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Stability Indicating UPLC Method Development and Validation of Furosemide and Spironolactone in Bulk Dosage Form

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Abstract

A simple, sensitive and specific stability indicating UPLC method was developed for simultaneous estimation of Furosemide and Spironolactone in bulk and tablet dosage forms. Effective separation was achieved by BEH C_{18} column (100mm×2.1mm,1.7µm) with mobile phase containing a mixture $NaH_2PO_4.H_2O$ and acetonitrile (57:43 v/v) adjusted with ortho phosphoric Acid. The run time was 6 min at the flow rate of 0.25ml/min. Then detection was performed at a wavelength of 235nm. The injection volume was 1.10µl and the column was maintained at 40ºC. The stress conditions such as acid, base, oxidative, thermal and Photo stability were applied as per ICH guidelines to determine the stability of drugs in different environmental conditions. The Retention time of Furosemide and Spironolactone were found to be 1.639 and 5.154 respectively. The developed Method was linear in the concentration range. All the Method validation parameter were within the limits and the degradation product were all resolved for the Furosemide and Spironolactone. The newly developed UPLC method for Assay determination of Furosemide and Spironolactone was found to be capable of giving faster retention time and maintained good resolution. The method was completely validated showing satisfactory data for all parameters listed. The method exhibited an excellent performance in terms of sensitivity and speed. Its stability indicates method for rapid analysis of Furosemide and Spironolactone in Bulk and Tablets.

Keywords: Furosemide, Spironolactone, Ortho Phosphoric Acid, UPLC, Stability, Validation.

1. Introduction

Ultra performance liquid chromatography (UPLC) is a recent developed technique in liquid chromatography that enables significant in separation time and solvent consumption. Literature indicates the UPLC system allows a fold decreases the analysis time as compared to HPLC system using 5µm particle size analytical columns about 3-fold decrease in analysis time in comparison with 3µm particle size analytical columns without comparison on overall separation of compounds.

Furosemide 4-Chloro-N-furfuryl-5-sulfamoylanthranilic acid, furosemide secreted into the tubular fluid via the organic acid pathway. The drug acts along the entire nephron (sites 2 and 3) primarily on the thick ascending limb of the loop of Henle with the exception only of the distal site where Na^+ is exchanged for K^+ and H^+. It blocks the Na^+-k^+-$2CL^-$ symport. It's empirical formula of furosemide is $C_{12}H_{11}C_1N_2O_5S$ and its molecular weight is 330.745g/mol. Furosemide is available in 20,40 and 80mg divided in 12 hours for adults.

Spironolactone7α-Acetylthio-17α-hydroxy-3-oxopregn4-ene-21-carboxylicacid, Spironolactone competitively inhibits aldosterone binding to the MR, thereby interfering with reabsorption of sodium and chloride ions and the associated water[3]. Spironolactone empirical formula is $C_{24}H_{32}O_4S$ and its molecular weight is 416.57g/mol. It is available in 25 to 100 m2g orally per day in single or divided doses for 5 days later dose may be adjusted accordingly. The duration of action of furosemide (loop diuretics) is 4-5hrs whereas spironolactone is 72 -96hrs.

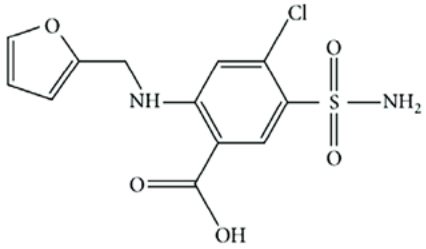

Figure 1: Furosemide

Based on our literature review, various methods were developed by HPLC for determination of Furosemide and Spironolactone. The already developed methods were carried out both individually and in combination with Furosemide and Spironolactone with other drugs. No single method was developed for the estimation of Furosemide and Spironolactone using UPLC. Hence a stability indicating rapid, accurate and valid UPLC method for the simultaneous determination of Furosemide and Spironolactone for combined dosage form in generic drug development was developed and validated.

