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RESEARCH ARTICLE

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

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ABSTRACT

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^H 4.5.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.

Key words: Inertsil C18, Spironolactone and Furosemide, RP-HPLC

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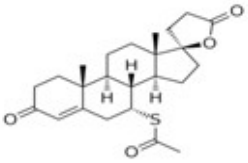
CONTENTS

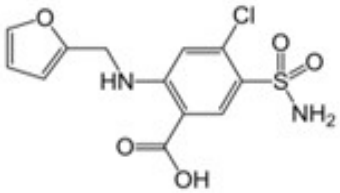
1. Introduction

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.



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.



Furosemide

2. Materials and Methods

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

Spironolactone and Furosemide, KH_2PO_4, water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Chromatographic conditions

Trial-6

Table 1.Chromatographic condition

Parameters	Description
Flow rate	1ml min^{-1}
Column	kromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer:Methanol P^H 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
Wavelength	254 nm
Injection volume	20µl
Run time	10min

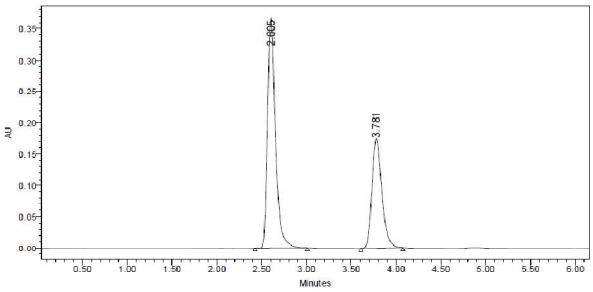


Fig.no.1Chromatogram of Trail-6

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 Saxagliptin and Dapagliflozin 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.

(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 Saxagliptin and Dapagliflozin (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 Saxagliptin e and Dapagliflozin 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 Saxagliptin and Dapagliflozin 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 Saxagliptin and Dapagliflozin 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 Saxagliptin and Dapagliflozin (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:

Limit of Detection: (For Saxagliptin):

Accurately weigh and transfer 10 mg of Saxagliptin 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 Dapagliflozin)

Accurately weigh and transfer 10mg of Dapagliflozin **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 Saxagliptin)

Accurately weigh and transfer 10 mg of Saxagliptin 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 Dapagliflozin)

Accurately weigh and transfer 10mg of Dapagliflozin 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 discussion

System Suitability

Table 1: Results of system suitability parameters for Saxagliptin and Dapagliflozin

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

Precision:

Table 2: Results of method precession for Saxagliptin

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 3: Results of method precession for Dapagliflozin

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Intermediate precession (ruggedness):

Table 4: Results of Intermediate precision for Saxagliptin

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table 5: Results of Intermediate precision for Dapagliflozin

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Accuracy:

Table-6: Accuracy (Recovery) Data for Saxagliptin

%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-7 accuracy (recovery) data for Dapagliflozin

%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%	

Linearity:

Table-8 Area of different concentration of Dapagliflozin

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-9 Area of different concentration of Saxagliptin

