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Rp-HPLC method development and validation of finerenone in bulk drug and its formulations

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Abstract

I have done research and achieved the method development & validation of highly accurate, sensitive, precise, rapid gradient system RP-HPLC method of finerenone in both bulk drug & pharmaceutical fixed dosage forms. The separation was achieved on (Agilent) C18 column (4.6 mm*250 mm, 5µm) having this configuration with particle size 5µm & using mobile Methanol: 10 Mm Citric Acid with ratio (38:62) having flow rate 1.0 ml/min. Detection of finerenone was carried out at 258nm. The total chromatographic analysis time per sample was about 15min and retention time of overall analysis is 6.9 min. The response exhibited a linear relationship with concentration in range 98-102 mcg/ml for finerenone correlation coefficient is 0.999 and %RSD is 0.126. The method was validated for accuracy, precision, specificity, linearity & sensitivity. Validation studies demonstrated that the method is simple, specific, rapid, reliable & reproducible.

Keywords: Finerenone; Chronic Kidney Disease; Validation; High performance liquid chromatography; KERENDIA

1. Introduction

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. Aldosterone is a mineralocorticoid hormone involved in the regulation of blood pressure, sodium reabsorption, and potassium excretion. In 1943, agonism of the MR along with increased salt was shown to be associated with malignant hypertension, which could progress to inflammation and fibrosis of organs. Binding of aldosterone, an MR agonist, to the MR causes a conformational change, which dissociates the receptor from inactivating chaperone proteins. The active MR translocates to the nucleus along with a complex of other coactivators to induce transcription of a number of genes.

Finerenone differs greatly from other steriodal moneralocorticcoid receptor anatagonist like spironolactone and epleronone due to its unique physicochemical, pharcokinetic & pharmacological features.

Drug Profile: Chemical name is 4*S*)-4-(4-Cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide **[Fig 1].**It is a minerlocorticoide receptor antagonist having molecular formula $C_{21}H_{22}N_4O_3$.

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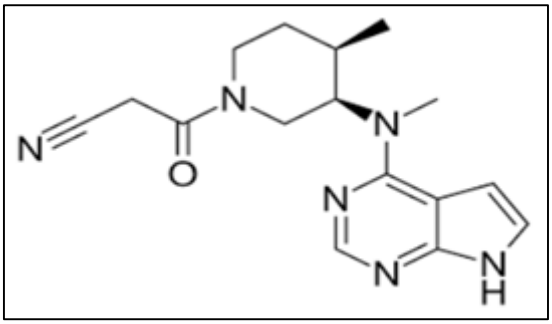

Figure 1 Structure of Finerenone

2. Materials and methods

2.1. Chemicals and Reagents

Acetonitrile, Methanol Water (HPLC Grade), 0.1 %OPA, 10Mm Citric acid.

2.2. Chromatography Instrument

HPLC was selected as analytical technique for estimation of Finerenone.

2.2.1. Instruments

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 250mm; 5µm), Quaternary Gradient (G130A) S.NO.DE9180834) pump, a 20µl injection loop and UV (DAD) G13148 S.NO. DE71365875 Absorbance detector and running Chemstation 10.1 software.

2.3. Preparation of Standard Sample

Weigh accurately about 10 mg of Finerenone Standard and transferred into 10 ml of volumetric flask add about 10 ml of methanol,shaked to dissolved and volume Was made upto the mark using methanol (Concentration of Finerenone is1000 µgm/ml)

2.4. Formulation Preparation

Weigh accurately about 25.6 mg of Finerenone and transffered into 10 ml of volumetric flask, add about 10 ml of methanol, shaked to dissolved and volume was made upto the mark using methanol (Concentration is 1000 µgm/ml)

2.5. Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well defined mathematical transformation, proportional to the concentration of analyst in samples within a given range.

2.6. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyst.

The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyst have been added. The accuracy is calculated from the test results as the percentage of analyst recovered by the assay.

2.7. Precision

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test.

