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Stability-indicating UPLC method for determination frusemide, spironolactone and their degradation products in active pharmaceutical ingredients and pharmaceutical dosage form

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ABSTRACT

A simple, precise, accurate isocratic stability indicating ultra-performance liquid chromatographic (UPLC) method was developed for quantitative determination of Frusemide, Spironolactone in bulk sample and solid pharmaceutical dosage form in the presence of degradation products. Isocratic RP-UPLC separation was achieved on Waters Aquity Bridge Ethylene Hybrid (BEH) C_{18} (100mm×2.1mm, 1.7µ) column using a mobile phase containing a mixture of sodium dihydrogen phosphate monohydrate (adjust pH 3 with ortho-phosphoric acid) and acetonitrile (57:43) (v/v) at flow rate of 0.25 ml min^{-1}. The detection was carried out at 235nm by using Tunable Ultra-Violet (TUV) detector. The runtime was 7 min for performing the analysis in which two main compounds were separated. The developed method was validated as per ICH guideline with respect to linearity, precision, accuracy and robustness. Degradation products produced as a result of stress studies did not interfere with the detection of frusemide and spironolactone; therefore, the assay can be considered as stability-indicating.

KEYWORDS

Frusemide;
Spironolactone;
UPLC;
Validation study;
Stress study.

INTRODUCTION

However, Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reduction in separation time and solvent consumption[1]. Literature indicates that the UPLC system allows about a nine fold decrease in analysis time as compared to the conventional HPLC system using 5µm particle size analytical columns, and about a threefold decrease in analysis time in comparison with 3 µm particle size analytical columns without compromising overall separation[2, 3]. These particles operate at elevated mobile phase with linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis. Because of its speed and sensitivity, this technique is gaining considerable attention in pharmaceutical analysis[4]. In this present work UPLC has been applied to the method development and validation study of assay determination of Frusemide and Spironolactone in bulk drug.

The chemical formula of Frusemide is 4-chloro-N-furfuryl-5-sulphamoylanthranilic acid as shown in Figure 1a.

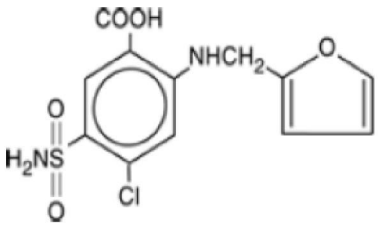

Figure 1a

Frusemide is an anthranilic acid derivative, is a potent diuretic of rapid action, used in treatment of edema associated with hypertension, congestive heart failure, pulmonary and renal disease, cirrhosis of the liver in humans, and normally administered as tablets or intravenous and intramuscular injectables. Numerous analytical methods are available for the assay of Frusemide in pharmaceutical preparations that include titrimetric[6], spectrophotometric[7, 8], 1H-NMR[9], and HPLC methods[10,11].

The chemical formula of Spironolactone is 7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17β-carbolactone as shown in Figure 1b.

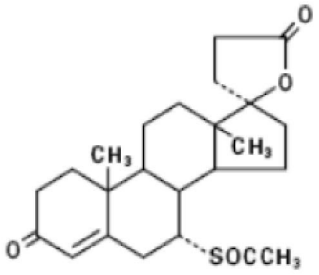

Figure 1b

HPLC methods[12, 13], spectrophotometric methods[14], gas chromatographic method[15] has been developed for the determination of spironolactone and/or its metabolites in plasma or serum. There are many methods reported for the determination of Frusemide, Spironolactone and with other drug by Micellar Liquid Chromatography[16], HPLC/MS/MS[17], TLC[18], and UHPLC method[19]. The aim of present work is to develop an efficient novel method for simultaneous estimation of Frusemide and spironolactone by UPLC as no method is available in combination for commercial dosage form by UPLC. Also their findings promoted us to develop simple, sensitive method in combination for Frusemide and Spironolactone along with their stability indicating properties by forced degradation studies.

EXPERIMENTAL

Chemicals and materials

Frusemide and Spironolactone were obtained from Sanofi Aventis Pharma ltd. (Ankleshwar India). HPLC grade Acetonitrile, Sodium dihydrogen phosphate monohydrate Orthophosphoric acid, Sodium Hydroxide (0.1N,4N solution), Hydrochloric acid (0.5N solution), Hydrogen peroxide (3% v/v solution) were purchased from Merck India Ltd, high purity water was used of Millipore.

