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REVIEW ON ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FINERENONE BY RP-HPLC

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ABSTARCT
Finerenone, a novel non-steroidal mineralocorticoid receptor antagonist, has gained significant attention for the treatment of chronic kidney disease and heart failure in patients with type 2 diabetes. The accurate quantification of finerenone in pharmaceutical formulations and biological matrices is crucial for ensuring its quality, efficacy, and safety. Reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as a preferred analytical technique owing to its robustness, sensitivity, and reproducibility. This review highlights the strategies employed in the development and optimization of RP-HPLC methods for finerenone analysis, covering critical parameters such as mobile phase composition, column selection, detection wavelength, and system suitability criteria. Method validation aspects, including specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness, are thoroughly discussed in accordance with ICH guidelines. Additionally, the review emphasizes the challenges encountered during method development and offers insights into future perspectives for enhancing the analytical performance for finerenone assessment.

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

INTRODUCTION

Finerenone is an antagonist of mineralocorticoid receptors that is non-steroidal and selectively targets these receptors without showing notable binding or effects on androgen, progesterone, estrogen, and glucocorticoid receptors. Research conducted on animals has demonstrated that the attachment of finerenone to mineralocorticoid receptors diminishes inflammation and fibrosis, while phase 2 clinical studies indicated a decrease in albumin levels in urine. Aldosterone, a type of mineralocorticoid hormone, plays a crucial role in managing blood pressure, reabsorbing sodium, and expelling potassium from the body. In 1943, it was observed that the activation of mineralocorticoid receptors along with higher salt intake was linked to severe hypertension, which can lead to inflammation and fibrosis in various organs. When aldosterone binds to its receptor, it triggers a structural alteration that separates the receptor from its inactivating chaperone proteins. This active mineralocorticoid receptor then moves into the cell nucleus with a group of other coactivators to stimulate the expression of various genes.

Finerenone stands apart significantly from other steroidal mineralocorticoid receptor blockers like spironolactone and eplerenone because of its distinct physicochemical, pharmacokinetic, and pharmacological properties. It functions as a blocker for the mineralocorticoid receptor aimed at reducing the risk of ongoing decline in the glomerular filtration rate, the development of end-stage kidney disease, cardiovascular-related deaths, heart attacks, and hospital stays due to heart failure in adults with chronic kidney disease stemming from type II diabetes. Initially, individuals with kidney problems would be given spironolactone or eplerenone to address the mineralocorticoid receptor. Spironolactone has limited selectivity and a weak affinity for the receptor; it detaches quickly and may affect the androgen, progesterone, and glucocorticoid receptors. Eplerenone[1] is noted for its higher selectivity and extended duration of action. Following this, more selective nonsteroidal mineralocorticoid blockers like apararenone, esaxerenone, and Finerenone were introduced. To this point, Finerenone stands as the sole nonsteroidal mineralocorticoid receptor antagonist to be approved by the FDA. It received FDA approval on July 9, 2021, which was soon after followed by EMA approval on March 11, 2022.

Pharmacokinetics

Absorption, Distribution and Excretion

Absorption

In individuals without health issues, the average absolute bioavailability of finerenone after a subcutaneous

injection at a dose of 1-2 mg/kg is near to 100%. The absorption of finerenone is linear, indicating that it scales with the amount administered. Following a subcutaneous dose, peak plasma anti-Xa activity usually occurs within three to five hours. The highest levels of anti-Factor Xa levels achieved 1.16 IU/mL following an immediate subcutaneous injection of 1 mg/kg, given twice a day after an initial 30 mg intravenous administration.

After a treatment period of 3 to 4 days, a consistent level is achieved with a peak concentration of 1.2 IU/mL. The thrombin generation curve's area under the curve (AUC) was recorded at 305 +/- 48.8.

Route of Elimination

The main metabolites M2, M3 (47.8%), and M4 represented the bulk of the dosage detected in urine; under 1.3% of the dosage appeared in urine as the original unaltered compound. Merely 0.2% of the dosage was eliminated as the unchanged parent compound, while the balance found in feces was recognized as the M5 metabolite. The M1 metabolite accounted for less than 1.5% of the total dosage recovered from both urine and feces.

Volume of Distribution

IJIRT 166111 The distribution volume for finerenone at steady state is 52.6L.

Clearance

The estimated clearance rate stands at 0.74L/h. More frequent side effects include:

- Confusion.
- Irregular heartbeat.
- Nausea or vomiting.
- Increased nervousness.
- Tingling or numb sensations in hands, feet, or lips.

