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Research

A New Analytical Method Development And Validation For Quantitative Estimation Of Spironolactone And Furosemide In Bulk And Tablet Dosage Form By Using Rp-Hplc

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Spironolactone and Furosemide, in its pure form as well as in pharmaceutical dosage form. Chromatographic separation was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.2 (40:60v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 272 nm. The retention time of the Spironolactone and Furosemide was 2.781, 4.048 ±0.02min respectively. The proposed method was validated for various ICH parameters like linearity, limit of detection, limits of quantification, accuracy, precision, range and specificity. The method produce linear responses in the concentration range of 5-25mg/ml of Spironolactone and 9.375-46.875 mg/ml of Furosemide. The method precision for the determination of assay was below 2.0%RSD. The proposed method is applicable to routine analysis of Spironolactone and Furosemide in bulk and pharmaceutical formulations.

Keywords: Spironolactone, Furosemide, RP-HPLC, Accuracy, Robustness.

INTRODUCTION

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

Qualitative analysis is the identification of elements, species and/or compounds present in sample.

Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control (QC) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

Fundamental and applied research The chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.

Analytical techniques There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various analytical techniques. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. Atomic, molecular spectrometry and chromatography, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. Spectrometric techniques may involve either the emission or absorption of electromagnetic radiation over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. The most important atomic and molecular spectrometric techniques and their principal applications are listed in Table.No.2.

Chromatographic techniques provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called hyphenation, provides a powerful means of separating and identifying unknown compounds.

MATERIALS AND METHODS

Spironolactone from Sura labs, Furosemide from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Spironolactone and Furosemide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15ml of Spironolactone and 0.2812ml of Furosemide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Optimized chromatographic conditions

Instrument used :Waters HPLC with auto sampler and PDA
Detector 996 model.
Temperature : 40ºC
Column : Symmetry C18 (4.6×150mm, 5µ)
pH : 4.2
Mobile phase : Methanol: TEA buffer pH 4.2 (40:60v/v)
Flow rate : 1ml/min
Wavelength : 272nm
Injection volume : 10 □l
Run time : 6 min

Validation

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 400 ml (40%) of Methanol and 600 ml of TEA buffer (60%) a were mixed and degassed in digital Ultrasonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Methanol: TEA Buffer pH 4.2 (40:60)
Column : Symmetry C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 272 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 6 minutes

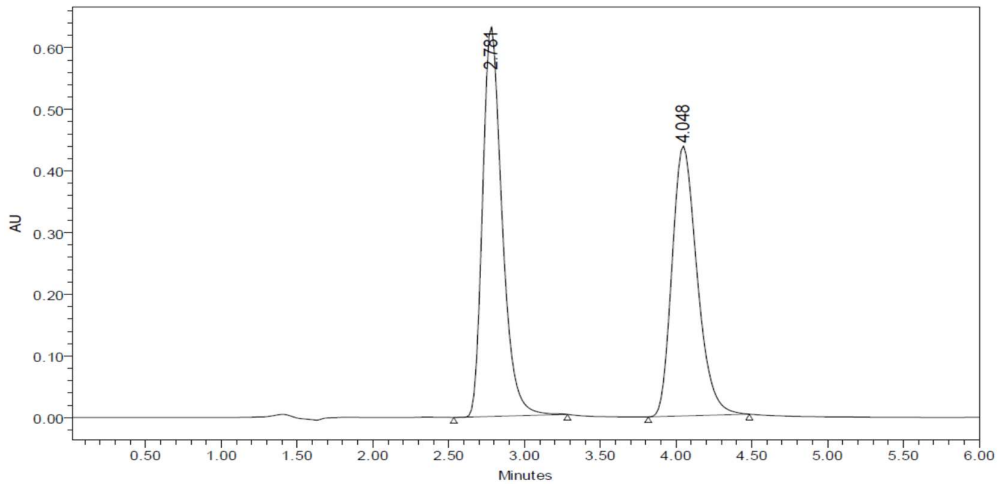

Fig 1: Results of Optimized Chromatogram

Table 1: Peak Results for Optimized Condition

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Spironolactone	2.781	2774027	299752		1.2	6314
2	Furosemide	4.048	2533532	210321	4.6	1.3	5521