UPLC and chromatographic conditions

Chromatography was performed by using Waters ACQUITY system comprising of quaternary pumps, auto injector, column compartment and Tunable UV detector. The system was controlled by use of EMPOWER software. Compounds were separated on BEH C_{18} (100mm×2.1mm, 1.7µm). The column temperature was maintained at 40°C. The mobile phase was $NaH_2PO_4.H_2O$ (pH 3 with orthophosphoric acid): Acetonitrile (57:43) at a flow rate of 0.25mLmin^{-1}. Before use of mobile phase was filtered through a 0.22µm Nylon filter and degassed in an ultrasonic bath. The injection volume was 10µL and detection was done at 235 nm.

Preparation of sample solution

Stock solution

A stock solution was prepared by dissolving an appropriate amount of Frusemide (20mg) and Spironolactone (50mg) in 100 ml of diluents $NaH_2PO_4.H_2O$ (pH 5 with NaOH): Acetonitrile (58:42)]. Final concentration of 0.02 mgmL^{-1} of Frusemide and 0.05 mgmL^{-1} of Spironolactone were used for assay determination.

Procedure for force degradation study

Initial degradation was attempted to stress condition of acid (0.5N HCL at 60°C), base (0.1N NaOH at 60°C), Oxidation (3% H_2O_2 at 60°C), heat (60°C) and UV light (254 nm) to evaluate the ability of proposed method to separate Frusemide and Spironolactone from its degradation product. Study Period for Acid and Base was 1h; for oxidation 5 h; and for heat and light was 1 day.

(a) Analysis of the tablet dosage form

Twenty tablets were weighed and transferred into

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a clean, dry mortar. Powder equivalent to 20mg of Frusemide and 50mg of Spironolactone drug were dissolved in 50 ml of diluent. Solution was placed in ultrasonic bath for 15 min. The final volume was made up to 100 ml with diluent and filtered through 0.22µm nylon membrane filter. Dilute further to obtain final concentration of approximately 0.02 $mgmL^{-1}$ of Frusemide and 0.05 $mgmL^{-1}$ of Spironolactone as shown in TABLE 1.

TABLE 1: Analysis of tablet formulation

Analyte	Label Claim (mg/tablet)	Amount Found (mg/tablet)	%RSD
Frusemide	20	19.390	1.369
Spironolactone	50	48.596	1.871

(b) Method validation

The described method has been validated for assay by UPLC determination as per ICH guideline[20].

(c) Precision

The repeatability of drug sample was checked by six fold analysis of 0.02mg ml^{-1} of Frusemide and 0.05mg ml^{-1} of Spironolactone. The RSD (%) of peak area was calculated for each injection.

Inter and intra-day variation and analyst variation was studied to determine intermediate precision of the proposed method.

(d) Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the true value; and the value found. The accuracy of assay method for frusemide and spironolactone was evaluated in triplicate at three concentrations, (80%, 100%, and 120%) on drug product and recovery was calculated for each added concentration.

(e) Linearity

Linearity of the assay test method was determine in the range from 80 to 120 % of the respective working concentration (100% test concentration) using at least 5 different concentration distribution throughout the entire range.

(f) Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage. By introducing deliberately small changes in mobile phase pH (±0.2), flow rate (±0.1ml/min), column dimension and Mobile phase composition (±2%).

RESULT AND DISCUSSION

Optimization of chromatographic condition

A new UPLC analytical method for the simultaneous determination of frusemide and spironolactone was utilized. The main objective of the developed method was to separate frusemide and spironolactone and its degradation products and to elute both the drugs as symmetrical peaks. To obtain the best chromatographic conditions, the mobile phase was optimized to provide sufficient selectivity and sensitivity which efficiently separated frusemide and spironolactone and its degradation product formed during stress degradation studies. Isocratic mode is used for the separation. $NaH_2PO_4.H_2O$ (pH 3.0 with ortho phosphoric acid): Acetonitrile (57:43) at the detection wavelength of 235 nm were found to be the most suitable because it afforded better resolution and shorter run time. Different columns were evaluated and the Waters Acquity BEH C_{18}, 100mm×2.1mm, 1.7µm analytical column