Drug Profile[2]: The chemical designation is (4S)-4-(4-Cyano-2-methoxyphenyl)-5-ethoxy 2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide [Fig.1]. It functions as an antagonist to the mineralocorticoid receptor and has the molecular composition of C21H22N4O3.

HPLC

High-performance liquid chromatography, often known as high-pressure liquid chromatography, is a technique employed for distinguishing, identifying, and quantifying active substances in the areas of biochemistry and analytical methods. This approach is often employed to recognize, obtain, and assess each element within a mixture.[3] The fundamental concept of this technique centers on the varying rates of movement within the column, which are affected by how the sample is divided between the stationary and mobile phases.[4]

Below are the steps that go into developing an HPLC method

1) Recognize the drug molecule's physicochemical characteristics.
2) Chromatographic conditions are selected.
3) Provide the analytical technique.
4) Sample preparations
5) Optimizing the method
6) Method validation.[5]

Development of an analytical method

In situations when conclusive approaches are lacking, fresh methodologies are being developed to assess the innovative product. To decrease the value other than time for increased precision and strength, innovative approaches are created to examine the existence of either pharmacopoeial or non-pharmacopoeial products. Through test runs, these approaches have been refined and found to be valid. Alternative strategies are developed and implemented to swap the current process with all available benefits and drawbacks inside the comparative laboratory data.[6]

Method Validation

The process of showing, via laboratory experiments, that the performance standards of a testing technique align with its intended analytical purpose is known as method validation. Every new or modified method is required to be validated to ensure that it yields consistent and dependable results when used by various individuals across one or more laboratories employing the same apparatus. The results obtained from method validation, which are crucial for any effective analytical procedure, can be utilized to evaluate the quality, repeatability, and dependability of the analytical findings.[7]

Parameters of analytical method validation

1) Accuracy
2) Precision
3) Specificity
4) Detection Limit
5) Quantitation limit
6) Linearity
7) Range
8) Stability
9) Robustness
10) Ruggedness
11) System Suitability.[8]

Need for development and validation of analytical method

The existing strategy might be excessively expensive, requiring too much labor or energy, or could lack automation feasibility. Current techniques may suffer from high error rates, be susceptible to contamination, or show inconsistencies. For regulatory or scientific purposes, it might be essential to adopt an alternative method for verifying analytical information that was originally obtained through recognized procedures. There may be instances where a specific analyte within a certain sample structure lacks an adequate method.

HPLC Method Development

Preparation of Standard Solution

Accurately weigh and place 10 mg of the Finerenone reference standard into a dry and clean 10 mL volumetric flask. Add around 7 mL of Methanol and apply sonication to ensure complete dissolution while removing any trapped air. Subsequently, bring the flask up to the mark with the same solvent. Then, measure 0.3 mL of this stock solution and move it to another clean 10 mL volumetric flask, and dilute it to the mark using Methanol.

Preparation of Sample Solution

Determine the mean weight of the powder and measure 10 mg of Finerenone into a clean, dry 10 mL volumetric flask. Add approximately 7 mL of Diluent and use sonication until it fully dissolves, then complete filling the flask to the designated line with the same solvent. Following this, take 0.3 mL of this prepared stock and transfer it into a separate 10 mL volumetric flask, filling it to the line with Methanol.

Inject the resulting samples under various chromatographic settings, record the generated chromatograms, and note the conditions that facilitate the best peak elution. These factors will be applied to carry out validation processes in line with ICH standards.

Mobile Phase Optimization

Initially, several mobile phases were tested, including Methanol:Water and ACN:Water in varying proportions. The optimal mobile phase.[9] was determined to be ACN:Methanol (80:20% v/v), which provided the best separation results.

Optimization of Column

The methodology was evaluated using various C18 columns, including Symmetry, Zodiac, and Xterra. The Symmetry ODS C18 (4.6 × 250 mm, 5 µm) column turned out to be the best option because of its excellent peak shape and resolution[10] at a flow rate of 1 mL/min. Preparation of Mobile Phase: Carefully quantify 800 mL (80%) of HPLC-quality Acetonitrile and 200 mL (20%) of Methanol. Thoroughly mix these substances and expel any trapped gas by employing a digital ultrasonic bath for 15 minutes. Finally, filter the resultant mixture through a 0.45 µm membrane using vacuum filtration before use.

Method Validation

System Suitability

Carefully weigh out and transfer 10 mg of the Finerenone reference standard into a clean, dry 10 mL volumetric flask. Add approximately 7 mL of the diluent, then apply sonication to ensure that the substance fully dissolves. Next, incorporate additional solvent to achieve the final desired volume, forming the stock solution.