From the above chromatogram it was observed that the Spironolactone and Furosemide peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

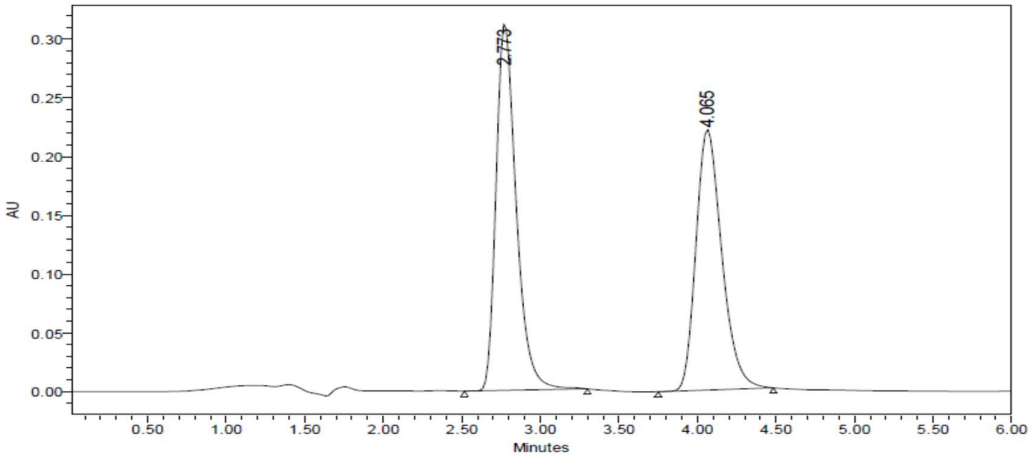

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Spironolactone	2.773	2770123	282157		1.6	5011
2	Furosemide	4.065	2522041	251068	3.3	1.5	5947

Resolution between two drugs must be not less than 2., Theoretical plates must be not less than 2000.
Tailing factor must be not less than 0.9 and not more than 2., It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Peak results for assay standard of Spironolactone

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Spironolactone	2.782	2762937	357421	1.3	6344.7
2	Spironolactone	2.766	2774613	388745	1.3	6344.2
3	Spironolactone	2.767	2762937	399854	1.3	6300.1
4	Spironolactone	2.795	2774613	386542	1.3	6344.7
5	Spironolactone	2.768	2776429	364121	1.3	6344.2
Mean			2770306			
Std. Dev.			6767.495			
% RSD			0.2			

Table 4: Peak results for assay standard of Furosemide

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Furosemide	4.049	2540214	236741	4.6	1.3	5937.7
2	Furosemide	4.025	2541284	226745	4.7	1.3	5008.8
3	Furosemide	4.029	2534375	210326	4.6	1.3	5937.7
4	Furosemide	4.067	2526189	226741	4.7	1.3	5008.8
5	Furosemide	4.030	2546248	231494	4.7	1.3	5990.7
Mean			2537662				
Std. Dev.			7677.647				
% RSD			0.3				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Sample)

Table 5: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	Spironolactone	2.764	2732203	294531		1.3	6314	1
2	Furosemide	4.012	2507543	216321	4.6	1.3	5954	1
3	Spironolactone	2.767	2751843	286473		1.3	6369	2
4	Furosemide	4.016	2509101	216354	4.6	1.3	5944	2
5	Spironolactone	2.764	2744776	312684		1.3	6329	3
6	Furosemide	4.013	2515628	206571	4.6	1.3	5990	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Spironolactone in pharmaceutical dosage form was found to be 100. 9%.