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

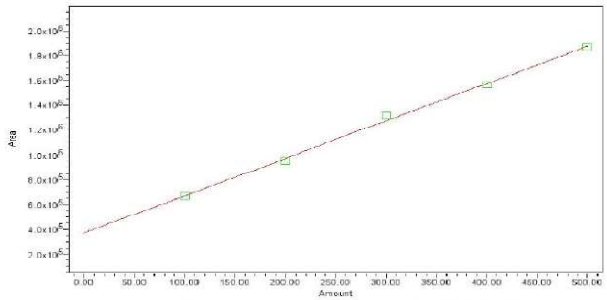


Figure 2: Calibration graph for Saxagliptin at 225 nm

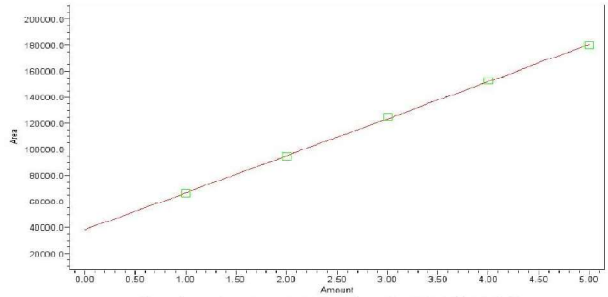


Figure 3: Calibration graph for Dapagliflozin at 225 nm

Table-10Analytical performance parameters of Saxagliptin and Dapagliflozin

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

Limit of Detection for Saxagliptin and Dapagliflozin

Table-11 Results of LOD

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

Limit of Quantification (LOQ):

Table no-12 Results of LOQ

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

Robustness:
Variation in Flow

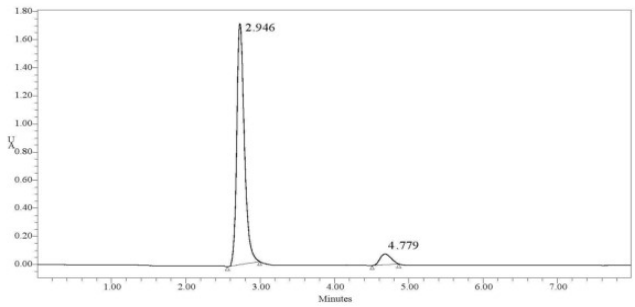


Figure 4 chromatogram snowing less flow of 0.6ml/min

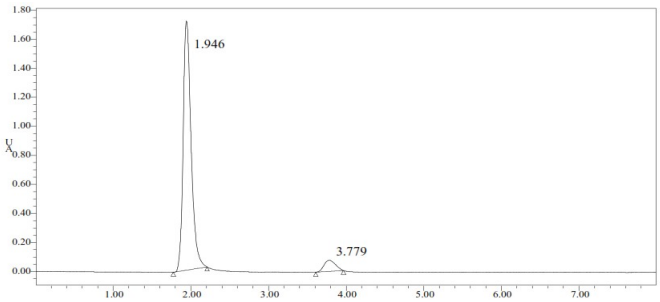


Figure 5: chromatogram snowing more flow of 1.0ml/min

Table-13 Flow Rate (ml/min) data for Saxagliptin

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-14 flow rate (ml/min) data for Dapagliflozin

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

6.3.7.2 Variation of Mobile Phase Organic Composition:

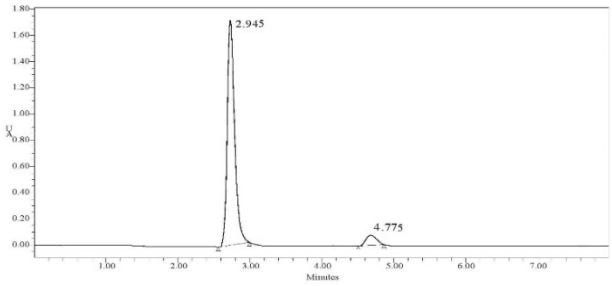


Figure 6: Chromatogram showing less organic composition

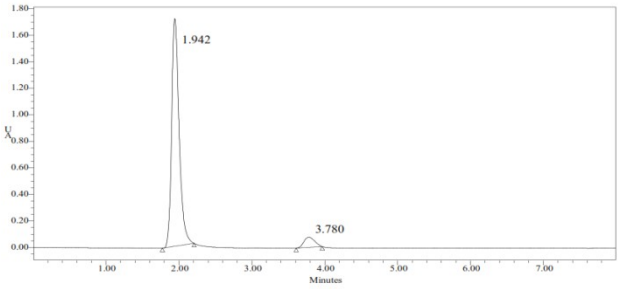


Figure 7: Chromatogram showing more organic composition

Table -15 Change in Organic Composition in the Mobile Phase for Saxagliptin

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 -16 Change in Organic Composition in the Mobile Phase for Dapagliflozin

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^H 4.5.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.

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