RESEARCH ARTICLE

Development of a Robust and Reliable RP-HPLC Method for the Estimation of Finerenone in Tablet Dosage Form

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Received: 25th January, 2024; Revised: 10th March, 2024; Accepted: 08th May, 2024; Available Online: 25th June, 2024

ABSTRACT

An advanced analytical method was explored, specifically tailored for estimating finerenone, a vital drug found in tablet formulations intended to reduce the risk of chronic kidney disease in patients with type-2 diabetes. This sophisticated technique employs an Inertsil ODS 3V C18 column (5 µm particle size x 250 x 4.6 mm) for precise separation of analyte. The mobile phase consists of an ammonium dihydrogen phosphate buffer (pH 4.5) and acetonitrile (60:40), which is a carefully balanced mixture that flows consistently at 1.0 mL/min. UV detection is achieved at a wavelength of 238 nm. Finerenone's characteristics are unveiled with a brief retention time of 4.06 minutes, highlighting the efficiency of the method. The method demonstrates linearity within the concentration range of 2.5 to 15 µg/mL, offering a comprehensive scope for accurate analysis. This robust reversed-phase high-performance liquid chromatography (RP-HPLC) method undergoes thorough validation following International Council for Harmonisation (ICH) guidelines, ensuring its reliability. Notably, the accuracy is impressive, with a mean recovery falling comfortably within the acceptable range of 99.76 to 101.09%. In summary, our analytical approach stands out for its simplicity, precision, sensitivity, rapidity, and robustness in estimating finerenone in tablet formulations.

Keywords: Finerenone, RP-HPLC, Method development, Validation.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.2.15

How to cite this article: Chepyala S, Medidi S, Malik JK. Development of a Robust and Reliable RP-HPLC Method for the Estimation of Finerenone in Tablet Dosage Form. International Journal of Drug Delivery Technology. 2024;14(2):703-708.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Diabetes mellitus (DM) is a chronic, progressive metabolic disorder and a significant global public health issue. It is characterized by impaired insulin secretion and/or insulin resistance, resulting in consistently high blood glucose levels.[1] Despite causing over a million deaths annually and ranking ninth in terms of mortality, DM has become a major concern in the 21st century. Projections suggest that by 2030, approximately 578 million people worldwide will have diabetes, with an additional 398 million at high risk of developing the condition.[2] The incidence of DM has quadrupled globally in the past three decades, with around three-quarters of those affected living in low- and middle-income countries.[3] Type 2 diabetes (T2DM) is the most common type, accounting for over 90% of all cases.[4] It affects 1 in 11 adults, with T2DM making up 90% of all instances, and the body either does not respond adequately to insulin or does not produce enough insulin, leading to hyperglycemia. Prolonged hyperglycemia increases the risk of heart attacks, kidney failure, nerve damage, and retinopathy. Among these complications, chronic kidney disease (CKD) is prevalent in persons with both types of diabetes, affecting approximately 40% of these cases. Hyperglycemia triggers the creation of reactive oxygen species and activates different pathways to generate products like protein kinase C, polyol, hexosamine, and advanced glycation end products (AGE). This inflammatory response leads to increased vascular permeability and fibrosis. Podocytopathy causes albuminuria, and systemic and intra-glomerular hypertension worsens proteinuria.[5] CKD is diagnosed by persistent albuminuria for at least three months (≥30 mg/g of creatinine or mg per day) and a progressive decline in the glomerular filtration rate (<60 mL/min/1.73 m^2).[6] Numerous studies have shown that early treatment can significantly slow down or even halt the progression of this disorder.[7] Fibrosis and inflammation in diabetic kidney disease (DKD) are primarily driven by mineralocorticoid receptor (MR) signaling, which is aided by glucocorticoid activation.

Finerenone (FNR) is a recently developed non-steroidal mineralocorticoid receptor antagonist (MRA) that has received authorization from the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2021/22 for the treatment of CKD in adults with T2DM. It lowers the risk of hospitalization for heart failure, nonfatal heart

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attacks, cardiovascular death, and loss of renal function. FNR is a white to yellow crystalline powder with the IUPAC name "(4S)-4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide" (see Figure 1). FNR is potent, selective, and minimally excreted in urine. It does not form active metabolites and has a short half-life of 2 to 3 hours.[8]

