

A REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF SELECTED CARDIOVASCULAR DRUG FINERENONE BY DIFFERENT ANALYTICAL TECHNIQUES

**Dr. Santhosh Illendula^{*1}, Vusala Tejaswi², Varipally Sai Sindhu², Vootkuri Nihathi²,
Jetamoni Mahesh², Kurumeti Divya², Mothe Praneeth², Dr. Suthakaran Raj³**

^{*1}Associate Professor & HOD, Department of Pharmaceutical Analysis, Vijaya college of Pharmacy, Munaganoor (V), Hayathnagar (M), Hyderabad, Rangareddy (Dt), Telangana (St), India, 501505.

²Under Graduate Pharmacy Scholar, Vijaya college of Pharmacy, Munaganoor (V), Hayathnagar (M), Hyderabad, Rangareddy (Dt), Telangana (St), India, 501505.

³Professor & Principal, Vijaya college of Pharmacy, Munaganoor (V), Hayathnagar (M), Hyderabad, Rangareddy (Dt), Telangana (St), India, 501505.

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***Corresponding Author**

Dr. Santhosh Illendula

Associate Professor & HOD,
Department of Pharmaceutical
Analysis, Vijaya college of
Pharmacy, Munaganoor (V),
Hayathnagar (M), Hyderabad,
Rangareddy (Dt), Telangana (St),
India, 501505.



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ABSTRACT

Instrumental methods of analysis are analytical techniques that use scientific instruments to identify, separate, and quantify chemical substances in pharmaceutical formulations. These methods provide rapid, accurate, sensitive, and precise analysis compared with conventional analytical techniques. Analytical method development involves optimization of parameters such as solvent selection, wavelength, mobile phase, flow rate, and chromatographic conditions to obtain accurate, precise, and reproducible results. The developed method is validated according to ICH guidelines for linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). Finerenone acts by selectively blocking the mineralocorticoid receptor (MR) and thereby inhibits aldosterone-mediated inflammatory and fibrotic pathways in the kidney and cardiovascular system. It reduces renal inflammation, fibrosis, and cardiovascular risk associated with CKD and T2DM. Finerenone is primarily

used to reduce the risk of kidney disease progression and cardiovascular events in appropriate patients with CKD associated with T2DM. Finerenone is indicated to reduce the risk of kidney disease progression and cardiovascular events.

KEYWORDS: Finerenone, Instrumental analysis, Analytical method development, Method validation, Mineralocorticoid receptor antagonist, Accuracy, LOD, LOQ.

INTRODUCTION

Analytical method development

Analytical method development is the process of establishing a suitable procedure for the identification and quantification of a drug. It involves selecting appropriate analytical techniques and optimizing experimental conditions. Parameters such as mobile phase, solvent, wavelength, flow rate, temperature, and column are optimized. The developed method should provide accurate, precise, specific, and reproducible results. It is essential for assessing the quality, purity, strength, and stability of pharmaceutical products. The method is generally validated according to applicable regulatory guidelines before routine use.

Criteria for the development of new analytical method

Drug analysis is the basis for the determination of the product. Very often, there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopeia's. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors.

The new drug or drug combination may not be official in any pharmacopeia's. A proper analytical procedure for the drug may not be available in the literature due to patent regulations. Analytical methods may not be available for the drug in the form of formulation excipients. Analytical methods for a drug in combination with other drugs may not be available. Analytical methods for the quantitation of the drug in biological fluids may not be available. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedure and these may not be reliable.

Steps for analytical method development

The procedure for analytical method development follows a set of steps as below:

Purpose of Analytical Method

Development In the pharmaceutical industry, analytical method development gives important information on the potency of a drug, the drugs' bioavailability, the drugs' stability and also its effects. In the very first step, the purpose of conducting any Analytical Method Development is established.

Highlighting of Steps

In the second step of Analytical Method Development, the steps involved in the development are recorded in a laboratory book.

Characterization of the Analyte

In this step, both the biological and chemical properties in addition to the physical properties of the analyte are collected. After that, the analyte is obtained and stored according to its specific requirements. The methods for analysis are then recorded with an example being the chromatography technique which employs different methods such as the High-Performance Liquid Chromatography.

