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Development and validation of a new analytical rp-hplc method for the quantitative estimation of finerenone in api form and marketed pharmaceutical dosage form

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ABSTRACT

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Finerenone in bulk form and marketed formulation. Separation of Finerenone was successfully achieved on a Symmetry ODS C18 (4.6 x 250mm, 5µm) column in an isocratic mode of separation utilizing Acetonitrile: Methanol in the ratio of 80:20% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 272nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 10-50mcg/mL for Finerenone. The correlation coefficient was found to be 0.999 for Finerenone. The LOD and LOQ for Finerenone were found to be 1.1µg/mL and 3.2µg/mL respectively. The proposed method was found to be good percentage recovery for Finerenone, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: Finerenone, RP-HPLC, Accuracy, ICH Guidelines.

INTRODUCTION

Finerenone, or BAY 94-8862, is a mineralocorticoid receptor antagonist indicated to reduce the risk of sustained decline in glomerular filtration rate, end stage kidney disease, cardiovascular death, heart attacks, and hospitalization due to heart failure in adults with chronic kidney disease associated with type II diabetes mellitus. Patients with kidney disease would originally be given [spironolactone] or [eplerenone] to antagonize the mineraclocorticoid receptor. Spironolactone has low selectivity and affinity for the receptor; it dissociates quickly and can also have effects at the androgen, progesterone, and glucocorticoid receptors. Eplerenone[1] is more selective and has longer lasting effects. More selective nonsteroidal mineralocorticoid antagonists such as [apararenone], [esaxerenone], and Finerenone were later developed. So far, Finerenone is the only nonsteroidal mineralocorticoid receptor antagonist to be FDA approved. Finerenone was granted FDA approval on 9 July 2021, followed by the EMA approval on 11 March 2022. Finerenone[2] is a Nonsteroidal Mineralocorticoid-Receptor Antagonist. The mechanism of action of Finerenone is as a Mineralocorticoid Receptor Antagonist. Finerenone is a non-steroidal mineralocorticoid receptor antagonist indicated to reduce the risk of sustained decline in glomerular filtration rate, end stage kidney disease, cardiovascular death, heart attacks, and hospitalization due to heart failure in adults with chronic kidney disease associated with type II diabetes mellitus. It has a moderate duration of action as it is taken once daily and a wide therapeutic window as patients were given doses from 1.25 mg to 80 mg in clinical trials. Patients should be counseled regarding the risk of hyperkalemia. The IUPAC Name of Finerenone[3] is (4S)-4-(4-cyano-2-methoxy phenyl)-5-ethoxy-2, 8-dimethyl-1, 4-dihydro-1, 6-naphthyridine-3-carboxamide. The Chemical Structure of Finerenone is follows

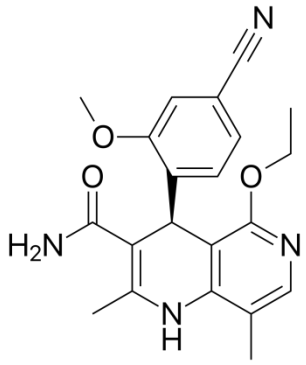

Fig 1: Chemical Structure of Finerenone

Experimental methods

Table 1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	Labindia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table 2: Chemicals Used

S.No	Chemical	Brand Names
1	Finerenone (Pure)	Local Market
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

Hplc method development

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Finerenone 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 0.3ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution

Take average weight of the Powder and weight 10 mg equivalent weight of Finerenone sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

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[32-33].

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase[4] was optimized to ACN: Methanol 80:20% v/v) respectively.

Optimization of Column

The method was performed with various C_{18} columns like Symmetry, Zodiac and Xterra. Symmetry ODS C18 (4.6 x 250mm, 5µm) Column was found to be ideal as it gave good peak shape and resolution[5] at 1ml/min flow.

