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An Isocratic RP-HPLC Method Development and Validation for the Quantitative Estimation of Finerenone in Solid Dosage Forms

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Abstract:
An accurate, sensitive, precise, quick isocratic reverse phase HPLC (RP-HPLC) method has been developed and validated for quantification of Finerenone in bulk and pharmaceutical tablet dosage forms. With acetonitrile as the organic solvent, the best separation was achieved on a 250 mmx 4.6 mm i.d, 5µ-particle size Inertsil®-Octadecyl-silyl-3V-Reverse-Phase-C_{18}-column with 0.03M Ammonium Acetate in water: Acetonitrile (20:80 v/ v) in the isocratic mode of elution as mobile phase solvent at a speed of 1.0 mL.min^{-1}. UV detection was at 230 nm. Retention time of Finerenone was 10.9 minutes. With a correlation coefficient of about 0.9999, peak-response was obtained as function of concentration over the range of 80 to 240 µg/ ml for Finerenone. Percentage assay of Finerenone was shown to be 109.42 %. Finerenone had a limit of detection of 0.1 µg/ ml and a limit of quantification (LOQ) of 0.3 µg/ ml. The presence of excipients in the formulation had no effect on the assay method. The procedure is appropriate for use in QC- laboratories since it is economical and precise.

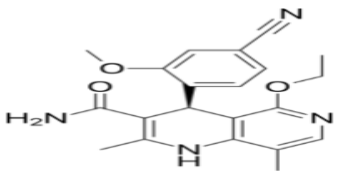
Keywords: Finerenone, Kerendia, RP-HPLC, Isocratic, Ammonium acetate, Acetonitrile.

INTRODUCTION

Finerenone, a non-steroidal mineralocorticoid (aldosterone) receptor antagonist (MRA) sold under the brand name Kerendia, is indicated to lower the risk of eGFR decline in end stage kidney disease, to reduce the risk of kidney function decline, kidney failure, cardiovascular death, non-fatal heart attacks, and hospitalization for heart failure in adults with chronic kidney disease associated with type 2 diabetes and to treat the symptoms of Chronic Kidney Disease [1]. Common side effects include hyperkalemia (high levels of potassium), hypotension (low blood pressure), and hyponatremia (low levels of sodium). So far, Finerenone is the only nonsteroidal mineralocorticoid receptor antagonist to be FDA approved [2].

Finerenone, chemical name is (4S)-4-(4-cyano-2- methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide [3].

Figure 1: Structure of Finerenone



Finerenone is a selective non-steroidal selective mineralocorticoid receptor (MR) antagonist (unlike spironolactone) with no significant affinity or activity at androgen, progesterone, estrogen, and glucocorticoid receptors. Finerenone's binding to the mineralocorticoid receptor prevents binding of MR coactivators, which in turn prevents pro-inflammatory and pro-fibrotic gene transcription [4]. Clinical trial data shows that blocking the mineralocorticoid receptor reduces mortality and morbidity in patients with chronic severe congestive heart failure with an ejection fraction ≤35% [5].

Finerenone is a white to yellow crystalline powder. It is practically insoluble in water; and sparingly soluble in 0.1 M HCl, ethanol, and acetone [6]. Each Kerendia tablet contains 10 mg or 20 mg of. It has a molecular formula of $C_{21}H_{20}N_4O_3$ and a molecular weight of 376.4g/ mol [7].

Hardly very few techniques for the determination of Finerenone in oral fixed dosage form have been published [8-14]. Furthermore, no official or preliminary monograph on this analyte has been published in any of the compendial pharmacopoeias. The goal of this study was to develop a simple, accurate and efficient RP-HPLC method to estimate Finerenone in unit dosage forms for oral administration. The validation of the devised approach is also addressed in this study, as per ICH standards [15].

EXPERIMENTAL

Chemicals and Reagents:

- 99%, Finerenone pure was acquired from Dr. Reddy's Laboratories Ltd, Hyderabad, India.
- Acetonitrile- Fine-Chemicals of HPLC- Grade -Rankem
- Ammonium Acetate, 85% (v/v), Qualigen-Fine chemicals.
- HPLC Grade water, Rankem-Fine chemicals.