2.8. Repeatability

Precision of the system was determined with the sample. Two replicates of sample solution containing 50µg/ml of Finerenone were injected and peak areas were measured and %RSD was calculated it was repeated for two times result.

2.9. Robustness

The mobile phase composition was changed in (±1 ml/ min^{-1}) proportion and the flow rate was (Fig No:41,42) of Methanol in the mobile phase composition (±1 ml/ min^{-1}) and the change in detection wavelength (±1 ml/ min^{-1}) and the effect of the results were examined.(Fig No: 43,44)and (Fig No:45,46) it was performed using 20µg/ml solution of Finerenone in triplicate

2.10. Detection Limit

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as,

$$DL = 3.3\ \sigma/S$$

Where,
σ = the S.D. of the y-intercepts of regression lines.
S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

2.11. Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as,

$$QL = 10\ \sigma/S$$

Where,

σ = the S.D. of the y-intercepts of regression lines.
S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

2.12. Ruggedness

The degree of reproducibility of test result obtains by the analysis of same sample under variety of Condition. Such as different analyst, laboratory, Different instrument.

3. Results and discussion

3.1. Analytical of Method Validation

3.1.1. Linearity

From Finerenone standard stock solution, different working standard solution (10-50µg/ml) for HPLC prepared in mobile phase 20µl of sample solution was injected into the chromatographic system using mixed volume loop injector. Chromatograms were recorded. The area for each concentration were recorded (Table 1). The Calibration curves are shown in [Fig.2]

3.1.2. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed Tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed. Statistical validation of recovery studies shown in (Table 2).

3.2. System suitability parameters : (repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Finerenone system suitability parameters were studied. The result shown in below (Table 3) [Fig. 3]

3.2.1. Precision

The method was established by analyzing various replicates standards of Finerenone. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in (Table 4) respectively.

3.2.2. Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in(±1 ml/min^{-1}) proportion and the flow rate was varied by of optimized chromatographic condition. The results of robustness studies are shown in (Table 5).Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

3.2.3. Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope The limit of detection (LOD) may be expressed as:

$$LOD = 3.3\ (SD)/S$$

$$= 3.3\ X\ 1.24/\ 29.85$$

$$= 0.1367$$

Where, SD = Standard deviation of Y intercept

S = Slope

The LOD of Finerenone was found to be 0.1367 (µg/mL) analytical methods that concluded.

3.2.4. Limit Quantification :

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

$$LOQ = 10\ (SD)/\ S$$

$$=10\ X\ 1.24/\ 29.85$$

$$= 0.4145$$

Where, SD = Standard deviation Y intercept

S = Slope

The LOQ of Finerenone was found to be 0.4145 (µg/mL) analytical methods that concluded.

3.2.5. Ruggedness

The degree of reproducibility of test result obtains by the analysis of same sample under variety of Condition. Such as different analyst, laboratory Different instrument.

Table 1 Linearity of Finerenone

Sr. No.	Concentration µg/ml	Area Finerenone
1	10	291.98
2	20	594.28
3	30	878.95
4	40	1187.99
5	50	1487.99

