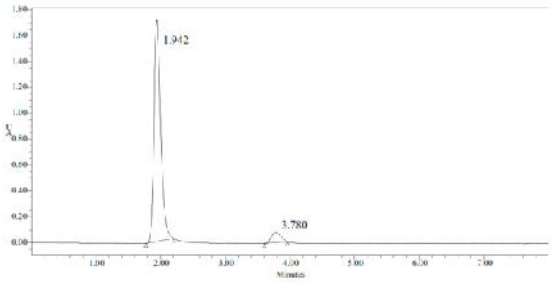

Fig 8: Chromatogram showing more organic phase ratio

Table No 2: Results of system suitability parameters for spironolactone and Furosemide

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**spironolactone**	2.5	124505	213642		1.2	4673.4
2	**Furosemide**	3.9	1308495	154566	6 0	1.3	6090.3

Table No 3: Results of method precession for spironolactone and Furosemide

Injection	Area	
	Spironolactone	Furosemide
Injection-1	1302729	123149
Injection-2	1302947	123766
Injection-3	1303236	124271
Injection-4	1303977	124691
Injection-5	1309759	124956
Average	1304529.8	124162.7
Standard Deviation	2961.1	725.6
%RSD	0.2	0.6

Table No 4: Results of Intermediate precision for spironolactone and Furosemide

Injection	Area	
	Spironolactone	Furosemide
Injection-1	1300148	122487
Injection-2	1304520	122626
Injection-3	1305937	122632
Injection-4	1306476	122702
Injection-5	130871	122962
Average	1305070.2	122681.8
Standard Deviation	3061.8	174.8
%RSD	0.2	0.1

Table No 5: Accuracy (recovery) data for spironolactone

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table No 6: Accuracy (recovery) data for Furosemide

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	

150%	177940	14.2	14.45	99.68%	

Table No 7: Area of different concentration of spironolactone

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table No 8: Area of different concentration of Furosemide

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Table No 9: Analytical performance parameters of spironolactone and Furosemide

Parameters	spironolactone	Furosemide
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table No 10: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Spironolactone	52	152	2.9
Furosemide	52	156	3

Table No 11: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Spironolactone	52	522	10.03
Furosemide	52	524	10.1

Table No 12: Flow Rate (ml/min) data for spironolactone

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table No 13: Flow rate (ml/min) data for Furosemide

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table No 14: Change in Organic Composition in the Mobile Phase for spironolactone

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3

2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table No 15: Change in Organic Composition in the Mobile Phase for Furosemide

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Spironolactone and Furosemide was done by RP-HPLC. The Phosphate buffer was p^H4.5.and the mobile phase was optimized with consists of Phosphate buffer:Methanol P^H 4.5(20:80 v/v). Kromasil C18 (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1ml min^{-1}. the linearity range of Spironolactone and Furosemide were found to be from 100-500 µg/ml of Spironolactone and 1-5µg/ml of Furosemide . Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Spironolactone and Furosemide. LOD and LOQ were found to be within limit.The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

5. References

[1] http://www.wikipedia.com/ Furosemide.,.

[2] www.drugbank.ca/drugs/DB00285.

[3] Sawant Ramesh L., Bharat Anjali V , Tanpure Kallyani D., Jadhav Kallyani A, A New Rp-Hplc Method Development For Simultaneous Estimation Of Salbutamol Sulphate, Theophylline And Furosemide., International Journal of Pharma Sciences and Research., Vol. 6 No.4 Apr 2015, ISSN : 0975-9492.

[4] Rahul CR, Jigisha Patel, Mayank Bapna, Development and validation of analytical method for estimation of Spironolactone in oral suspension., J. Chem. Bio. Phy. Sci. Sec. B, May 2014 – July 2014; Vol.4, No.3; 2196-2204.

[5] Chandrakanth.Vadloori, Venkat Tallada, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Spironolactone and Frusemide in Bulk and Pharmaceutical Dosage Form., Journal of Pharmacy Research Vol.5, Issue 8.August 2012, 3998-4000.

[6] Hardik Patel, Sagar Solanki, Simultaneous Estimation Of Furosemide And Spironolactone In Combined pharmaceutical Dosage Form By Rp-HPLC.,Asian journal of pharmaceutical & clinical research., Vol 5, Issue 3, 2012, ISSN-0974-2441.

[7] Vijay R. Ram , Pragnesh N. Dave and Hitendra S. Joshi, Development and Validation of a Stability-Indicating HPLC Assay Method for Simultaneous Determination of Spironolactone and Furosemide in Tablet Formulation., Journal of Chromatographic Science ., 2012;50:721– 726.

[8] Dr. Kealey and P.J Haines, Analytical Chemistry, 1stedition, Bios Publisher, (2002), PP 1-7.

[9] A.BraithWait and F.J.Smith, Chromatographic Methods, 5thedition, Kluwer Academic Publisher, (1996), PP 1-2.

[10] Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.