Using a pipette, take 0.3 mL of the Finerenone stock solution and transfer it to a 10 mL volumetric flask, then top it off with Methanol to the specified mark. Execute five injections of the standard solution into the HPLC system and document the peak area for each injection. The %RSD for the peak area among the five repetitions should adhere to the specified limits.

Specificity

Preparation of Standard Solution

Precisely weigh out and deposit 10 mg of the Finerenone reference substance into a clean, dry 10 mL volumetric flask. Add 7 mL of the diluent and sonicate until completely dissolved, then amend the volume using the same solvent to prepare the stock solution.

With a pipette, dispense 0.3 mL of the stock solution into a 10 mL volumetric flask and top it off to the line with methanol.

Preparation of Sample Solution

Precisely weigh out and deposit 10 mg of the Finerenone reference substance into a clean, dry 10 mL volumetric flask. Add 7 mL of the diluent and sonicate until completely dissolved, then amend the volume using the same solvent to prepare the stock solution.

With a pipette, dispense 0.3 mL of the stock solution into a 10 mL volumetric flask and top it off to the line with methanol.

Conduct five distinct injections of the reference solution and three distinct injections of the sample solution. Calculate the assay following the outlined formula.

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

Linearity

Carefully weigh out 10 mg of the Finerenone standard and transfer it into a clean, dry 10 ml volumetric flask. Introduce about 7 ml of diluent[11] and sonicate the mixture until completely dissolved. After that, fill the flask with the same solvent up to the calibration mark. (This constitutes the stock solution).

Preparation of Calibration Levels

- Level I (10 ppm): Take 0.1 mL of the initial solution and transfer it into a 10 mL volumetric flask, then add the diluent until the flask reaches the specified mark.
- Level II (20 ppm): Take 0.2 mL of the initial solution and transfer it into a 10 mL volumetric flask, then add the diluent until the flask reaches the specified mark.
- Level III (30 ppm): Take 0.3 mL of the initial solution and transfer it into a 10 mL volumetric flask, then add the diluent until the flask reaches the specified mark.
- Level IV (40 ppm): Take 0.4 mL of the initial solution and transfer it into a 10 mL volumetric flask, then add the diluent until the flask reaches the specified mark.
- Level V (50 ppm): Take 0.5 mL of the initial solution and transfer it into a 10 mL volumetric flask, then add the diluent until the flask reaches the specified mark.

Place each concentration level into the chromatographic system and document the peak area. Construct a graph that depicts the peak area versus concentration (X-axis: concentration, Y axis: peak area) and find the correlation coefficient.

Precision

Repeatability

Preparation of Finerenone Product Solution for Precision

Carefully weigh and transfer 10 mg of the Finerenone standard into a clean, dry 10 mL volumetric flask. Add approximately 7 mL of the diluent, then utilize sonication to ensure complete dissolution, and fill to the mark with the same solvent (Stock solution).

Conduct six injections of the standard solution and document the peak areas during the HPLC analysis. The %RSD for the six repeated injections should stay within the established acceptable limits.

Intermediate Precision (Ruggedness)[12]

Precision was assessed on various days under identical circumstances.

- Analyst 1: A series of six injections of the reference solution was conducted, and the %RSD stayed within the specified limits.
- Analyst 2: The reference solution was injected six times, and the %RSD conformed to the outlined standards.

Accuracy

Preparation of Accuracy Samples

- 50% Standard Stock Solution: Take 0.15 mL of the concentrated solution and transfer it into a 10 mL volumetric flask. After that, add the diluent until the liquid reaches the marked line on the flask.
- 100% Standard Stock Solution: Take 0.3 mL of the stock solution and place it into a 10 mL volumetric flask, then add diluent until it hits the calibration mark.
- 150% Standard Stock Solution: Take 0.45 mL of the stock solution and place it in a 10 mL volumetric flask, then complete it to the mark with diluent.

Perform three repetitions for each concentration level (50%, 100%, and 150%) while adhering to the defined optimal parameters. Record the chromatograms and analyze the peak responses. Establish the Amount detected and Amount introduced for Finerenone and calculate both the individual[13] recovery and the average recovery metrics.

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

Preparation of LOD Solution (0.597 µg/mL)

Carefully weigh and transfer 10 mg of the Finerenone working standard into a clean, dry 10 mL volumetric flask. Add 7 mL of methanol, then sonicate the mixture until it is fully dissolved, and top it off to the calibration line with the same solvent. Next, take 0.00597 mL of this prepared stock solution and move it to a 10 mL volumetric flask, diluting to the mark using methanol.

Preparation of LOQ Solution (1.811 µg/mL)

Carry out the same procedure as described earlier, utilizing 0.01811 mL of the original solution instead.

Robustness

The strength of the approach was evaluated by altering various analytical parameters: Effect of Flow Rate Variation.