Chromatographic-Instrument:

Quantitative RP- HPLC was carried out on a Waters 2996 high-performance liquid chromatograph with a variable wavelength PDA detector module, which included an automated injector with a 20 µl injection volume and a quadra-pump. The column utilized was a Reverse Phase Inertsil Octa Decyl-S-3V-C_{18} column (250mmx 4.6 mm internal diameter with particle size 5µm). Empower Software was installed on the HPLC equipment. The column temperature was maintained ambient and eluted over 20.0 minutes at a mobile solvent speed of 1.0 $ml.min^{-1}$ under isocratic conditions. The mobile phase used was 0.03M Ammonium Acetate in water: Acetonitrile (20:80 v/v). It was degassed and filtered via 0.45µm Nylon membrane filters before use. For the analyte, UV detection at 230 nm was used as wavelength of detection with a PDA detector. Mobile phase was used as diluent to make the standard dilutions. Finerenone eluted at 10.9 minutes.

Preparation of the Primary Standard Drug solutions: To make the primary standard stock solution, 200 mg of Finerenone was dissolved in a volumetric flask (100mL) and diluted with the diluent (mobile phase), sonicated for 15 minutes and diluted up to 100ml with the diluent to get the primary standard stock solution containing 2000 µg/ ml of Finerenone.

Preparation of Working Standard Drug Solution: After adding 5 ml of the primary working standard solution to the 50 ml volumetric flask, the flask was filled with 50 ml of the diluent. This resultant mixture, which includes 200 µg/ ml of Finerenone, was suitable for use as a working standard solution. The stock solutions were kept in a cool, dark place that was controlled to be 4 °C.

Sample Preparation: After measuring the weight of each individual tablet, the average weight of twenty Kerendia ® pills was calculated. After crushing the tablets into a powder form a sample containing 200-mg of Finerenone was obtained, which was then weighed, shifted to a 100 ml pre-

calibrated-measuring flask, and dissolved in a blend of 0.03M Ammonium Acetate in water: Acetonitrile with a volumetric ratio of (20:80 v/ v). After being sonicated in diluent and strained via Whattman 41 filter paper, the resultant primary working sample solution has 2000 µg/ml of Finerenone. After quantitatively transferring 5ml of the filtrate to a 50 ml pre-calibrated-measuring flask, the diluent was added to bring the volume of the solution to 50 ml. This served as a working testing solution having 200 µg/ml of Finerenone. The stock solution was kept in a dark place at 4 °C.

RESULTS AND DISCUSSION

The purpose of this research was to create a chromatographic technique for the quantifiable determination of fixed-dose of Finerenone.

Optimized Chromatographic Conditions:

Elution solvents: 0.03M Ammonium Acetate in water: Acetonitrile (20:80 v/ v).
Elution mode: Isocratic
Column: Inertsil ODS C-18-3V (250 x 4.6mm, 5µm particle size)
Flow rate: 1.0 ml/ min
Injection volume: 20 µl
Detector: Photo diode array (PDA)
Wavelength (λ_{max}): 230nm
Column temperature: Ambient
Diluent: Mobile phase
Run time: 20 minutes
Retention time: 10.9 mins

Linearity: Aliquots of Finerenone working stock solutions was placed in various 10 ml volumetric flasks and the volume was made up to the 10 ml with the mobile phase, yielding in final strengths of 80 to 240 µg/ ml (Table 2). The peak areas and retention time of the drug solution (loaded at 20 µl) were measured thrice in the column. Using a PDA-detector set at 230 nm, a linearity-graph was generated by plotting peak areas-vs- Finerenone concentrations in µg/ ml.