Definition of Requirements

Requirements for the method development of the analysis are done and recorded. All the materials, reagents and instruments are procured those are required for the analysis of the sample.

Review of Literature and Previous Methods

All literature information related to the specific analyte e.g. a specific drug is assessed for any biological, chemical and chemical properties regarding the analyte. Reference is then made from journals, books and any other publications.

Choosing an Analytical Method

From the information obtained from the literature during the literature review, a specific methodology is modified to cater for accurate output and also because methods change with the requirements of the analyte. If there are no previous methods in the literature being reviewed regarding the analyte, the procedure goes on uninterrupted.

Setting up of Instruments

Appropriate instruments for the analytical method development are set up in the laboratory by each of the instruments standard operating procedures. Standard Operating Procedures usually abbreviated as SOP's are a set of instructions or steps to aid in performing a specific procedure in a laboratory set up. They are usually universal and standardized for ease of use in any laboratory set up.

Optimization of the Method

In carrying out the optimization of the analytical method, parameters are changed individually depending on the arising interests. Optimization of an analytical method is done in reference to a systematic and procedural plan while making sure to critically follow all the documented steps.

Analytical Figures of Merit Documentation

Documentation of the analytical figures of merit decided upon is done. These analytical figures of merit include quantification limits, detection limits, analysis time frame, operational costs and sample preparation.

Development Method Evaluation

The resultant product of analysis should give a desirable result as expected in the identification of the analyte.

Sample Estimation, Quantitative Demonstration and Analysis of Samples

Estimation of an analyte with an example being a drug in a matrix sample containing the analyte is done here.

Analytical method validation

It establishes documented evidence that the procedure adapted for a test is fit for the intended purpose in terms of quality, reliability, and consistency of results.

Parameters to be checked for method validation

Selectivity/specificity

Selectivity or specificity is the ability of an analytical method to measure the desired analyte accurately in the presence of other substances. It ensures that impurities, excipients, and degradation products do not interfere with the analysis. A specific method provides a clear and reliable response for the analyte. It is evaluated by comparing blank, placebo, standard,

and sample preparations. Thus, specificity ensures the reliability and accuracy of the analytical results.

Precision

Precision is the degree of agreement among individual test results obtained from repeated measurements of the same sample. It indicates the reproducibility and consistency of an analytical method. Precision is generally evaluated by repeatability, intermediate precision, and reproducibility. It is commonly expressed as the percentage relative standard deviation (%RSD) of the obtained results. A precise analytical method produces results that are closely similar under specified experimental conditions

Accuracy

Accuracy is the closeness of the measured value obtained by an analytical method to the true or accepted reference value. It indicates how correctly the method determines the actual amount of analyte present in a sample. Accuracy is commonly evaluated by recovery studies using known concentrations of the analyte. The results are generally expressed as percentage recovery of the added or known amount. A method is considered accurate when the obtained results are close to the true value.

Linearity

Linearity is the ability of an analytical method to produce test results that are directly proportional to the concentration of analyte in a given range. It indicates the method's capability to obtain reliable results at different concentrations. Linearity is generally evaluated by analyzing a series of standard solutions at different concentration levels. A calibration curve is plotted between the concentration of analyte and the corresponding analytical response. A method is considered linear when the calibration curve shows a suitable correlation between concentration and response.

Range

Range is the interval between the upper and lower concentration of analyte for which the analytical method has suitable accuracy, precision, and linearity. It defines the concentration limits within which the method can be reliably applied. The range is established using results obtained from linearity, accuracy, and precision studies. It depends on the intended use of the analytical method and the concentration of the analyte in the sample. A suitable range ensures reliable and consistent measurement of the analyte.

Limit of detection and limit of quantitation

Limit of Detection (LOD) is the lowest concentration of an analyte that can be detected but not necessarily quantified accurately.

Limit of Quantification (LOQ) is the lowest concentration of an analyte that can be quantitatively determined with suitable accuracy and precision.