In the pharmaceutical industry, an efficient analytical method is necessary for individual drug analysis or combination with other drugs. A survey of the literature revealed that only a few reported methods are available for estimating finerenone by reverse phase high-performance liquid chromatography (RP-HPLC).[9-13] However, these methods are not sensitive or accurate enough to quantify FNR at low concentrations. Additionally, they are expensive, time-consuming, and not suitable for routine daily analysis. Therefore, we chose a cost-effective UV-coupled HPLC technique, considering its widespread availability in laboratories with limited financial resources that need to regularly monitor FNR in its formulation. Taking these factors into account, this study aims to develop and establish an RP-HPLC method for quantifying FNR in tablet formulation following the International Council of Harmonization (ICH) guidelines under Q specification.[14]

MATERIALS AND METHODS

Chemicals and Reagents

In this study, high-grade HPLC reagents and solvents were utilized. The solvents used were CH_3CN (acetonitrile), $NH_4H_2PO_4$ (Ammonium dihydrogen orthophosphate), which were provided by SD Fine Chem Ltd., located in Mumbai, India. The API for finerenone was obtained from Ascentyo BioSciences in Hyderabad, India.

Instruments

The study was conducted using the Shimadzu HPLC system (LC-20AD series) coupled with a UV detector (SPD-20A). Data acquisition was done using Empower software version 2. Chromatography was performed using an Inertsil ODS 3V column (dimensions: 250 × 4.6 mm, 5 µm). Weighing was done using an analytical balance from Mettler Toledo.

Chromatographic Conditions

The chromatographic conditions for the analysis were as follows: A mobile phase consisting of a volumetric ratio of 60:40 ($NH_4H_2PO_4$: CH_3CN) was used in isocratic mode. The analysis was conducted at an ambient temperature with a flow rate of 1.0 mL/min for the mobile phase. Each run involved injecting 20 µL of the sample into the HPLC. The UV detector was set to a detection wavelength of 238 nm to detect FNR in the effluents from the column (Figure 2).

Buffer Preparation

To prepare the diluent, 5.75 g of $NH_4H_2PO_4$ was dissolved in 1000 mL of methanol. Formic acid was used to adjust the pH to 4.5. The solution was then filtered using a nylon membrane filter (0.45 µm) and degassed before being used as the diluent in the HPLC analysis.

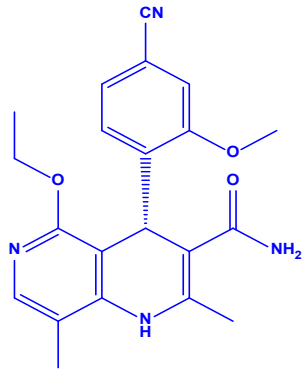

Figure 1: Structure of finerenone

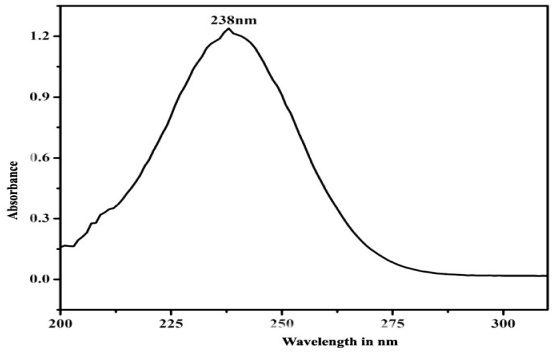

Figure 2: UV spectrum of finerenone

Preparation of Standard Stock Solution (STD Solution)

In a volumetric flask (VF) with a capacity of 100 mL, 10 mg of FNR powder was added. The flask was then filled with the diluent and sonicated for 20 minutes. Diluent was used to adjust the volume up to 100 mL. Next, 1.0 mL of the solution was transferred to a 10 mL VF and filled with 10 mL of diluent to achieve a concentration of 10 µg/mL of FNR.

Preparation of Sample Solution

About 20 film-coated tablets of Kerendia ™ (10 mg dose) were weighed. The average weight was determined and crushed into powder form, and a 10.0 mg equivalent of the powder was transferred into a 100 mL VF. The flask was filled with the diluent and subjected to sonication for 20 minutes. Next, 1.0 mL of the resulting solution was transferred into a 10 mL VF and diluted with 10 mL of diluent to achieve a concentration of 10 µg/mL of FNR.

Analytical Method Validation

System suitability

System suitability tests were conducted to ensure the reliability of the HPLC system. Six injections at a concentration of 10 µg/mL were made to measure column efficiency, plate count, and tailing factor. The results met the predetermined criteria, confirming the consistency of the system and its adherence to specified limits.