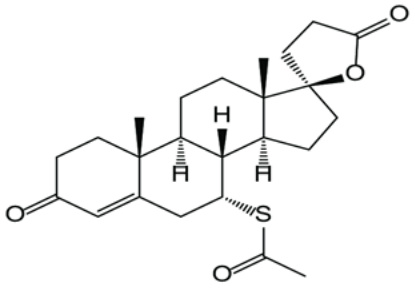

Figure 2: Spironolactone

2. Method and Materials

Samples of Furosemide and Spironolactone were received from Sanofi Aventis pharma Ltd. HPLC-Grade acetonitrile and ortho phosphoric acid were purchased from Merck India Ltd. High purity water was obtained from Millipore MilliQ plus water purification system.

3. Chromatographic Conditions

Assay was performed by using Waters Acquity UPLC system equipped with quaternary solvent manager, sample manager and tunable Ultraviolet/Visible detector. Different mobile phases and other chromatographic conditions were tried in order to find the best conditions for the separation of the Furosemide and Spironolactone. The optimized method was developed and chromatographic separation was achieved by using a Waters Acquity BEH C_{18} column (100mm×2.1mm,1.7m) with Mobile phase containing a mixture $NaH_2PO_4.H_2O$ and Acetonitrile (57:43 v/v) adjusted with ortho phosphoric Acid. The run time was 6 min at the flow rate of 0.25ml/min. The detection was performed at wavelength of 235nm.The injection volume was 1.10µl while the column was maintained at 40ºC.

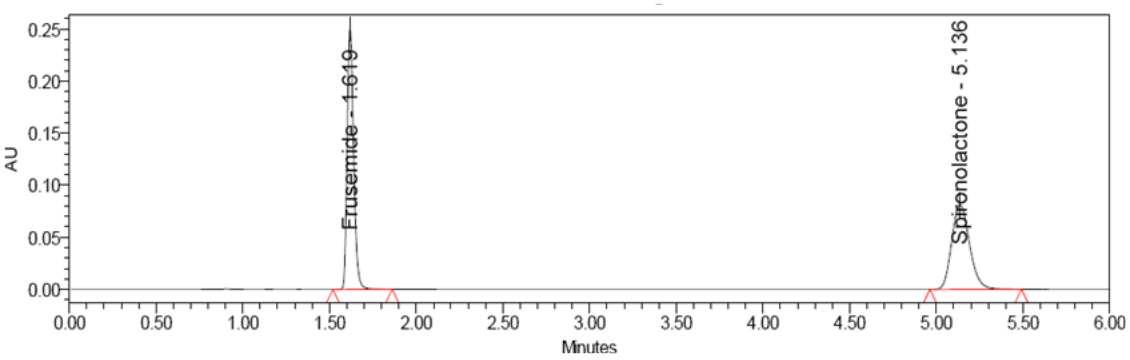

Figure 3: A optimized chromatogram of Furosemide and Spironolactone

4. Preparation of Standard Solution

Weigh accurately about 20mg of Furosemide working standard and 50mg of Spironolactone working standard and dilute to 100ml with diluent [$NaH_2PO_4.H_2O$: Acetonitrile (57:43 v/v)]. Dilute to get the final concentration of 0.02mg/ml of Furosemide and 0.05mg/ml of Spironolactone.

5. Preparation of Sample Solution

Accurately weigh 20 tablets collectively and determine the average weight. Grind the tablet homogenously with the aid of mortar and pestle. Weigh approximately 220mg of the tablets powder and transfer into 100ml volumetric flask. Add 70ml of diluents and keep it in Ultrasonic bath for 15 min. Then cool and dilute up to the mark with the same diluent. Dilute to make the final solution containing 0.02mg/ml of Furosemide and 0.05mg/ml of Spironolactone.