Different Analytical techniques**Liquid chromatography-mass spectrometry (LC-MS)**

Liquid Chromatography–Mass Spectrometry (LC-MS) is a powerful analytical technique used for the identification and quantification of chemical compounds. It combines the separation capability of liquid chromatography with the detection and identification capabilities of mass spectrometry. In LC-MS, liquid chromatography separates the components of a sample based on their chemical properties, and the separated compounds are introduced into the mass spectrometer for analysis. The mass spectrometer detects compounds based on their mass-to-charge ratio (m/z). LC-MS offers high sensitivity, selectivity, accuracy, and precision, making it suitable for complex samples. It is widely used in pharmaceutical, biomedical, environmental, and food analysis. The technique is particularly useful for the analysis of drugs, metabolites, impurities, and degradation products. LC-MS can detect and quantify compounds present at very low concentrations.

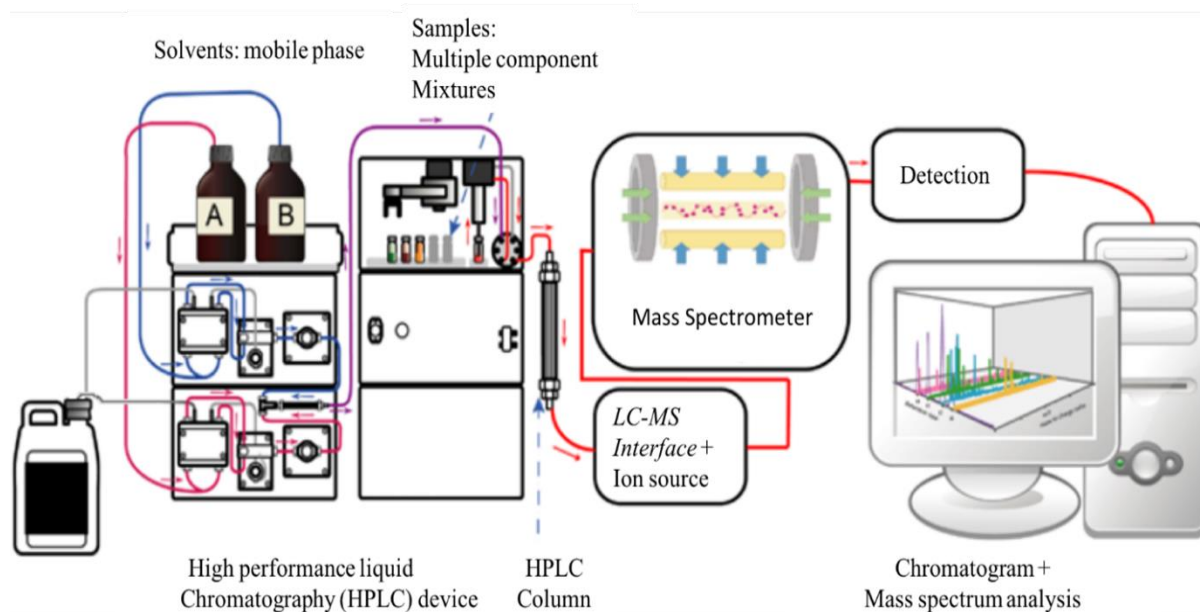


Fig. 1: LC-MS Instrumentation.

Ultra Performance Liquid Chromatography

UPLC stands for Ultra Performance Liquid Chromatography, a technique used in liquid chromatography analysis. It's an improvement on HPLC (high-performance liquid chromatography) and offers better resolution and sensitivity, shorter run times, and the ability to analyse more samples in less time. Ultra-performance liquid chromatography (UPLC), developed in 2004 by Waters, is a modern liquid chromatographic system, which is simply the modification of HPLC.

The smaller particle size of sorbent (diameter less than 2 μm) in UPLC offers better resolution, speed, and sensitivity compared with HPLC. The basic principle of UPLC for the separation of components in a matrix is same as HPLC, the main difference is in the particle size of sorbent of the column, which is less than 2 μm . The small particles in UPLC require a high pressure (6000 psi) to work with. A significant decrease in the particle size of stationary phase below 2.5 μm provides a remarkable increase in the effectiveness of the column, which does not reduce on increasing the linear speed or rate of flow. The use of speed, particles with a small radius, and maximum number of resolvable peaks (peak capacity) comprehends the efficiency together with resolution. UPLC cuts the mobile phase volume consumption by at least 80% compared to HPLC with a very short runtime (even less than 1 min).

