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DEVELOPMENT AND VALIDATION OF A NEW ANALYTICAL RP-HPLC METHOD FOR THE ESTIMATION OF FINERENONE IN BULK AND MARKETED FORMULATION

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ABSTRACT

A novel, simple, accurate, precise, sensitive and specific analytical RP-HPLC method was developed and validated for the quantitative estimation of Finerenone in bulk drug and marketed pharmaceutical dosage form. The Chromatographic separation was achieved on an Symmetry ODS C_{18} (4.6×250mm, 5µm) analytical column using mobile phase composition of methanol and Phosphate Buffer in ratio of (35: 65% v/v) that was set at a flow rate of 1.0µl/min with detection of 235 nm. The retention time of Finerenone was found to be 3.006min. The drug was analyzed by following the guidelines of International conference on Harmonization (ICH). This drug showing linearity in the concentration range of 6-14µg/ml and the correlation coefficient showing R2 = 0.9996. The % Recoveries showing within the limits. The presentation of the method was validated according to the present ICH guidelines for accuracy, precision and robustness, Linearity, limit of quantification, limit of detection linearity.

KEYWORDS: Finerenone, RP-HPLC, Method Development, Accuracy, Precision.

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.[1] 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 is more selective and has longer lasting effects.[2] 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. Finerenone is a non-steroidal selective mineralocorticoid receptor (MR) antagonist with no significant affinity or activity at androgen, progesterone, estrogen, and glucocorticoid receptors. Animal studies have shown that Finerenone binding to the MR reduces inflammation and fibrosis, and phase 2 clinical trials showed a reduction in albuminuria.[3] The IUPAC name of (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 shown in fig-1.

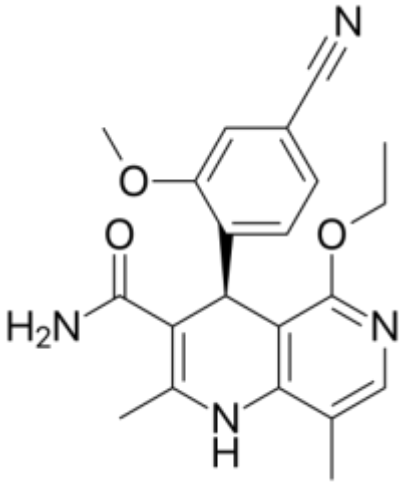

Fig. 1: Chemical Structure of Finerenone.

EXPERIMENTAL METHODS

Table 1: Instruments used.

S.No.	Instruments and Glass wares	Model
1	HPLC	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2	pH meter	Lab India
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)	Kerendia Tab
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 0.1ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions[4] and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.[25,30]

Mobile Phase Optimization

Initially the mobile phase tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase[5] was optimized to Methanol: Phosphate Buffer in proportion 35:65% v/v.

Optimization of Column

The method was performed with various C18 columns like, X- bridge column, Xterra, and C18 column. Symmetry ODS C18 (4.6 x 250mm, 5µm) was found to be ideal as it gave good peak shape and resolution[6] at 1ml/min flow.

Preparation of Potassium dihydrogen Phosphate (KH_2PO_4) buffer (pH-3.6)

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication.

Preparation of mobile phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in 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 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 0.1ml of the above Finerenone stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

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 0.1ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution

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 0.1ml of Finerenone above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

Inject the three replicate injections of standard and sample solutions,[7] and calculate the assay 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 and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (6ppm of Finerenone)

Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (8ppm of Finerenone)

Take 0.8ml of stock solution,[8] in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (10ppm of Finerenone)

Take 0.1ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (12ppm of Finerenone)

Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (14ppm of Finerenone)

Take 0.14ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.[9]

Procedure

Inject each level into the chromatographic system 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.[10]

Precision

Repeatabilit

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).

Further pipette 0.1ml of the above Finerenone stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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.[11]

Intermediate Precision

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

Procedure

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).

Further pipette 0.05ml of the above Finerenone stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

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).

Further pipette 0.1ml of the above Finerenone stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.[12]

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).

Further pipette 0.15ml of the above Finerenone stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Finerenone and calculate the individual recovery and mean recovery values.[13]

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).