Specificity and selectivity

The specificity and selectivity of the method were confirmed by the ability to detect FNR in the sample without interference.

The chromatogram of the FNR reference standard showed a positive result, while the blank (containing only the diluent) showed no response or interference. Figures 3 and 4 show the chromatograms of the standard and blank injections, respectively.

Linearity

The linearity of FNR was evaluated by preparing dilutions ranging from 2.5 to 15 µg/mL from the standard stock solution. Peak area responses were measured for each concentration in the HPLC analysis.

Precision

Precision was assessed by conducting six repeated injections of the STD solution, which resulted in a low %RSD of 0.9, indicating high precision and consistent results. Similarly, six injections of the sample solution showed a %RSD of 1.0, within the acceptable limit of 2.0% for precision, demonstrating the reliability of the method in sample analysis.

Intermediate precision

Intermediate precision (IP) was evaluated by having two analysts use separate HPLC instruments in different labs on different days to analyze the STD solution. Despite time-based variations, both obtained nearly identical assay results, with a negligible difference of 0.2% and an RSD below the acceptable limit of 2.0% on both days.

Accuracy or recovery studies

The accuracy of the HPLC method was verified through a triplicate recovery study. FNR at concentrations of 5, 10, and 15 µg/mL was injected into pre-analyzed samples. The mean recovery percentage was computed from these studies to validate accuracy.

Robustness

The robustness was assessed by deliberately adjusting the flow rate and wavelength. These variations did not significantly alter the chromatogram, tailing factor, or plate count, indicating that the method remains robust and unaffected by fluctuations in flow rate and wavelength, ensuring accuracy and precision.

Analysis of marketed formulation

Assay of the proposed method was achieved by injecting the standard as well as test sample solutions both having the concentration of 10 µg/mL of FNR respectively. A detailed procedure for the preparation of both the solutions is given in the methods section.

RESULTS AND DISCUSSION

Solubility Studies

Initially, the solubility of FNR was determined. It was found that FNR was freely soluble in methanol, sparingly soluble in acetonitrile, and poorly soluble in water. Based on these observations, a diluent consisting of a mixture of CH_3CN and CH_3OH in a ratio of 60:40 was selected.

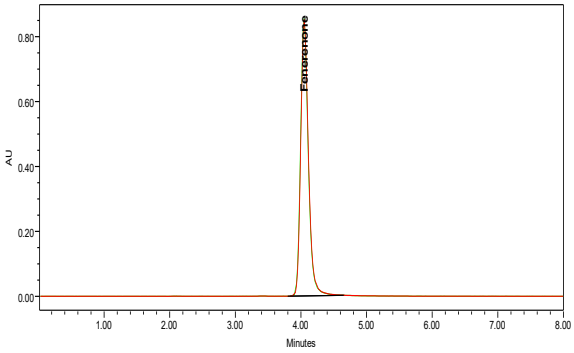

Figure 3: Optimized chromatogram of the standard

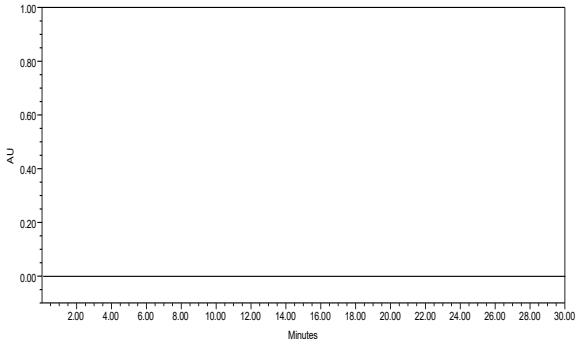

Figure 4: Blank chromatogram

Optimization of Chromatographic Method

The chromatographic method was optimized by fine-tuning various parameters to meet specific criteria and establishing an HPLC method with a quick runtime (less than 5 minutes) and high resolution (RS > 2). Different mobile phases were tested, and the blend of ammonium dihydrogen orthophosphate buffer (pH 4.5) and acetonitrile (60:40 v/v) demonstrated superior outcomes, delivering symmetrical peak shapes and excellent resolution for FNR. The ideal detection wavelength at 238 nm is aligned with the prominent absorption signal of FNR, making it suitable for analysis. This rigorous parameter optimization ensured the development of an efficient HPLC method that meets desired performance benchmarks.

