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Analytical Method Validation of Simultaneous determination of Spironolactone and Furosemide in tablet formulation and its Statistical evaluation

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ABSTRACT

The objective of current study was to Statistical Comparison for Precision and Intermediate Precision study for Analytical Method Validation of Spironolactone and Furosemide in tablet formulation and developed easy, exact and correct isocratic stability indicating reversed phase HPLC assay method and validated for determination of Spironolactone and Furosemide in solid pharmaceutical dosage forms. Isocratic RP-HPLC separation was achieved on an SGE make 150× 4.6mm SS Wakosil II 5C18RS 5 µm column (Part Number: 206610 and Serial Number: A01-063) using mobile phase of Acetonitrile- Ammonium acetate buffer (50:50, v/v) at a flow rate of 1.1 ml/min and the detection was carried out at 254 nm using photo-diode array detector. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The method was linear in the drug concentration range of 40-160 µg/ml with a correlation coefficient 0.9977 and 0.9953 for Spironolactone and Furosemide respectively. The precision (RSD) amongst six-sample preparation was 0.87% and 1.1 % for Spironolactone and Furosemide respectively. For repeatability and intermediate precision (RSD) amongst six-sample preparation was 0.46 % and 0.20 % for Spironolactone and Furosemide respectively. As result shown that for furosemide, % RSD was 1.12% and in ANOVA *study Significance F* value found 0.625502408 and for spironolactone Precision study and Intermediate precision study % RSD was 0.68, in ANOVA *study Significance F* value found 0.905843808.

***Keywords*:** Statistical Comparison; Precision and Intermediate Precision study; Analytical Method Validation; Spironolactone; Furosemide; Tablet Formulation

1. INTRODUCTION

Stress testing is a part of developmental strategy under the ICH requirements and is carried out under more severe conditions than accelerated conditions. These studies serve to give information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies [1-3]. It is suggested that stress testing should include the effect of temperature, light, oxidizing agents as well as susceptibility across a wide range of pH values. It is also recommended that analysis of stability sample should de done through the use of a validated stability testing methods.

1. 1. Introduction of drug

1. 1. 1. Introduction of furosemide

Furosemide is chemically 4-Chloro-2-(furan-2-ylmethylamino)-5-sulfa moylbenzoic acid (Figure 1). Its CAS number is 54-31-9. Its molecular formula is $C_{12}H_{11}ClN_2O_5S$ having molecular weight 330.74gm/mole. 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 [4]. This loop diuretic is commonly used for the treatment of renal diseases, congestive heart failure and hypertension [5].

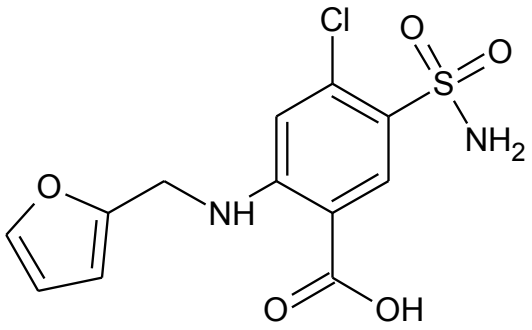

Figure 1. 4-Chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid.

1. 1. 2. Introduction of spironolactone

Spironolactone is chemically 7α-Acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone (Figure 2). Its molecular formula is $C_{24}H_{32}O_4S$ having molecular weight 416.58 gm/mole. Spironolactone inhibits the effect of aldosterone by competing for intracellular aldosterone receptors in the distal tubule cells (it actually works on aldosterone receptors in the collecting duct). This increases the excretion of water and sodium, while decreasing the excretion of potassium. Spironolactone has a fairly slow onset of action, taking several days to develop, and similarly the effect diminishes slowly. Spironolactone has anti-androgen activity by binding to the androgen receptor and preventing it from interacting with dihydro testosterone [6]. Various publications are available regarding determination method of Spironolactone and Furosemide but most of the methods are applicable to alone Spironolactone or Furosemide in pharmaceutical dosage form or in biological fluids. Potentiometric [7], colorimetric estimation [8], thin-layer chromatography [9], fluorometrically [10], complexation [11], flow injection chemiluminescence method [12], proton nuclear magnetic resonance spectroscopic [13] and HPLC methods [14] are reported. Only four methods are reported for the simultaneous determination of spironolactone and furosemide. One method is reported for HPLC analysis. [15] As far as our knowledge is concern, no method for the determination of spironolactone and furosemide in combine dosage forms has been published. The previous published methods are not directly applicable for this issue and need more investigation for method development and validation.