Accuracy: The accuracy of the method was found by evaluating the drug recovery using the standard-spiking method. To assess if the analyte contained in the formulation caused positive or negative interventions, known amounts of the drug equivalent to 12 percent standard drug solution was added to 80 percent, 100 percent and 120 percent of the target test concentrations of the formulation. Each set-of-addition was replicated thrice at each dilution level. The results were compared to a competent reference standard after extraction of sample preparation. The percentage of analyte recovered by the assay was used to assess the accuracy. Table 3 shows the results of accuracy investigations on standard solution and process-related impurity; recovery measurements suggest that the procedure was accurate.

Precision: Quality-control samples in 100 % (w/v) dilution were used to assess intraday and inter-day precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability was indicated by the low RSD value (1%) as given in Table 4.

Limits of Detection and Quantification: The method's LOD was set at the lowest concentrations of active pharmaceutical component with a signal-to-noise (S/N) ratio of around 3 (LOD). The lowest active therapeutic medication concentration that can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ) was also determined.

Method Applicability: The newly created method was evaluated by applying it to pharmaceutical tablets for the estimation of Finerenone.

Optimization of Chromatographic Conditions:

An isocratic RP- HPLC procedure for assaying the active ingredients was developed due to lack of an easy, economical, reproducible, and quick-to-use method for the determination of Finerenone concentrations in formulary matrices. The effect of various HPLC technique variables was examined on the result of the study to optimize the chromatographic parameters, various proportions of CH_3CN-KH_2PO_4, CH_3CN-H_2O, CH_3COONH_4-H_2O and CH_3CN: *O*-H_3PO_3 buffer were tested. After several early investigatory tests with CH_3CN: KH_2PO_4 *O*-H_3PO_3, K_2HPO_4 *O*-H_3PO_3 (0.1%) binary system at the proportion of 60:40 v/ v and (20:80 v/ v), finally 0.03M Ammonium Acetate in water: Acetonitrile (20:80 v/ v) was chosen over other mobile phases because it resulted in improved resolution of active component. This procedure gave the good detection of analyte after multiple exploratory & investigatory trail runs. The active pharmaceutical analyte had excellent UV sensitivity and was interference-free at 230 nm. The analyte peak was highly defined and showed no incidence of tailing under these conditions. The set of conditions previously noted in this article were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Method Validation Tests [16]:

Method precision (RSD percent), method accuracy (recovery percent & % RSD,), linearity range (r^2) and LOD & LOQ were explored as recommended method validation characteristics.

Linearity: With a correlation coefficient of 0.9995, the graph of chromatographic-peak areas of the analyte versus respective concentration was shown to be linear in the concentration range of 80-240 µg/ ml for Finerenone (Table 2). The least square fit data of linear regression analysis derived from the measurements is given in Table 1. Finerenone is y = 89869x. Table 1 presents the regression parameters for this technique that include slope, intercept, and % RSD. These findings suggest that there was a significant correlation (Figure 3).

Accuracy: Individual recovery of analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 116.75%, 87.66 % and 95.91 % for Finerenone respectively demonstrating the method's accuracy. The % RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results (Table 3)

Precision: Table 4 summarizes the intraday and inter day fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value (less than 1%). These results show that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs.

Limit-of-Detection & Limit-of-Quantifications: Finerenone has a limit of detection of 0.1 µg/ ml and a limit of quantification (LOQ) of 0.3 µg/ ml. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can be used to detect and quantify the analyte over a wide concentration range.

Specificity: The Retention time for Finerenone was determined to be 10.9 minutes, according to the representative chromatogram given in Figure 2. When the pharmaceutical tablet matrices were evaluated, no indication of excipient interference signal was observed in the respective retention time of the chromatogram. It indicates that the analyte was not disturbed of probable merging peaks. As a result, this technique can be employed with certainity.