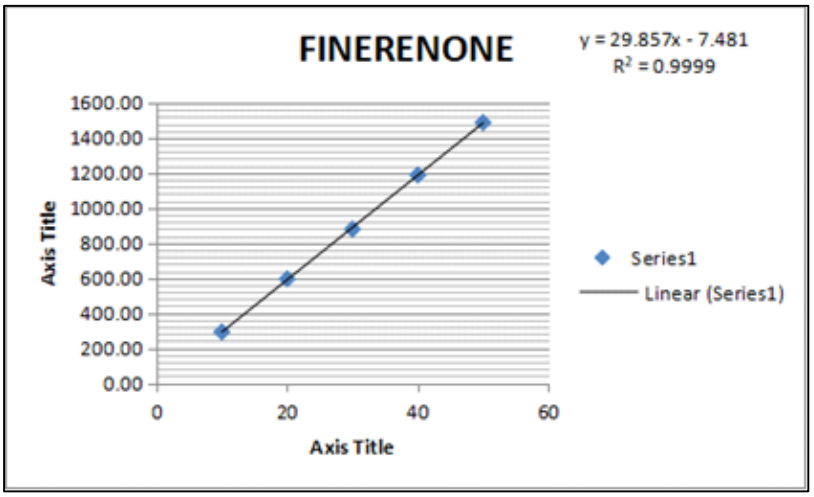

Figure 2 Calibration curve of Finerenone

Table 2 Result of Recovery data for Finerenone

Drug	Sr No.	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Area. Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
HPLC method	1	80%	10	8	18.07±0.03	8.07± 0.03	100.83±0.04
	2	100%	10	10	20.13±0.04	10.1±0.043	101.2± 0.43
	3	120%	10	12	22.01±0.06	12.01±0.06	100.09± 0.56

*Average Mean of 3 Readings

Table 3 Repeatability studies on Finerenone (HPLC)

Sr.No.	Concentration of Finerenone(mg/ml)	Peak area	Amount found (mg)	% Amount found
1	50	1486.63	50.03	100.07
2	50	1485.358	50.01	100.05
		Mean	50.02	100.06
		SD	0.90	0.90
		%RSD	0.06	0.06

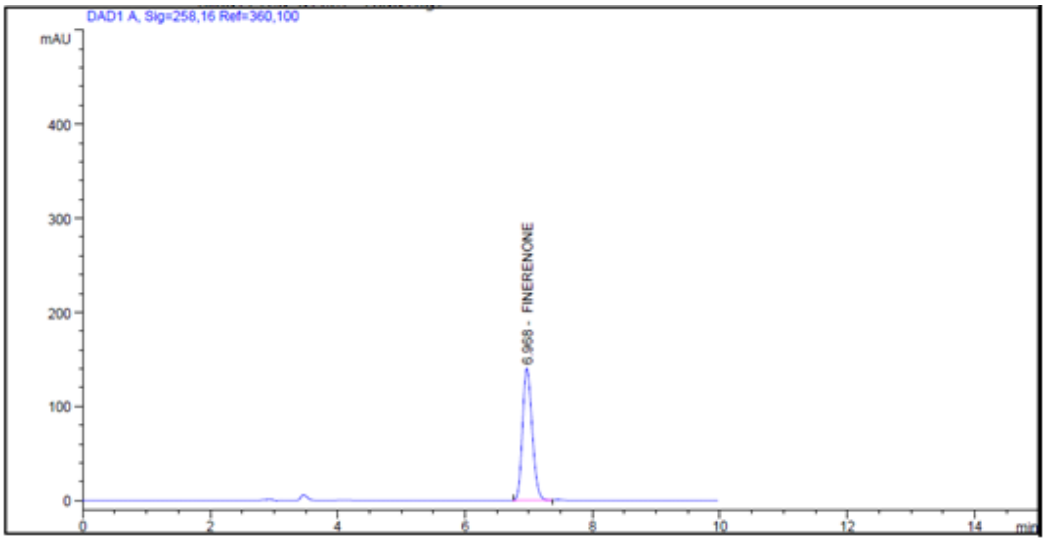

Figure 3 Chromatogram of System suitability No- 2

Table 4 Result of Intraday and Inter day Precision for Finerenone HPLC

Concⁿ (µg/ml) HPLC	Intraday Precision			Interday Precision		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
10	289.54±1.14	99.51	0.39	291.93±6.01	100.30	0.27
40	1188.11±1.83	100.13	0.15	1192.04±2.73	100.46	0.23
50	1485.54±2.55	100.04	0.17	1484.90±0.56	99.99	0.04

Table 5 Result of Robustness Study of Finerenone

Parameters	Conc.(µg/ml)	Amount of detected(mean ±SD)	%RSD
Mob-phase composition(39ml+61ml)Methanol + Buffer	50	1785.22±0.27	0.02
Mob-phase composition(37 ml+63ml) Methanol + Buffer	50	1860.06±1.64	0.09