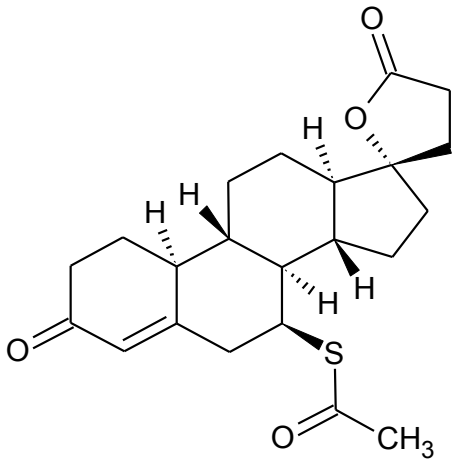

Figure 2. 7α-Acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone.

2. EXPERIMENTAL

2. 1. Materials

Spironolactone and Furosemide standard of was provided by Alembic Pharmaceuticals Ltd., Baroda (India). Spironolactone and Furosemide tablets containing 50 mg Spironolactone and 20 mg Furosemide and the inactive ingredient used in drug matrix were obtained from market. HPLC grade acetonitrile and water were obtained from Spectrochem Pvt. Ltd., Mumbai (India). Analytical grade ammonium acetate, hydrochloric acid, glacial acetic acid, sodium hydroxide pellets and 30% v/v hydrogen peroxide solution were obtained from Ranbaxy Fine Chemicals, New Delhi (India).

2. 2. Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10ATvp binary pump, a SPD-M10Avp photo-diode array detector and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-VP 6.13 SP2, Shimadzu).

2. 3. Chromatographic conditions

Chromatographic analysis was performed on a SGE make SS Wakosil II 5C18RS column (150mm x 4.6mm i.d., 5 µm particle size) column. The mobile phase consisted of acetonitrile – 0.01M ammonium acetate buffer pH 3.9 (50: 50, v/v). To prepare the buffer solution, 0.7708 g ammonium acetate were weighed and dissolve in 1000 ml HPLC grade water and then adjusted to pH 3.9 with glacial acetic acid. Mobile phase was filtered through a 0.45µm nylon membrane (Millipore Pvt. Ltd. Bangalore, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai). The flow rate of the mobile phase was adjusted to 1.1 ml/min and the injection volume was 20 µl. Detection was performed at 254nm.

2. 4. Standard preparation

Spironolactone standard stock solution containing 500µg/ml was prepared in a 100 ml volumetric flask by dissolving 50.00 mg of Spironolactone and then diluted to volume with diluent. Further take 10 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 100µg/ml). And for a Furosemide standard stock solution containing 200µg/ml was prepared in a 100 ml volumetric flask by dissolving 20.00 mg of Furosemide and then diluted to volume with diluent. Further take 10 ml of this stock solution in 50 ml volumetric flask and make up to mark with diluent (this standard solution of 40µg/ml).

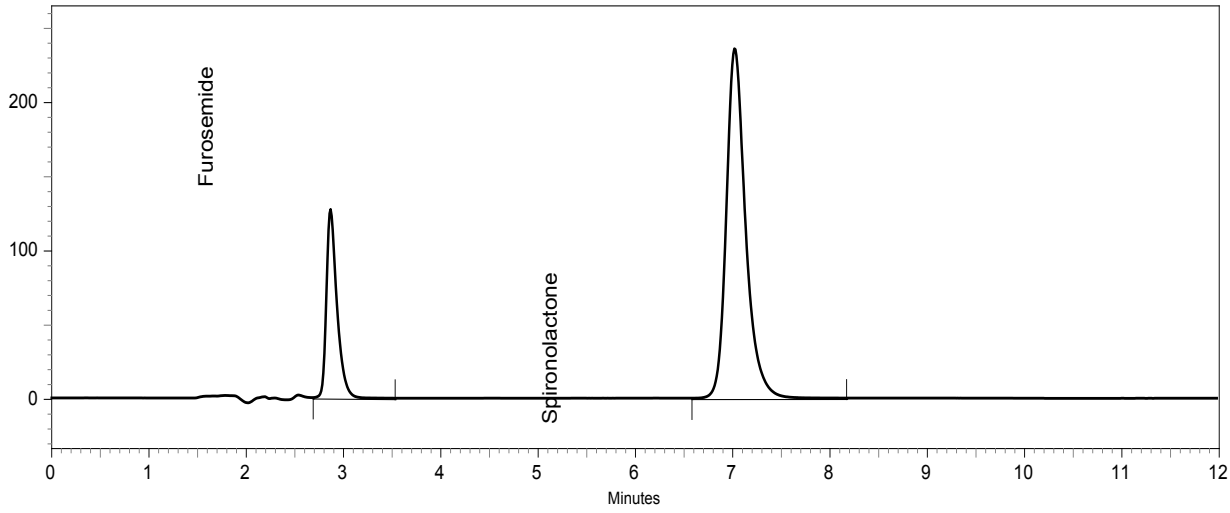

Figure 3. Chromatogram of standard preparation.