Preparation of Mobile Phase

Accurately measured 800 ml (80%) of HPLC Acetonitrile and 200 ml of Methanol (20%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

Method validation

Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Finerenone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Finerenone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution

Take average weight of the Powder and weight 10 mg equivalent weight of Finerenone sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Inject the five replicate injections of standard and inject the three replicate injections sample solutions and calculate the assay[6] by using formula:

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

Linearity

Accurately weigh and transfer 10 mg of Finerenone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents[7] and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Finerenone): Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (20ppm of Finerenone): Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (30ppm of Finerenone): Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (40ppm of Finerenone): Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (50ppm of Finerenone): Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Inject each level into the chromatographic system[8] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability

Preparation of Finerenone Product Solution for Precision

Accurately weigh and transfer 10 mg of Finerenone working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision[9] (also known as Ruggedness[10]) of the method, Precision was performed on different days by maintaining same conditions.

Analyst 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

For Preparation of 50% Standard Stock Solution: Accurately weigh and transfer 10 mg of Finerenone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.15ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For Preparation of 100% Standard Stock Solution: Accurately weigh and transfer 10 mg of Finerenone working standard

into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
For Preparation of 150% Standard Stock Solution: Accurately weigh and transfer 10 mg of Finerenone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Take 0.45ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions[11]. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Finerenone and calculate the individual recovery[12] and mean recovery values.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Preparation of 0.597µg/ml solution (LOD)

Accurately weigh and transfer 10 mg of Finerenone 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 0.00597ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of 1.811µg/ml solution (LOQ)

Accurately weigh and transfer 10 mg of Finerenone 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 0.01811ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Robustness

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard Solution

Accurately weigh and transfer 10 mg of Finerenone working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Effect of Variation of Flow Conditions

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. ACN: Methanol was taken in the ratio and 75:25, 85:15 instead of 80:20, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Method Development

Several concurrent trails developed the proposed method to establish the preferred chromatographic conditions, which would be helpful to conduct a complete validation study[13]. The mobile phase for consisting of Acetonitrile and methanol (80:20 v/v) at 1-mL/min flow rate and detection wavelength 272 nm was optimized, which gave sharp peak, minimum tailing factor with short run time for Finerenone. The retention time[14] for Pravastatin was found to be 3.155 minutes (Figure 2).

Optimized Chromatographic Condition

Column	: Symmetry ODS C18 (4.6 x 250mm, 5µm)
Column temperature	: Ambient
Wavelength	: 272 nm
Mobile phase ratio	: ACN: Methanol (80:20% v/v)
Flow rate	: 1.0mL/min
Injection volume	: 20 µl
Run time	: 8 minutes

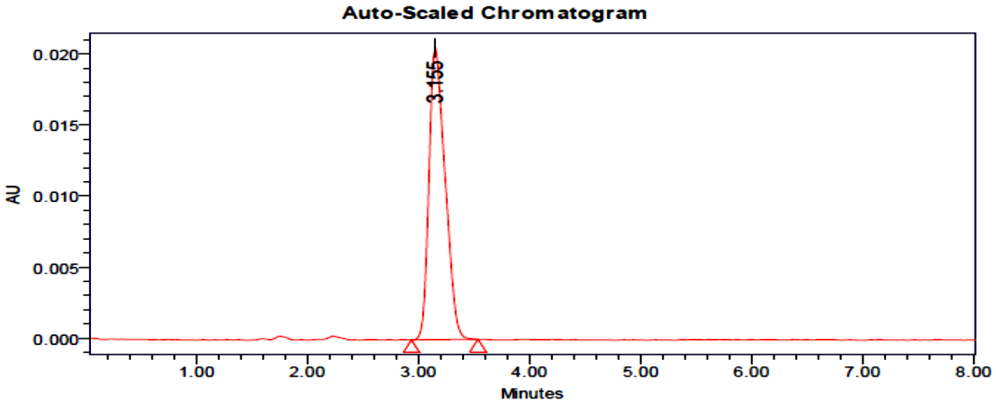

Fig 2: Optimized Chromatographic Condition of Finerenone

Validation of Analytical Method

Validation of the developed HPLC method[15] was carried out according to the International Council on Harmonization (ICH) guidelines Q2 (R1) for linearity and range, accuracy, precision, LOD and LOQ, specificity and robustness by the following procedure.

System Suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters[16] like theoretical plates, resolution and asymmetric factor were evaluated. The results were presented in Table 3.

Table 3: Results of system suitability for Finerenone

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Finerenone	3.192	225645	20584	6286	1.38
2	Finerenone	3.146	225847	20965	6358	1.39
3	Finerenone	3.123	228656	20758	6285	1.41
4	Finerenone	3.167	228547	20859	6278	1.40
5	Finerenone	3.158	229658	20968	6395	1.42
Mean			227670.6			
Std. Dev.			1810.899			
% RSD			0.795403			

Specificity

The specificity[17] was studied to examine the presence of interfering components, while in the comparison of Chromatograms; there was no interference from blank and standard Chromatogram.

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

= 99.24%

The % purity[18] of Finerenone in marketed pharmaceutical dosage form was found to be 99.24%.

Linearity

Linearity was performed by preparing a standard solution of Finerenone at different concentration levels, i.e., 10–50 µg/mL. The absorbance was measured at 272 nm. Linearity[19] was proven by regression analysis by the least square method. The correlation coefficient and linearity results were presented in Table 4, and the linearity curve[20] was represented in Figure 3.

Table 4: Data for Linearity of Finerenone

Concentration µg/ml	Average Peak Area
10	78683
20	146545
30	213584
40	279895
50	346568

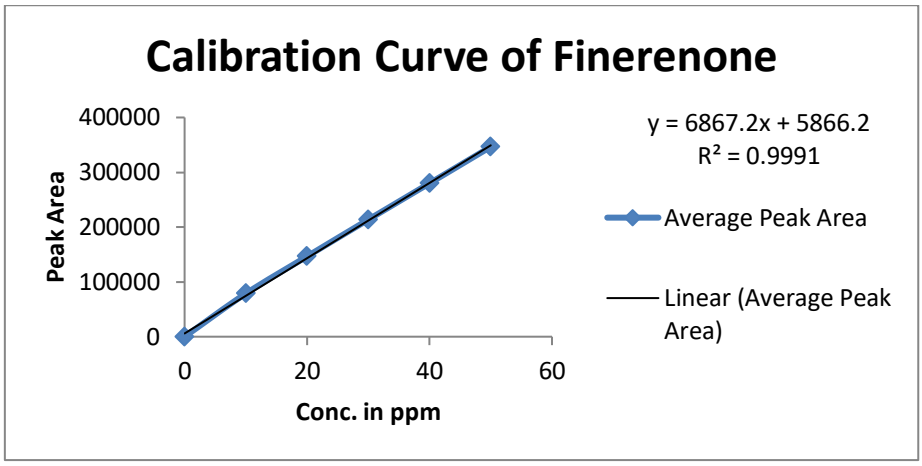

Fig 3: Calibration Curve of Finerenone

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Finerenone is a straight line.

Y = mx + c
Slope (m) =6867
Intercept (c) = 5866
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient[21] is 0.99 or greater.
Correlation Coefficient (r) is 0.99, and the intercept is 5866. These values meet the validation criteria.

Precision

Precision[22-25] was studied to find out intra-day and inter-day variation in the test methods of Finerenone for 6 times on the same day and different day. The intra-day and inter-day precision[26] obtained was %RSD (<2.0) indicates that the proposed method is quite precise and reproducible, and results are shown in Table 5 and 6 & 7.