Further pipette 0.1ml of the above Finerenone stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl 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. Methanol: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead (35:65), remaining conditions are same. 10µl of the above sample was injected and chromatograms,[14] were recorded.

RESULTS AND DISCUSSION

Development of Analytical Method

Optimized Chromatographic Conditions

Mobile phase ratio	: Methanol Phosphate Buffer (35:65) V/V
Column	: Symmetry ODS C18 (4.6×250mm, 5µm)
Column temperature	: Ambient
Wavelength	: 235nm
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 8min

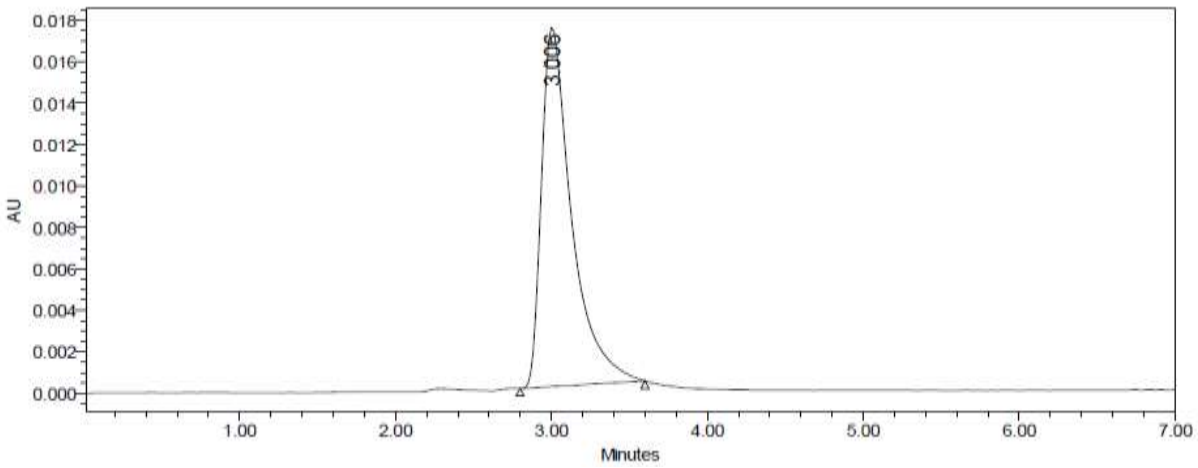

Fig. 2: Optimized Chromatographic Condition.

Method Validation
System Suitability
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	3.008	1652847	185647	6589	1.24
2	Finerenone	3.005	1653658	186254	6587	1.26
3	Finerenone	3.001	1654521	185475	6584	1.28
4	Finerenone	3.000	1653564	186594	6582	1.29
5	Finerenone	3.001	1658745	185684	6895	1.24
Mean			1654667			
Std. Dev.			2355.764			
% RSD			0.142371			

Specificity
The ICH documents define specificity.[15] as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitates Finerenone in drug product.

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

The % purity,[16] of Finerenone in pharmaceutical dosage form was found to be 99.86%.

Linearity
Chromatographic Data for Linearity Study
Table 4: Data for Linearity of Finerenone.

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

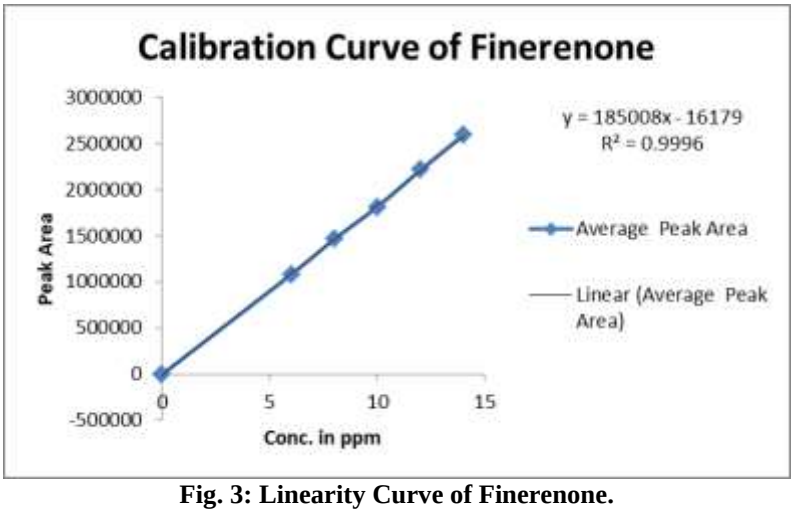

Fig. 3: Linearity Curve of Finerenone.