Instrumentation :

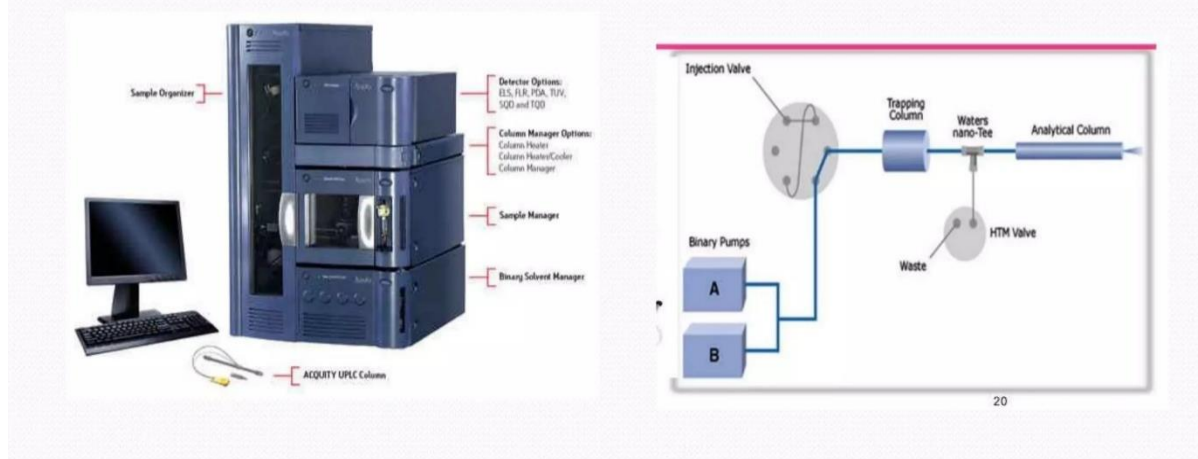


Fig. 2: UPLC Instrumentation.

High-Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for the separation, identification, and quantification of components in a mixture. It works by passing a liquid mobile phase containing the sample through a column packed with a stationary phase under high pressure. Different components interact differently with the stationary phase and are separated based on their chemical properties. The separated compounds are detected using a suitable detector, such as a UV or PDA detector. HPLC provides high accuracy, precision, sensitivity, and reproducibility. It is widely used in pharmaceutical analysis for the determination of drugs, impurities, and degradation products. HPLC is also useful for quality control, stability studies, and formulation analysis. Due to its reliability and versatility, HPLC is an important technique in pharmaceutical research and development.