System Suitability Parameters

The precision of the HPLC system was assessed by determining the %RSD (relative standard deviation) from six replicate injections of the standard solution. A requirement was set for the RSD not to exceed 2% to be considered acceptable. Since the RSD of the standard solution fell within this limit, indicating precision within the specified criterion, the system proved reliable for accurate FNR quantification in samples. These findings are detailed in Table 1.

FNR exhibited a retention time (RT) of 4.06 minutes, confirming its effective separation and timely detection. Through meticulous selection of mobile phase composition, optimal wavelength, and parameter fine-tuning, the HPLC method for FNR analysis was tailored to meet the desired criteria: swift analysis and satisfactory resolution. This

rigorous optimization ensures reliable and efficient FNR quantification, meeting stringent analytical demands.

Linearity

A linearity graph was generated for FNR, with concentration in µg/mL on the *x*-axis and area under the curve (AUC) on the *y*-axis. Within the range of 2.5 to 15 µg/mL, a linear correlation was observed between drug concentrations and peak area responses. The results, depicted in Figure 5 and summarized in Table 2, highlight the importance of linearity in analytical methods. This characteristic ensures precise drug quantification across a wide range of concentrations based on the measurement of peak areas. The HPLC method exhibits excellent linearity within the specified concentration range, establishing a strong relationship between concentration and peak area response, which facilitates accurate FNR analysis.

Precision

The precision of the method, including the repeatability of both sample and standard preparations, was found to be satisfactory. Table 3 confirms these findings, demonstrating the reproducibility and reliability of the HPLC method in FNR analysis. This validation supports the routine use of the method for accurate quantification across various types of samples.

Table 1: System suitability parameters for finerenone

S. No.	*Parameter*	*Finerenone*	*Acceptance criteria*
1.	Retention time (RT)	4.06	--
2.	Theoretical plates (N)	3348	NLT 2000
3.	Tailing factor (T)	1.26	NMT 2.0
4.	Linearity range (µg/mL)	2.5-15 µg/ml	--
5.	Detection limit (µg/mL)	0.05	--
6.	Quantification limit (µg/mL)	0.15	--
7.	Regression data: Slope	141543	--
8.	Regression data: Intercept	45066	--
9.	Regression data: Correlation coefficient	0.999	--

Table 2: Linearity of finerenone

S.No.	*Drug*	*Values of X and Y variables*						*Correlation co-efficient*
1.	FNR	Variable	1	2	3	4	5	6	0.999
		X	2.5	5	7.5	10	12.5	15	
		Y	396757.4	728548	1134442	1480355	179 9011	2162304	

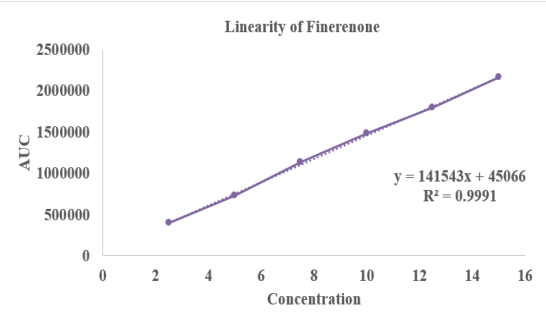

Figure 5: Linearity plot of finerenone

Intermediate Precision

The consistent performance of the HPLC method across diverse laboratories, instruments, and analysts, even on different days, emphasizes its robustness. Its intermediate precision affirms its suitability for routine use, ensuring consistently accurate and reliable results under varying experimental conditions. Table 4 displays the percentage RSD values for intermediate precision, and they are below 2.0%, confirming the precision of the method and instrument. This consistency guarantees dependable and minimally variable results, as evidenced by the acceptable recovery rate range of typically 98.75 to 98.90%.

Accuracy

The assay yielded a mean recovery percentage of 100.2%, signifying the accuracy of the HPLC method in quantifying the FNR content within the expected values. Results from Table 5, which displays spiked concentrations and mean recovery percentages, validate the reliability of the method

Table 3: Precision study

S.No.	*System precision*		*Method precision*	
	Rt	*AUC*	*Rt*	*AUC*
1.	4.04	1488459	4.04	1491332
2.	4.03	1491372	4.04	1488689
3.	4.04	1483875	4.04	1484769
4.	4.04	1474952	4.07	1484931
5.	4.04	1475443	4.07	1475696
Mean	4.03	1482820.2	4.05	1485083.4
SD	0.0	7456.1	0.0	5922.22
%RSD	0.1	0.5	0.1	0.40