6. Results and Discussion

Method Development and Optimization of Stability Studies

Method optimization was done by trial-and-error method in such a way to obtain a chromatogram with good resolution(R), USP tailing factor, USP count. Several trails were done by altering the mobile phases and flow rate and finally the method with BEH C_{18} column (100mm×2.1mm,1.7m) with Mobile phase containing a mixture $NaH_2PO_4.H_2O$ and Acetonitrile (57:43 v/v) and adjusted with ortho phosphoric Acid. The run time was 6 min at the flow rate of 0.25ml/min. Then detection was performed at wavelength of 235nm.The injection volume was 1.10µl and the column was maintained at a temperature of 40ºC. The stress condition such as Acid, Base, Oxidative, Thermal and Photo stability were used.

7. Discussion

The stability indicating UPLC assay Method plays a significant role in the qualitative and quantitative estimation of the drug. Till now only RP-HPLC methods were developed but not UPLC techniques was reported, hence attempts were made to develop an effective stability method. The RT in the reported method for Furosemide and Spironolactone were found to be 1.639 and 5.154 respectively. It reduces solvent consumption and analyte run time, hence rapid analysis of a greater number of samples can be done. The calculated and statistical results of the validation parameters were not out of the acceptance limit stated by ICH guidelines.

8. Specificity – Forced Degradation Studies

Specificity is the ability of the method to measure the analyte in the presence of its potential impurities. Stress studies were performed at an initial concentration 20mg/ml of Furosemide and 50mg/ml of Spironolactone to provide an indication of the stability indicating property. Since the conditions that cause instability and result in degradation products of the API cannot be predicted initially, one has to subject the API to a variety of stress conditions. Trial and error are needed to find the proper combination of stress agent concentration and time to effect

degradation, preferably in the range of 5-15 % Depending on the API, not every stress agent may affect degradation, but each agent has to be evaluated to determine whether degradation occurs or not. Typical degradative conditions involve hydrolysis, photolysis, acid/base reactions, and temperature. Achieving 100 % degradation would require too much effort and could be possibly cause secondary degradation. Secondary degradation products are the degradation products of the degradation products, which are not likely to be formed under normal storage conditions.

Table 1: Summary Results of Forced Degradation Experiments

Degradation	RT	Std. Peak	Peak Area	% Degrade
Peroxide				
FUR	1.56	626.21059	No Change	-
SPR	4.94	561.26090	531.94599	5.22%
Base				
FUR	1.56	625.27275	618.42071	1.09%
SPR	4.94	560.36399	526.68497	6.01%
Thermal				
FUR	1.56	628.46945	610.52237	2.85%
SPR	4.94	563.19118	545.93937	3.06%

Table 2: Results From System Suitability Tests

Parameters	Furosemide	Spironolactone
Retention time	1.639	5.154
USP Tailing factor	1.05	1.22
USP Resolution	-	39.2
%RSD	0.100	0.130

9. Method Validation

Method validation was performed as per ICH guidance for simultaneous determination of IB and FM in the formulations. The following validation parameters were addressed, linearity, precision, accuracy, specificity, sensitivity, robustness, ruggedness and specificity.

10. Linearity

The linear response of the UPLC system for Furosemide and Spironolactone was in the concentration range of 80µg/ml to 120µg/ml of the respective working concentration (100% test concentration) using at least 5 different concentration distribution throughout the entire range. The r^2 values for Furosemide and Spironolactone were 0.999 and 0.998 respectively. The values are shown in the (table no 3 and 4)

Table 3: Result of Linearity of Furosemide

S. No	Concentration (%)	Peak Area		Mean	%RSD
		1	2		
1	80	520.05699	519.56052	519.80875	0.0675359
2	90	586.72423	586.41693	586.57058	0.0370448
3	100	656.27238	656.86217	656.56728	0.0635189
4	110	720.92715	719.48538	720.20627	0.1415546
5	120	782.63450	781.61998	782.12724	0.09172088

Table 4: Result of Linearity of Spironolactone

S. No	Concentration (%)	Peak Area		Mean	%RSD
		1	2		
1	80	468.80266	468.46069	468.631675	0.05159901
2	90	529.22800	529.00362	529.11581	0.02998599
3	100	590.82938	591.32951	591.07945	0.0598304
4	110	650.81732	648.61713	649.71723	0.2394533
5	120	708.14033	707.22202	707.681175	0.09175646

11. Precision

For System precision, % RSD for each analyte peak area counts should be not more than 2.0 or as specified in under evaluation of system suitability in analytical test procedure.