Linearity
Chromatographic data for linearity study
Spironolactone

Concentration µg/ml	Average Peak Area
9.375	892464
18.75	1866364
28.125	2777423
37.5	3709213
46.875	4601317

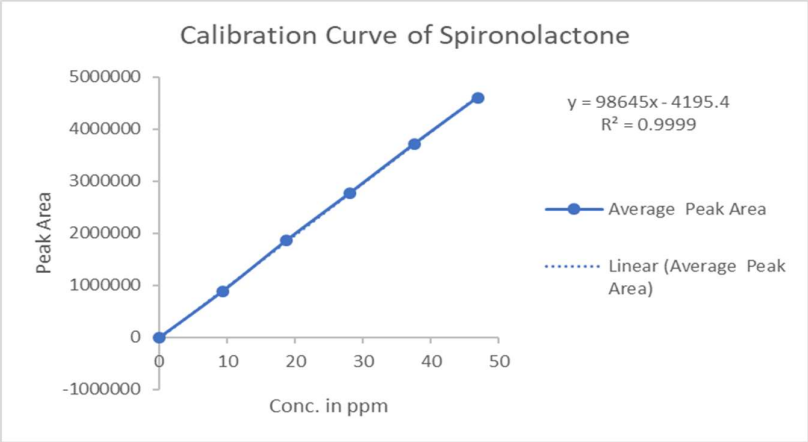

Fig 3: Calibration Graph for Spironolactone

Furosemide

Concentration µg/ml	Average Peak Area
5	920032
10	1752782
15	2521426
20	3326009
25	4217393

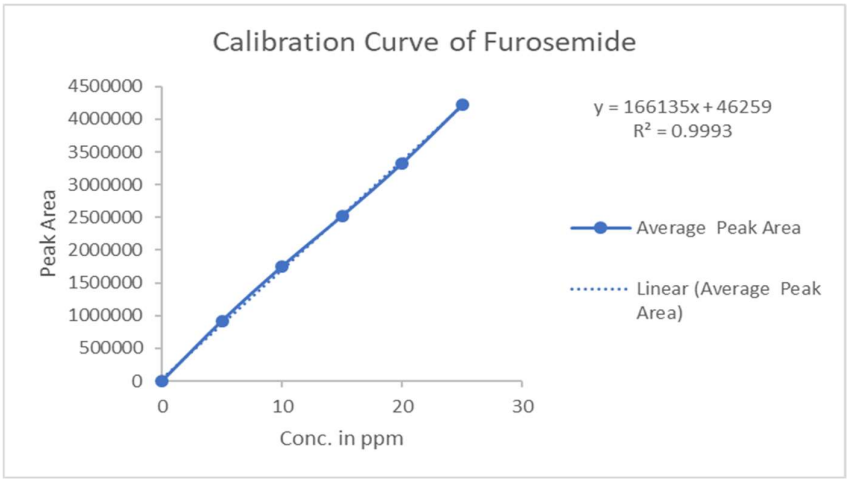

Fig 4: Calibration Graph for Furosemide

Repeatability

Table 6: Results of repeatability for Spironolactone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Spironolactone	2.766	2766870	294578	6684	1.3
2	Spironolactone	2.774	2771971	286541	6347	1.3
3	Spironolactone	2.770	2771958	302657	6674	1.3
4	Spironolactone	2.772	2780299	293412	6451	1.3
5	Spironolactone	2.771	2789695	283154	6678	1.3
Mean			2776159			
Std. Dev			8969.896			

% RSD	0.3

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 7: Results of method precision for Furosemide

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Furosemide	4.025	2534539	193240	5761	1.3	4.7
2	Furosemide	4.040	2539247	201647	5489	1.3	4.6
3	Furosemide	4.032	2544661	193472	5367	1.3	4.6
4	Furosemide	4.041	2548839	196475	5845	1.3	4.6
5	Furosemide	4.036	2558822	201394	5347	1.3	4.7
Mean			2545222				
Std. Dev			9329.852				
% RSD			0.3				

%RSD for sample should be NMT 2, The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 8: Results of Intermediate precision for Spironolactone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Spironolactone	2.781	2715421	294651	6647	1.3
2	Spironolactone	2.780	2778540	284123	6781	1.3
3	Spironolactone	2.782	2754247	274561	6984	1.3
4	Spironolactone	2.780	2780545	281241	6475	1.3
5	Spironolactone	2.782	2777021	286471	6647	1.3
6	Spironolactone	2.774	2780254	294512	6489	1.3
Mean			2764338			
Std. Dev			25974			
% RSD			0.9			

%RSD of five different sample solutions should not more than 2.