Effect of Flow Rate Variation

The sample was analyzed at flow rates of 0.9 mL/min and 1.1 mL/min, differing from the typical rate of 1 mL/min, while all other conditions were kept constant. Graphs of chromatography were recorded.

Effect of Mobile Phase Composition Variation

The sample underwent analysis using mobile phase mixtures of ACN and Methanol at ratios of 75:25 and 85:15, deviating from the 80:20 ratio. Chromatograms were documented and evaluated for differences.

RESULTS AND DISCUSSION

Method Development Numerous concurrent trials were performed to enhance the proposed methodology for identifying the optimal chromatographic settings that would aid in executing a comprehensive validation assessment.[14] The mobile phase, composed of Acetonitrile and methanol at an 80:20 volume ratio, was fine-tuned to a flow rate of 1 mL/min with a detection wavelength set at 272 nm, producing a distinct peak, a low tailing factor, and a short analysis time for Finerenone. The retention time[15] for Pravastatin was recorded at 3.155 minutes.

Validation of Analytical Method

The approach was confirmed according to ICH standards, examining system functionality, uniqueness, linear response, consistency, correctness, resilience, and stress testing.

System Functionality: Theoretical plates, separation, and tailing characteristics were within permissible thresholds.

Uniqueness: No disruptions from control samples or additives were noted. Linear Response: Demonstrated in the 10-50 µg/mL interval with a remarkable correlation coefficient ($R^2 = 0.99$).

Consistency: The percentage relative standard deviation values for both intra-day and inter day remained below 2.0, indicating dependability.

Accuracy: The percentage of recovery varied between 100.40% and 100.44%, which validates the precision.

LOD and LOQ: Set at 0.597 µg/mL and 1.811 µg/mL, correspondingly.

Robustness: Small changes in flow rate and the makeup of the organic phase had no substantial impact on the outcomes.

Forced Degradation Studies

Finerenone was subjected to multiple stress factors such as acidic, basic, oxidative, thermal, photolytic conditions, and contact with water, resulting in a degradation range of 6.69% to 19.77%.

Table 1: Reported Analytical Methods for the Analysis of Finerenone.

S.no	Author/Drug	Conditions	Findings
1.	Vijay Birappa Metkari et al.,	Stationary Phase: C18 (Agilent) Mobile Phase: Methanol: water(0.1% OPA) 45:55 Flow rate :0.7 ml/min Wavelength :230 nm	RT: 3.945. r 2: 0.999. Linearity: 2-10 µg/ml
2.	Jyothi Mirjapuram, Gade Sammaiah	Stationary Phase: Symmetry ODS C18 (4.6 x 250mm, 5µm) column Mobile Phase: Acetonitrile: Methanol in the ratio of 80:20% v/v Flow rate :1ml/min Wavelength :272 nm	Linearity:10-50µg/ml RT: 3.155 r 2: 0.99 LOD and LOQ: 0.597µg/ml and 1.811µg/ml
3	Mugdha Manoj Varade	Stationary Phase: (Agilent) C18 column Mobile Phase: Methanol: 10 Mm Citric Acid with ratio (38:62) Flow rate: 1.0 ml/min. Wavelength:258nm	LOD and LOQ: 0.1367µg/ml and 0.4145µg/ml Linearity:10-50 µg/ml RT: 6.9 r 2:0.999

FIGURE

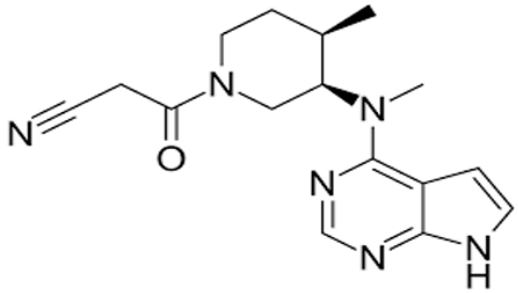

Figure 1: Structure of Finerenone.

CONCLUSION

In this research, a quick, easy, dependable, accurate, and linear HPLC method has been developed and confirmed for Finerenone, making it ideal for routine quality control evaluations. The parameters of the analytical technique and the solvents used in the mobile phase provided superb separation for Finerenone. Additionally, notable features of this method include a short analysis time and a retention period of about 8 minutes. The procedure was validated in accordance with ICH guidelines. It proved to be robust, offering consistent and precise results across different chromatographic conditions. Thus, the suggested RP-HPLC technique has been validated as simple, accurate, and reproducible for evaluating Finerenone in a timely manner. The validation findings showed positive results for all criteria assessed during the validation procedure. This developed method can be readily adopted by labs concentrating on quality control.

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