Table 1: Regression analysis & Operating-System Suitability Results:

Study-Parameter	Finerenone
Retention Time (min)	10.9
Peak areas	17997586
Percentage of peak areas	99.52
USP-Tailing	1.70
Theoretical Plates	4098.37
Resolution	7.1
Linear range in (µg/mL)	80-240
Limit-of-Detection in µg. mL^{-1}	0.1
Limit-of-Quantification in µg. mL^{-1}	0.3
Correlation-Coefficient (r^2)	1.000
Assay-in-Percentage (%)	109.42

Table 2: Summary of the standard calibration Curve for Linearity experiment

Calibration Standard Dilution Level	Concentration of Finerenone (µg/ ml)	Peak Area
40 %	80	7236505
60 %	120	10540388
80%	160	14471909
100 %	200	17885938
120 %	240	21466163

Table 3: Accuracy evaluation by Spike-analysis method

Accuracy study at 80% target level	Injection Number	Finerenone	
		Standard Soln.	Spiked Soln.
Kerendia -® tablet dosage form solution at 80% level was spiked with 12% of standard solution of API	1	14271647	16643953
	2	14290992	16620101
	3	14327301	16651636
	Mean area	14306370	16637147
	Std. Dev	42901	38315
	% RSD	0.22	0.19
	% Recovery		93.13%
80% of the target concentration is equivalent to 160 µg/ mL in 0.03M Ammonium Acetate in water: Acetonitrile (20:80 v/ v) as diluent.			
Accuracy study at 100% target level	Injection Number	Finerenone	
		Standard Soln.	Spiked Soln.
Kerendia -® tablet dosage form solution	1	17890939	19590043
	2	17928604	19626615

at 100% level was spiked with 12% of mixed standard solution of API's	3	18013594	19586141
	Mean area	17942559	19612075.3
	Std. Dev	66937	55244
	% RSD	0.05	0.31
	% Recovery		87.66
100% of the target concentration is equivalent to 200 µg/ ml in 0.03M Ammonium Acetate in water: Acetonitrile (20:80 v/ v) as diluent.			
Accuracy study at 120% target level	Injection Number	Finerenone	
		Standard Soln.	Spiked Soln.
Kerendia -® tablet dosage form solution at 120% level was spiked with 12% of mixed standard solution of API's	1	21506321	23192291
	2	21353647	23233487
	3	21417136	23204638
	Mean area	21421724	23216332
	Std. Dev	26398	24284
	% RSD	0.11	0.11
	% Recovery		95.91
120% of the target concentration is equivalent to 240 µg/mL in 0.03M Ammonium Acetate in water: Acetonitrile (20:80 v/ v) as diluent.			

Table 4: Evaluation of precision with-in-day and day-to-day analysis

Intra-Day Precision study of 100% standard dilution containing 200 µg/ mL of Finerenone			Inter-Day Precision study of 100% standard dilution containing 200 µg/ mL of Finerenone	
S. No	Finerenone		Finerenone	
	Ret. time	Peak area	Ret. time	Peak area
1	11.005	17926814	10.924	17738301
2	10.992	17951858	10.917	17719306
3	10.970	17932829	10.910	17742231
4	10.950	17947612	10.915	17728486
5	10.940	17950116	10.892	17726918
6	10.932	17945557	10.891	17742452
Average	10.965	17948886	10.908	17733371
Std. Dev	0.029	32367	0.014	44957
% RSD	0.27	0.3	0.12	0.33

Figure 2: Chromatogram of Finerenone 200µg/mL analyzed by optimized method.