For method precision % RSD of Six results should be not more than 2.0.

For Ruggedness (Intermediate Precision) % RSD of the six assay results should be not more than 2.0 and overall % RSD with method precision results should be not more than 2.0.

Table 5: Result of Intermediate Precision (Analyst 1/ Day 1)

Test No.	Furosemide Area	Spironolactone Area
1	654.20254	589.55549
2	654.51848	589.84959
3	656.32732	591.45961
4	654.21721	589.46931
5	654.92958	589.86783
6	654.98778	589.74415
Mean	654.86382	589.99099
SD	0.7919821	0.73685104
%RSD	**0.121**	**0.125**

Table 6: Result of Intermediate Precision (Analyst 2/ Day 1)

Test No	Furosemide Area	Spironolactone Area
1	635.85108	589.65586
2	634.26979	587.21523
3	633.27385	576.31669
4	635.90808	568.62622
5	635.67459	571.01385
6	636.38486	568.81520
Mean	635.227042	576.940508
SD	1.19376449	9.35855253
%RSD	**0.187**	**1.622**

12. Accuracy

% Drug recovery of drug at each level should be between 98.0 and 102.0.% and %RSD should be more than 2.0. The overall average should be between 98.0 and 102.0 % and %RSD should not more than 2.0.

13. Specificity

No peak should elute or interfere at retention time of analyte peak from blank and placebo solution.

The difference between the mean of triplicate preparations spiked with known impurities and mean result from method precision with respect to mean results of method precision should be not more than 2.0 %

Peak purity of analyte peak/s from spiked sample should pass.

14. Sensitivity

The LOD and LOQ values for Furosemide were determined as 15.605µg/ml and 47.288 µg/ml respectively.

The LOD and LOQ values for Spironolactone were determined as 8.584µg/ml and 26.014 µg/ml respectively.

15. Solution Stability and Mobile Phase Stability

A standard solution was stored in the chromatographic vials and in the volumetric flask. The solution was injected at 6 hours,12 hours,24 hours,48 hours with fresh preparation and the % variation in the response area with fresh solution was calculated and it should not be more than 2%.

Table 7: Parameters For Solution Stability

Variation	Frusemide (Vol. Flask)	Spironolactone (Vol. Flask)	Frusemide (Vial)	Spironolactone (Vial)
%variation (6 hr)	0.96	0.61	0.10	0.53
%variation(12hr)	0.12	1.45	0.33	0.07
%variation(24hr)	0.89	0.38	0.45	0.81
%variation(48hr)	1.84	0.19	2.03	1.09

16. Robustness

Slightly deliberate variations in mobile phase ratio, flow and absorbance maximum of the method could not produce the system suitability parameter beyond the acceptance limit. Overall % RSD should not be more than 2.0.

Table 8: Parameters For Robustness Study

Sr. No	Method Parameters Varied	Resolution	Frusemide	Spironolactone
			Tailing Factor	
1	Flow rate – 0.2 mL/min	38.7	1.22	1.05
2	Flow rate – 0.3 mL/min	39.2	1.22	1.07
3	Mobile Phase pH-2.9	40.9	1.21	1.05
4	Mobile Phase pH-3.1	40.5	1.22	1.06
5	Change in Column (50mm length)	26.5	1.26	1.05
6	Mobile Phase Composition	43.9	1.19	1.04

Conclusion

The newly developed UPLC method for Assay determination of Furosemide and Spironolactone was found to be capable of giving faster retention time and maintained good resolution. The method was completely validated showing satisfactory data for all parameters listed. The method exhibited an excellent performance in terms of sensitivity and speed. Its stability indicates method for rapid analysis of Furosemide and Spironolactone in Bulk and Tablet dosage form.

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Authors Contribution

Both the authors contributed equally in the manuscript preparation. Both authors have read and approved the manuscript.

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