2. 5. Test preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer into a 500 ml volumetric flask. About 50 ml of diluent was added and sonicated for a minimum 30 min. with intermittent shaking. Then content was brought back to room temperature and diluted to volume with diluent. The sample was filtered through 0.45µm nylon syringe filter. Further take 10 ml of this stock solution in 50 ml of volumetric flask and make up to mark with diluent. The concentration obtained was 100 µg/ml of Spironolactone and 40 µg/ml of Furosemide.

3. RESULT AND DISCUSSION

3. 1. Method validation

3. 1. 1. Specificity study

The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of checking the interference of the degradation products in the drug quantification for assay during the forced degradation study.

Table 1. Precision study for Analytical Method Validation of Furosemide Spironolactone.

Furosemide				**Spironolactone**			
Description	Mean area	Wt. (mg)	% Assay	Description	Mean area	Wt. (mg)	% Assay
Set 1	998819	1071.5	101.0	Set 1	3366197	1071.5	101.0
Set 2	999963	1070.5	101.1	Set 2	3379481	1070.5	101.4
Set 3	978857	1071.3	98.9	Set 3	3311436	1071.3	99.4
Set 4	975578	1070.3	98.6	Set 4	3366585	1070.4	101.0
Set 5	987644	1071.4	99.8	Set 5	3351654	1071.4	100.6
Set 6	1003532	1072	101.4	Set 6	3397057	1072	101.9
		Mean	100.1			Mean	100.9
		Stdev	1.19			Stdev	0.87
		% RSD	1.19			% RSD	0.87
Standard mean area	995588	Standard mean area		Standard mean area	3374356		
Standard wt. (mg)	19.8	Standard wt. (mg)		Standard wt. (mg)	49.8		
Test wt	1071.3	Test wt		Test wt	1071.3		
Label claim (mg)	20	Label claim (mg)		Label claim (mg)	50		
Average Wt. (mg)	217.8	Average Wt. (mg)		Average Wt. (mg)	217.8		

Table 2. Intermediate Precision study for Analytical Method Validation of Furosemide Spironolactone.

Furosemide				**Spironolactone**			
Description	Mean area	Wt. (mg)	% Assay	Description	Mean area	Wt. (mg)	% Assay
Set 1	1003949	1071.5	98.9	Set 1	3496814	1071.5	100.7
Set 2	1004029	1070.5	98.9	Set 2	3500878	1070.5	100.9
Set 3	1001844	1071.3	98.7	Set 3	3480761	1071.3	100.3
Set 4	1000111	1070.3	98.5	Set 4	3459055	1070.3	99.7
Set 5	1000388	1071.4	98.5	Set 5	3497922	1071.4	100.8
Set 6	999289	1072	98.4	Set 6	3480035	1072	100.3
		Mean	98.6			Mean	100.4
		Stdev	0.20			Stdev	0.46
		% RSD	0.20			% RSD	0.46
Standard mean area	1052789			Standard mean area	3577951		
Standard wt. (mg)	20.4			Standard wt. (mg)	50.7		
Test wt (mg)	1071.3			Test wt (mg)	1071.3		
Label claim (mg)	20			Label claim (mg)	50		
Average Wt. (mg)	217.8			Average Wt. (mg)	217.8		

Table 3. Statistical Comparison of Precision and Intermediate Precision study for Analytical Method Validation of Furosemide in tablet formulation.