Table 5: Results of Method Precision for Finerenone

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Finerenone	3.165	225645	20562	6125	1.36
2	Finerenone	3.163	225847	20645	6129	1.36
3	Finerenone	3.158	226542	20534	6135	1.35
4	Finerenone	3.167	226598	20564	6189	1.36
5	Finerenone	3.171	226584	20549	6138	1.35
6	Finerenone	3.181	226859	20685	6179	1.37
Mean			226345.8			
Std. Dev			482.1068			
%RSD			0.212996			

Intermediate Precision

Analyst 1

Table 6: Results of Ruggedness for Analyst 1 for Finerenone

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Finerenone	3.165	226534	20653	6235	1.35
2	Finerenone	3.163	226542	20598	6198	1.36
3	Finerenone	30158	225989	20653	6254	1.36
4	Finerenone	3.167	226512	20548	6281	1.35
5	Finerenone	3.171	226531	20653	6199	1.36
6	Finerenone	3.171	225898	20658	6253	1.35
Mean			226334.3			
Std. Dev.			304.2622			
% RSD			0.13443			

Analyst 2

Table 7: Results of Intermediate Precision Analyst 2 for Finerenone

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Finerenone	3.173	225487	20542	6253	1.35
2	Finerenone	3.134	225484	20532	6098	1.36
3	Finerenone	3.161	225364	20541	6254	1.35
4	Finerenone	3.174	226513	20534	6235	1.36
5	Finerenone	3.199	225487	20549	6199	1.36
6	Finerenone	3.199	226532	20451	6235	1.35
Mean			225811.2			
Std. Dev.			553.0524			
% RSD			0.244918			

Accuracy

The accuracy[27] of the method was determined by the standard addition method. A known standard drug was added to the fixed amount of pre-analyzed drug sample solution. The standard addition method was performed at three concentration levels in triplicate at 50%, 100%, and 150%. Percent recovery (Table 8) was calculated by comparing the peak area before and after adding the standard drug. Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 8: The Accuracy Results for Finerenone

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109283.3	15	15.060	100.40%	
100%	212732	30	30.124	100.413%	100.42%
150%	316263.3	45	45.201	100.446%	

Limit of Detection

The detection limit[28] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: = 0.597µg/ml

Limit of Quantitation

The quantitation limit[29] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: = 1.811µg/ml

Robustness

To demonstrate the method's robustness, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and organic content in the mobile phase. The results for robustness[30] are represented in Table 9.

Table 9: Results for Robustness of Finerenone

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	225645	3.155	6125	1.36
Less Flow rate of 0.9 mL/min	236586	3.488	6452	1.38
More Flow rate of 1.1 mL/min	219865	2.877	6098	1.42
Less organic phase	235848	4.705	6126	1.43
More organic phase	241245	2.090	6324	1.39

Forced Degradation Studies

Forced degradation study[31] is used to identify degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating the power of the analytical procedures used. The nature of stress testing will depend on the individual drug substance and the type of drug product involved. Finerenone were subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the conditions of acid and base

hydrolysis, oxidation, thermal, UV light as mentioned in ICH Q1A (R2).

Table 10: Results of Forced Degradation Studies for Finerenone

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	225645	0	100%	100%
2	Acidic	190015.65	15.79	84.21	100%
3	Basic	187353.04	16.97	83.03	100%
4	Oxidative	190985.92	15.36	84.64	100%
5	Thermal	183020.65	18.89	81.11	100%
6	Photolytic	181034.98	19.77	80.23	100%
7	Water	210549.34	6.69	93.31	100%

SUMMARY

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 272nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry ODS C18 (4.6 x 250mm, 5µm) because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Acetonitrile and Methanol (80:20% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 8.0min because analyze gave peak around 3.155 and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range of 10-50µg/ml of the Finerenone target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present research, a fast, simple, accurate, precise, and linear HPLC method has been developed and validated for Finerenone, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Finerenone. In addition, the main features of the developed method are short run time and retention time around 8 min. The method was validated in accordance with ICH guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions. Hence the proposed RP-HPLC method proved to be simple, accurate and reproducible for the determination of Finerenone in a reasonable run time. The method was validated showing satisfactory data for all the method validation parameters tested. The developed method can be conveniently used by quality control laboratories.

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