Table 4: Intermediated precision or ruggedness study

Analyst name	*Analyst I*			*Analyst II*		
Area of Std.	*1490012*			*1478876*		
S.No.	*Concentration (µg/mL)*	*AUC*	*Assay (%)*	*Concentration*	*AUC*	*Assay (%)*
1.	10	1486542	98.57	10	1480345	99.42
2.	10	1478129	98.31	10	1476890	98.54
3.	10	1480236	99.14	10	1481973	99.21
4.	10	1489714	99.07	10	1490284	99.35
5.	10	1488901	98.67	10	1487456	98.02
	Mean	1484704.4	98.75	Mean	1483389.6	98.90
	SD	5227.0	0.4	SD	5421.9	0.60
	%RSD	0.4	0.4	%RSD	0.4	0.60

Difference between mean assay of two different analysts = 0.2%

Table 5: Accuracy study

S. No.	*Level (%)*	*Amount added (µg/mL)*	*Mean (n = 5)*	*%Recovery*
1.	50	5	752200.3	99.95
2.	100	10	1480332	101.09
3.	150	15	2163301	99.76

Table 7: Analysis of marketed formulation

Commercial formulation	*Ingredients*	*Labeled amount (mg)*	*Amount found (mg)*	*Found %*
Kerendia™	Finerenone	10 mg	9.87 mg	98.70

Table 6: Robustness study

Parameters	*Variation*	*Mean peak area*	*%RSD*	*Tailing factor*	*No of theoretical Plates*
Wavelength minus	236 nm	1473 782	0.13	1.25	4934
Wavelength plus	240 nm	1474 319	0.13	1.29	4387
Flow rate minus	0.8 min/mL	14780 321	0.14	0.93	3841
Flow rate plus	1.2 min/mL	1489 901	0.13	0.93	3952
Organic phase ratio change (less)	Acetonitrile: Water (40:60)	1478 934	0.21	1.26	4482
Organic phase ratio change (more)	Acetonitrile: Water (80: 20)	1492 234	0.11	1.26	4258
Column change	Merck C_{18} column (250 mm × 4.6 mm × 5 µm)	1490 239	0.22	1.25	4367
Temperature minus	20°C	1478 934	0.13	0.85	4428
Temperature plus	30°C	1488 916	0.13	0.97	4937

for quantitative analysis, showcasing successful FNR recovery from varied spiked samples. This accuracy, as evidenced by the recovery falling within the acceptable range, confirms the satisfactory performance of the method in accurately measuring the FNR content.

Limit of Detection and Limit of Quantification

This method obtained the limit of detection (LoD) at 0.05 and the limit of quantification (LoQ) at 0.15 µg/mL. Overall, the developed method is suitable for reliable quantification of FNR in various samples within the specified concentration range.

Robustness

Table 6 presents the results from robustness studies, detailing the variations tested and their impact on the performance of the technique. The confirmed robustness of the HPLC method affirms its suitability for routine application, ensuring consistent and reliable results even under slightly altered operating conditions. Notably, insignificant changes in peak areas and retention times highlight the method's ability to deliver reliable outcomes across different conditions. In comparison to earlier reported analytical methods in the literature, this HPLC method excels with shorter retention times, enhanced theoretical plates (indicating improved resolution), and a mobile phase that promotes better separation of FNR from other constituents. Consequently, its increased efficiency and precision make it more suitable for the routine quantification of FNR across several sample types.

Analysis of Marketed Formulation

Six replicate sample solutions were injected into the HPLC system to measure peak area, enabling the determination of the amount of FNR present in the marketed product. The percentage content of FNR was calculated using the standard calibration curve and average peak area obtained from the regression equation. Based on the label claim, the drug's content, determined by averaging the values of the six sample solutions, fell within the allowed range of 90-110%. The study showcased the accuracy and practicality of the new RP-HPLC technique for FNR, indicating its suitability for routine analysis. The results are summarized in Table 7.

CONCLUSION

Following the ICH guidelines, an HPLC method was established and validated for FNR in dosage form. The method utilized a Shimadzu HPLC with UV detection at a wavelength of 238 nm. The injection volume was 20 µL, and an Inertsil ODS column was used with isocratic elution. The resulting method is rapid, robust, straightforward, precise, sensitive, and cost-effective. It offers key benefits such as a brief run time (below 5 minutes) and excellent resolution. The %RSD values for all validation parameters met the criteria, confirming the method's suitability for routine analysis of FNR in laboratories and quality control.

ACKNOWLEDGMENTS

The authors are thankful to Teja Educational Society, Hyderabad, for providing facilities to carry out this work.

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