Table 9: Results of Intermediate precision for Furosemide

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Furosemide	4.048	2506927	211541	5495	1.4	4.6
2	Furosemide	4.050	2504522	206141	5694	1.4	4.6
3	Furosemide	4.049	2541270	198641	5785	1.4	4.7
4	Furosemide	4.050	2507885	206741	5947	1.4	4.6
5	Furosemide	4.049	2504587	209487	5742	1.4	4.6
6	Furosemide	4.040	2504780	193481	5914	1.4	4.6
Mean			2511662				
Std. Dev			14572.01				
% RSD			0.5				

%RSD of five different sample solutions should not more than 2, The %RSD obtained is within the limit, hence the method is rugged.

Table 10: Results of Intermediate precision Day 2 for Spironolactone

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Spironolactone	2.764	2781856	294651	6647	1.3
2	Spironolactone	2.759	2761510	284123	6781	1.3
3	Spironolactone	3.015	2748811	274561	6984	1.3
4	Spironolactone	2.773	2790831	281241	6475	1.3
5	Spironolactone	2.765	2785112	286471	6647	1.3

6	Spironolactone	2.764	2781932	294512	6489	1.3
Mean			2775009			
Std. Dev			16222.05			
% RSD			0.5			

%RSD of five different sample solutions should not more than 2.

Table 11: Results of Intermediate precision for Furosemide

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Furosemide	4.015	2536301	211541	5495	1.4	4.6
2	Furosemide	4.007	2541972	206141	5694	1.4	4.6
3	Furosemide	4.323	2521259	198641	5785	1.4	4.7
4	Furosemide	4.065	2537081	206741	5947	1.4	4.6
5	Furosemide	4.020	2549869	209487	5742	1.4	4.6
6	Furosemide	4.015	2536301	193481	5914	1.4	4.6
Mean			2537131				
Std. Dev			9370.087				
% RSD			0.3				

%RSD of five different sample solutions should not more than 2. The %RSD obtained is within the limit, hence the method is rugged.

Accuracy

Table 12: The accuracy results for Spironolactone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1382603	14.0625	14.05	99. 9	99.8%
100%	2777270	28.125	28.1	99. 9	
150%	41448756	42.1875	42.06	99.6	

Table 13: The accuracy results for Furosemide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1306990	7.5	7.5	100	99.4%
100%	2510628	15	14.8	98.6	
150%	3777999	22.5	22.46	99.8	

Robustness

Table 14: Results for Robustness

Spironolactone

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
More Flow rate of 1.1 mL/min	2542012	2.516	6234	1.4
Less organic phase	2888515	3.326	6298	1.4
More organic phase	2541550	2.416	6287	1.2

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Furosemide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2533532	4.048	5521	1.3
Less Flow rate of 0.9 mL/min	2750214	5.319	5643	1.6
More Flow rate of 1.1 mL/min	2254107	3.649	5782	1.5

Less organic phase	2754017	5.318	5309	1.4
More organic phase	2215870	3.233	5580	1.51

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Spironolactone and Furosemide in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Spironolactone was found to be practically insoluble in water; soluble in chloroform, ethanol. Furosemide was found to be slightly soluble in ethanol, soluble in methanol, DMSO, and alkali hydroxides. Furosemide is practically insoluble in water. Methanol: TEA pH 4.2 (40:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Spironolactone and Furosemide in bulk drug and in Pharmaceutical dosage forms.

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