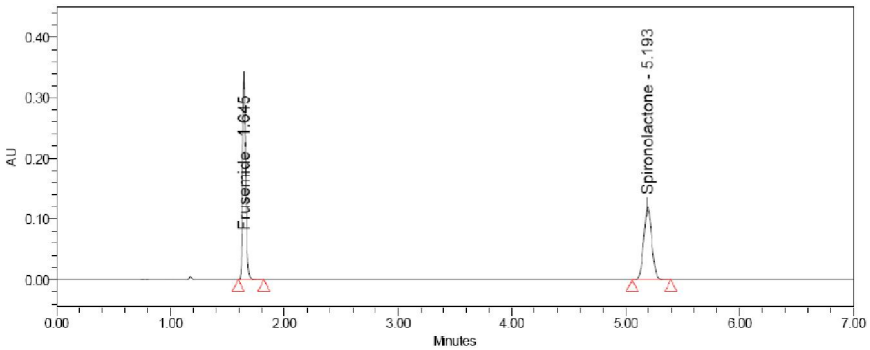

Figure 2 : Chromatogram of test preparation

was selected as it provides chromatographic performance and peak characteristics. Figure 2 represents the chromatograms of test preparation.

Chromatographic Performance

(a) Ratio of mobile phase component:

Best resolution without peak tailing was achieved in the ratio of $NaH_2PO_4.H_2O$: acetonitrile in (57:43) as compared to mobile phase contain acetonitrile: water. pH of Mobile phase:

The pH of the mobile phase was investigated in the pH range of 2-4. It was found that proper resolution with peak area ratio was achieved at pH 3.0

(b) Effect of flow rate:

The effect of different flow rate (0.2-0.5ml/min) on the separation of complex was tested and it was found that 0.25 ml/min was the most suitable flow rate in terms of retention time and symmetry of the peaks.

Validation of the method

System suitability parameters were studied by injecting five replicate injections of working standard solution of frusemide and spironolactone TABLE 2.

TABLE 2: System Suitability

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

TABLE 3: Results for validation parameters

Parameters	Frusemide	Spironolactone
Linearity & Range		
Range[μg]	16-24	40-60
Linearity equation	Y = 6.5827x-5.2166	Y = 5.987x-9.4554
Coefficient correlation [r^2]	0.999	0.999
Accuracy		
% Recovery [n=9]	100.61	100.60
% RSD	0.539	0.402
Precision [%RSD]		
Intra-Day [n=3]	0.20 - 0.42	1.21 - 1.32
Inter-Day [n=3]	1.48 - 1.75	0.23 - 0.75
Repeatability [n=6]	0.121	0.125
Ruggedness [%RSD]		
Analyst I [n=6]	0.429	1.329
Analyst II [n=6]	1.499	0.758

The correlation coefficient (r^2) obtained was greater than 0.999. The experimental results revealed that approximately 99-101% recoveries were obtained, therefore, based on the recovery data the estimation of related compound that are prescribed in this report has been demonstrated to be accurate for intended purpose and is adequate for routine analysis. %RSD was less than 2% in intraday and interday precision. So the proposed method is more precise TABLE 3.

Small but deliberate changes were made to the method conditions, but there were no marked changes in chromatographic behavior and content of the drug, as %RSD was less than 2%, indicating the method is robust TABLE 4.

Result from forced degradation studies

All forced degradation samples were analyzed at an initial concentration. Degradation was not observed when Frusemide and Spironolactone was subjected to acid hydrolysis and light effect. But when they were subjected to base hydrolysis Figure. 3 and thermal effect Figure. 4, both the drug undergoes degradation and incase of peroxide effect Figure.5 only Spironolactone was found to be degraded as shown in TABLE 5.