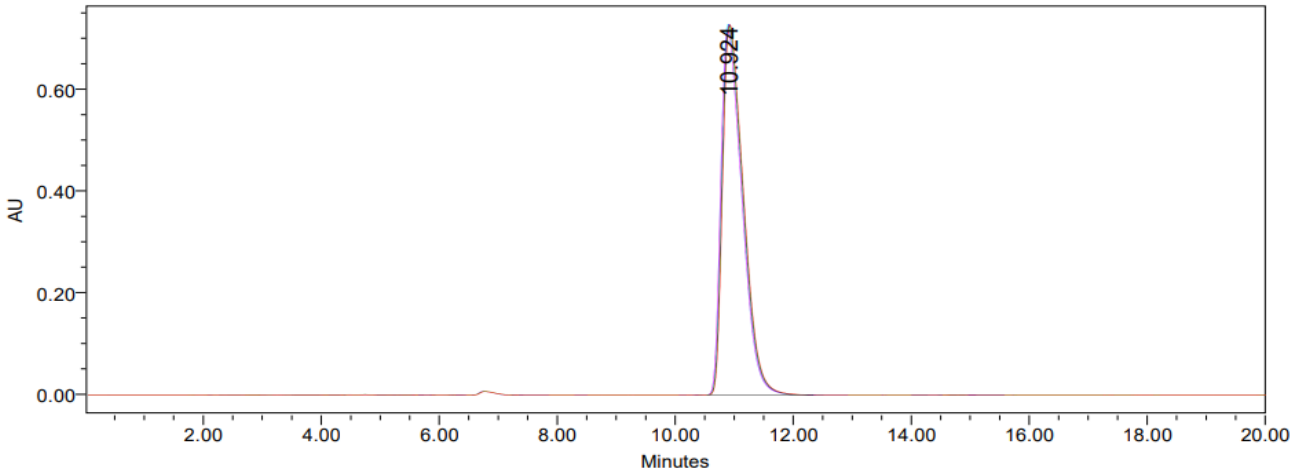
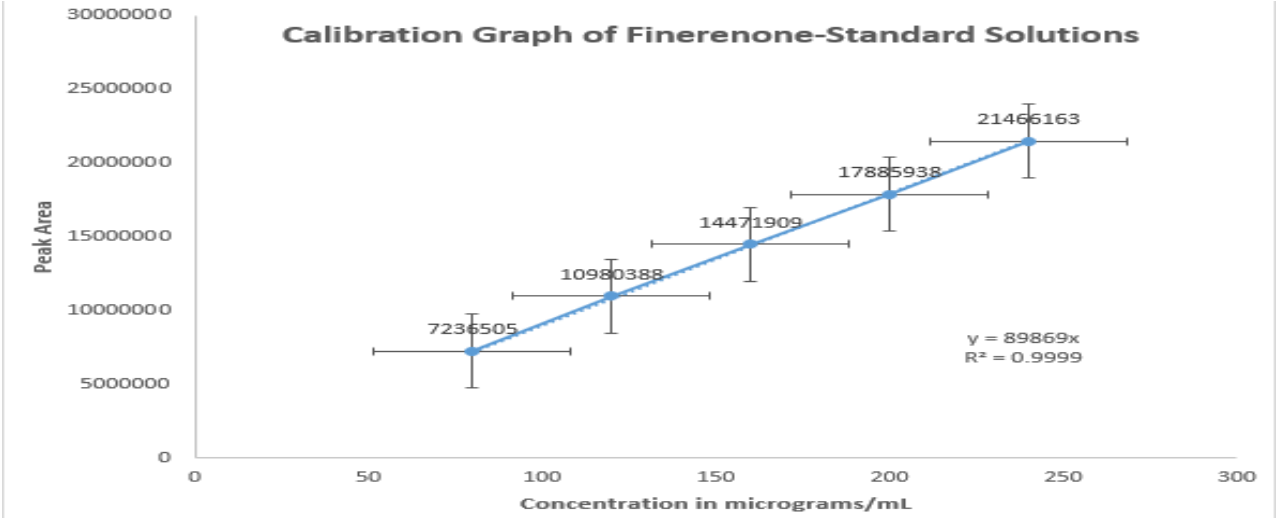


Figure 3: Linearity graph of Finerenone standard solution



CONCLUSION

In this study, an economical, efficient and commonly available HPLC method for the analysis of Finerenone in pharmaceutical matrices was devised. This method's key advantages are its significantly reduced cost, ease of use, reduced run time and ease of operation. All these features are critical in operation, especially when analyzing a large number of samples. The validation experiments demonstrated that the procedural approach has a large calibration concentration range, adequate precision & accuracy, and practically reliable sensitivity. The method can be used for regular analysis in formulation QC-studies and allows for a straightforward, selective, sensitive, and specific assessment of Finerenone.

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