	FUROSEMIDE					
Precision study	Set 1	101.0	For Precision		For Intermediate Precision	
	Set 2	101.1	Mean	101.35	Mean	100.3333
	Set 3	98.9	Standard Error	0.287228132	Standard Error	0.567255
	Set 4	98.6	Median	101.15	Median	99.8
	Set 5	99.8	Mode	#N/A	Mode	#N/A
	Set 6	101.3	Standard Deviation	0.703562364	Standard Deviation	1.389484
Intermediate precision study	Set 1	98.9	Sample Variance	0.495	Sample Variance	1.930667
	Set 2	98.9	Kurtosis	-1.398102234	Kurtosis	-1.40619
	Set 3	98.7	Skewness	0.478086455	Skewness	0.698096
	Set 4	98.5	Range	1.8	Range	3.5
	Set 5	98.5	Minimum	100.5	Minimum	98.8
	Set 6	98.4	Maximum	102.3	Maximum	102.3
	Mean	99.4	Sum	608.1	Sum	602
	Stdev	1.12	Count	6	Count	6
	% RSD	1.12	Confidence Level(95.0%)	0.738342213	Confidence Level(95.0%)	1.458172
	FUROSEMIDE SUMMARY OUTPUT					
Regression Statistics						

Multiple R	0.255205564					
R Square	0.06512988					
Adjusted R Square	-0.16858765					
Standard Error	0.234357888					
Observations	6					
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	0.015305522	0.015305522	0.278669	0.625502408	
Residual	4	0.219694478	0.05492362			
Total	5	0.235				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	93.99657614	8.815636944	10.66248267	0.000438	69.5204441	118.4727082
X Variable 1	0.046734418	0.088530383	0.527891288	0.625502	-0.19906533	0.292534165

3. 1. 2. Linearity

Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration (40, 60, 80, 100, 120, 140 and 160µg/ml). The peak areas versus concentration data were evaluated by linear regression analysis.

3. 1. 3. Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of Spironolactone and Furosemide test sample preparation and

calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing same procedure on the different day (interday) by another person under the same experimental condition.

3. 1. 4. STATISTICAL EVALUATION

Statistical evaluation is given in table no. 1, 2, 3 and 4. And Formula for assay calculation was

% Assay = Average Test area / Average std. area * Std. wt. * 405.48/441.94 / 250 * 250 / Test wt. / Label claim * Average wt. * Potency of std.

Table 4. Statistical Comparison of Precision and Intermediate Precision study for Analytical Method Validation of Spironolactone in tablet formulation.

	SPIRONOLACTONE					
Precision study	Set 1	101.0	For Precision		For Intermediate Precision	
	Set 2	101.4	Mean	101.35	Mean	100.3333
	Set 3	99.4	Standard Error	0.287228132	Standard Error	0.567255
	Set 4	101.0	Median	101.15	Median	99.8
	Set 5	100.6	Mode	#N/A	Mode	#N/A
	Set 6	101.9	Standard Deviation	0.703562364	Standard Deviation	1.389484
Intermediate precision study	Set 1	100.7	Sample Variance	0.495	Sample Variance	1.930667
	Set 2	100.9	Kurtosis	-1.398102234	Kurtosis	-1.40619
	Set 3	100.3	Skewness	0.478086455	Skewness	0.698096
	Set 4	99.7	Range	1.8	Range	3.5
	Set 5	100.8	Minimum	100.5	Minimum	98.8
	Set 6	100.3	Maximum	102.3	Maximum	102.3
	Mean	100.7	Sum	608.1	Sum	602
	Stdev	0.69	Count	6	Count	6

	% RSD	0.68	Confidence Level(95.0%)	0.738342213	Confidence Level(95.0%)	1.458172
	FUROSEMIDE SUMMARY OUTPUT					
Regression Statistics						
Multiple R	0.062853564					
R Square	0.00395057					
Adjusted R Square	-0.245061787					
Standard Error	0.497762288					
Observations	6					
ANOVA						
	df	*SS*	*MS*	*F*	*Significance F*	
Regression	1	0.003930818	0.003930818	0.015865	0.905843808	
Residual	4	0.991069182	0.247767296			
Total	5	0.995				
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	97.28805031	25.10439313	3.875339659	0.017912	27.58708089	166.9890197
X Variable 1	0.031446541	0.249662563	0.125956173	0.905844	-0.661727859	0.72462094

4. CONCLUSION

In this current study a new analytical method has been developed to be routinely applied to simultaneous determine Spironolactone and Furosemide in pharmaceutical dosage form. The developed procedure has been statistically evaluated for Precision study and Intermediate precision study. And as result shown that for furosemide, % RSD was 1.12% and in ANOVA *study Significance F* value found 0.625502408 and for spironolactone Precision study and Intermediate precision study % RSD was 0.68, in ANOVA *study Significance F* value found

0.905843808. So, it is concluded that current method passes through its *Significance value.* Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

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