TABLE 4 : Result for robustness parameters

Sr. No.	Method parameter 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 dimension	26.5	1.26	1.05
6	Mobile phase composition	43.9	1.19	1.04

TABLE 5: Results for force degradation study

Degradation	RT	Std. Peak	Peak Area	% Degrade
Peroxide Effect				
Frusemide	1.56	626.21059	No Change	-
Spironolactone	4.94	561.26090	531.94199	5.22%
Base Effect				
Frusemide	1.56	625.27275	618.42071	1.09%
Spironolactone	4.94	560.36399	526.68497	6.01%
Thermal Effect				
Frusemide	1.56	628.46954	610.52237	2.85%
Spironolactone	4.94	563.19118	545.93937	3.06%

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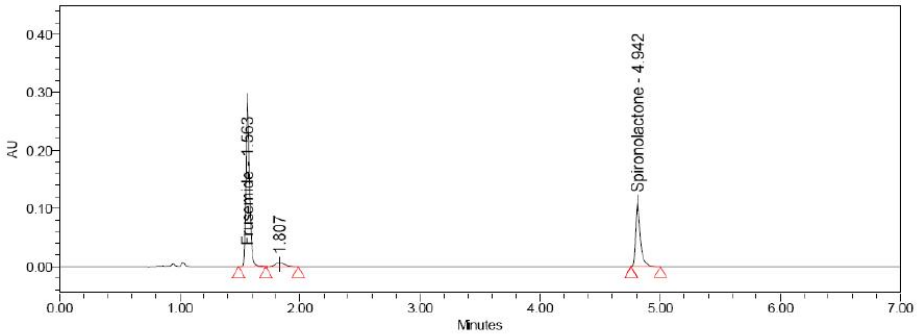

Figure 3 : Chromatogram of base hydrolysis

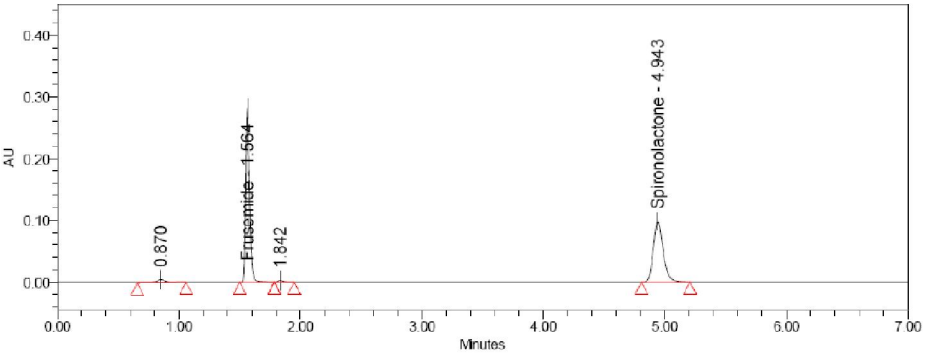

Figure 4 : Chromatogram of thermal effect

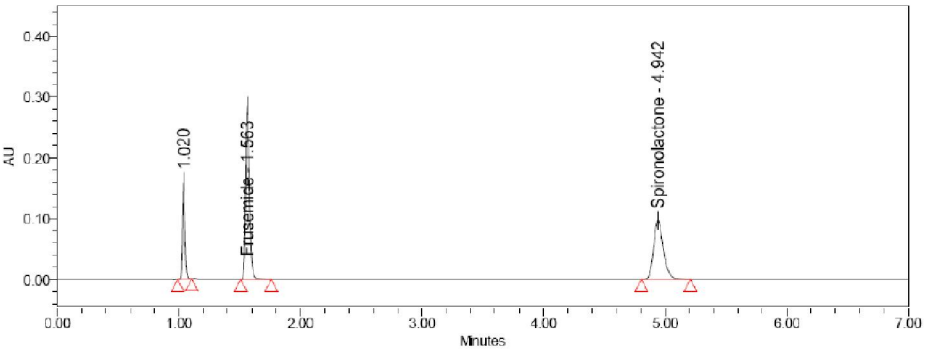

Figure 5 : Chromatogram of peroxide effect

CONCLUSION

A new UPLC assay method was developed for simultaneous determination of Frusemide and Spironolactone. The method validation results have proved that the method is selective, precise, accurate, robust and stability-indicating. This method exhibited an excellent performance in terms of speed and sensitivity. The total runtime was 7 min, within which both the drug were separated. This method can be successfully applied for the routine analysis as well as the stability study. Overall, the method provides a high throughput solution for determination of frusemide and spironolactone in tablet dosage form.

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