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) = 185008
Intercept (c) = 16179
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity,[17] is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 0.16179. These values meet the validation criteria.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.[18-19]

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 5: Results of Repeatability for Finerenone.

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Finerenone	3.008	1658954	186958	1.26	6785
2	Finerenone	3.000	1658745	187548	1.27	6854
3	Finerenone	3.013	1659865	189854	1.26	6852
4	Finerenone	3.006	1653254	186985	1.25	6784
5	Finerenone	3.001	1654781	189542	1.24	6895
Mean			1657120			
Std. Dev			2913.592			
%RSD			0.175823			

Intermediate Precision

The Intermediate Precision,[20] consists of two methods:-

Intra Day: In Intra Day process, the 50%, 100% and 150% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 50%, 100% and 150% concentration are injected at same intervals of time in different days.

Table 6: Results of Intra-Assay & Inter-Assay.

Conc. of Finerenone (API) (µg/ml)	Observed Conc. of Finerenone (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
50	49.38	0.56	49.45	0.56
100	100.17	0.71	99.70	0.77
150	150.89	0.89	149.91	0.85

Observations: The intra & inter day variation of the method was carried out for standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Finerenone revealed that the proposed method is precise.

Accuracy

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery[21] was calculated. The results are tabulated in table-7.

Table 7: The Accuracy Results for Finerenone.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

Limit of Detection for Finerenone

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected[22] but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation[23] of the response

S = Slope of the calibration curve

Result

= 1.2µg/ml

Quantitation Limit

The quantitation limit[24] 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,[26]

Result
= 3.6µg/ml

Robustness
The robustness,[27] was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Finerenone. The method is robust only in less flow condition. The standard of Finerenone was injected by changing the conditions of chromatography,[28] There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 8: Result of Method Robustness Test.

Change in parameter	% RSD
Flow (1.1 ml/min)	0.68
Flow (0.9 ml/min)	0.39
Temperature (27^0C)	0.54
Temperature (23^0C)	0.63
Wavelength of Detection (280 nm)	0.91
Wavelength of detection (270 nm)	0.93

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

Estimation of Finerenone in TABLET Dosage Form
Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with same solvent. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-9.

ASSAY
%
Assay=AT/AS×WS/DS×DT/WT×P/100×AW/LC×100

Where
AT = Peak Area of Finerenone obtained with test preparation
AS = Peak Area of Finerenone obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard

Results obtained are tabulated below
Table 9: Assay,[29] of Finerenone Tablets.

Brand Name of Capsules	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=5)	Assay + % RSD
Kerendia Tab (Bayer)	10mg	9.678 (± 0.476)	99.487 % (± 0.638)

RESULT AND DISCUSSION
The %Purity of Kerendia Tablets containing Finerenone was found to be 99.487 % (± 0.638).

Forced Degradation Studies
Results of Degradation Studies: The results of the forced degradation studies,[30] indicated the specificity of the developed method that has been developed. Finerenone were stable only in oxidation, photolytic and acidic stress conditions. The results of stability studies are given in the following Table-10.

Table 10: Results of Forced Degradation Studies of Finerenone API.

Stress Condition	Time (hours)	Assay of Active Substance	Assay of Degraded Products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	96.854	3.146	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	81.632	18.368	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	86.475	13.525	100.00
UV (254nm)	24Hrs.	97.866	2.134	100.00
3% Hydrogen Peroxide	24Hrs.	98.654	1.346	100.00

SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Finerenone, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS C18 (4.6×250mm, 5µm) column was preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Finerenone it is evident that most of the HPLC work can be accomplished in the wavelength range of 235 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 10µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Finerenone in different formulations.

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Finerenone API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity which can help in the analysis of Finerenone in different formulations.

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