Wavelength change 257nm	50	1885.2±9.19	0.49
Wavelength Change 259 nm	50	1979.07±7.28	0.37
Flow rate change(0.9ml)	50	2116.54±2.52	0.12
Flow rate change(1.1 ml)	50	1643.70±2.14	0.13

Table 6 Analysis of marketed formulation. (HPLC)

Sr.no	Amount present in mg	Area(I)	Amount found in mg	% Label claim
	FRN	FRN	FRN	FRN
1	20	586.7407	19.90692	99.53
2	20	587.7994	19.94239	99.71
Mean	-	587.27	19.92	99.62
SD	-	0.749	0.025	0.125
%RsD	-	0.127	0.126	0.126

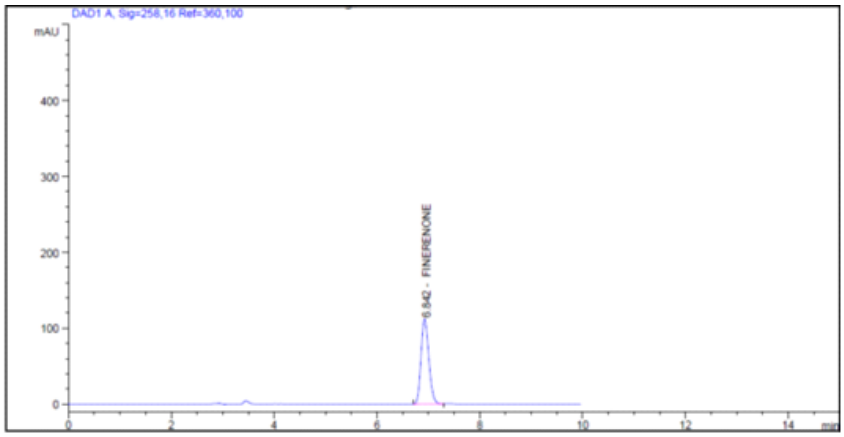

Figure 4 Chromatpgram of Analyst 1

Table 7 Result for Chromatogram of Analyst 1

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	6.961	586.74072	10487	0.84	-

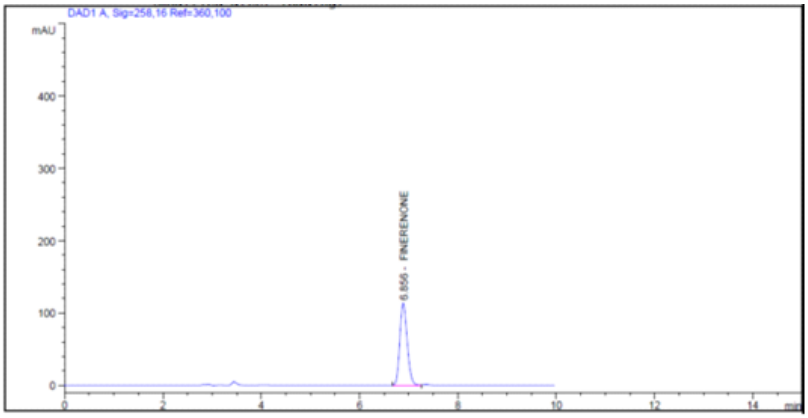

Figure 5 Chromatogram of Analyst 2

Table 8 Result for Chromatogram of Analyst 2

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	6.856	1189.6352	10236	0.80	-

Table 9 Tablet for %Lable claim

Sample	Label claimed	%Label claimed± SD	%RSD
Finerenone Tablet	Finerenone =10 mg	99.62± 0.125	0.126

4. Conclusion

In this research a simple HPLC method was developed for determination of Finerenone in bulk drug and fixed pharmaceutical formulation. The approach offers several advantages, including its simplicity, preciseness, ease of use and shorter run times which is particularly time saving when dealing with large number of samples. The validation tests demonstrated a method with a wide linear range, acceptable precision and accurarcy and reliable sensitivity. The developed method allows for easy,selective,sensitive and specific analysis of finerenone, making it suitable for routine use in pharmaceutical quality control.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declare no conflict of interest in constructing this manuscript.

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