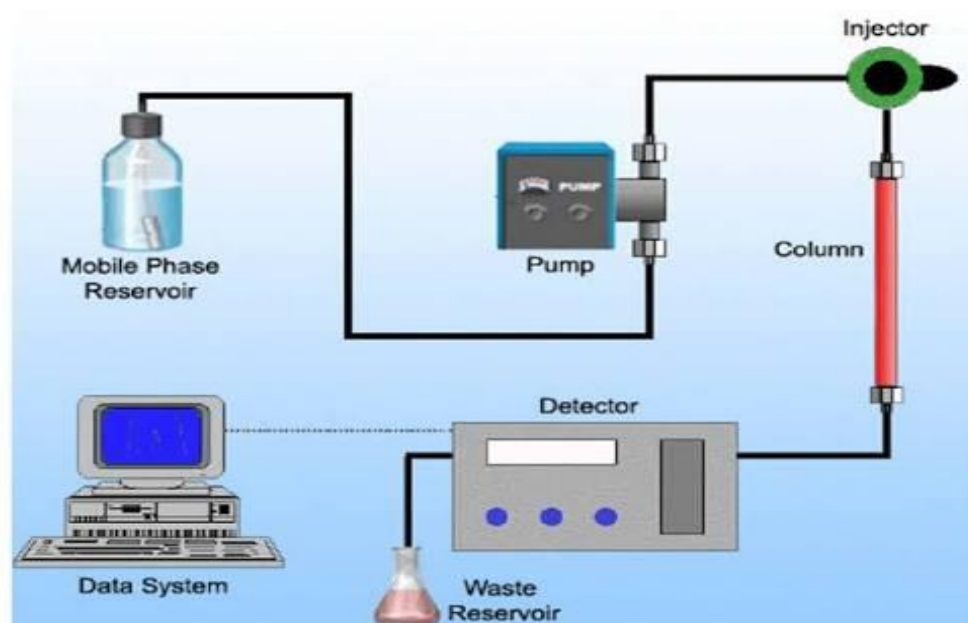


Fig. 3: HPLC Instrumentation.

Gas chromatography

Gas Chromatography (GC or GLC) is a commonly used analytic technique in many research and industrial laboratories for quality control as well as identification and quantitation of compounds in a mixture. GC is also a frequently used technique in many environmental and forensic laboratories because it allows for the detection of very small quantities. A broad variety of samples can be analysed as long as the compounds are sufficiently thermally stable and reasonably volatile.

In gas-solid chromatography, a solid adsorbent is utilised as the stationary and separational phase. In gas liquid chromatography with a stationary phase adsorption process Solid consists of a thin covering of immobile liquid with help and detachment. Through the process of breaking up. Gas liquid chromatography is the most generally used organization. The break up sample is first vaporized and also combined with the gas Mobile stage. In the stationary phase, quickly patches run mostly densely & in the stationary phase, the bottom answerable factors run quickly. The sample result stored in the gadget, which is together for distribution, enters the gas stream that passes through the division pipe called “column”. different factors are break up under the column. The detector calculates the number of factors leaving the column. To calculate a sample with an unknown concentration, a standard sample with a known concentration is fitted into the instrument.

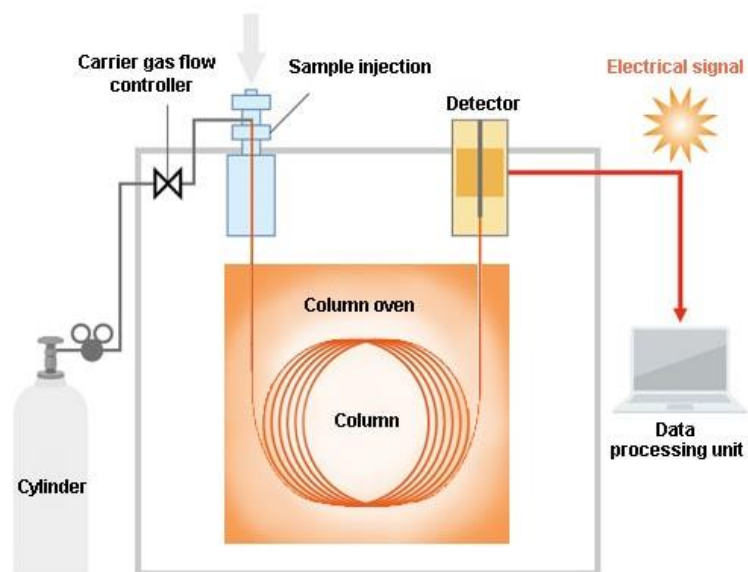


Fig.4: Gas Chromatography Instrumentation.

UV Spectroscopy

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. UV spectroscopy is a type of absorption spectroscopy in which light of the ultra-violet region (200-400 nm) is absorbed by the molecule which results in the excitation of the electrons from the ground state to a higher energy state.

UV Vis spectroscopy is a scientific technique used to measure the amount of light that is absorbed or transmitted by a sample at different wavelengths of ultraviolet (UV) and visible (Vis) light.

The process involves shining a beam of UV Vis light through the sample and measuring the amount of light that passes through it. By analysing the pattern of absorption and transmission of light, scientists can identify and quantify the components of the sample.

A unique relationship exists between the substance and its UV Vis spectrum when a substance absorbs the maximum light at a specific wavelength. This relationship can be used for:

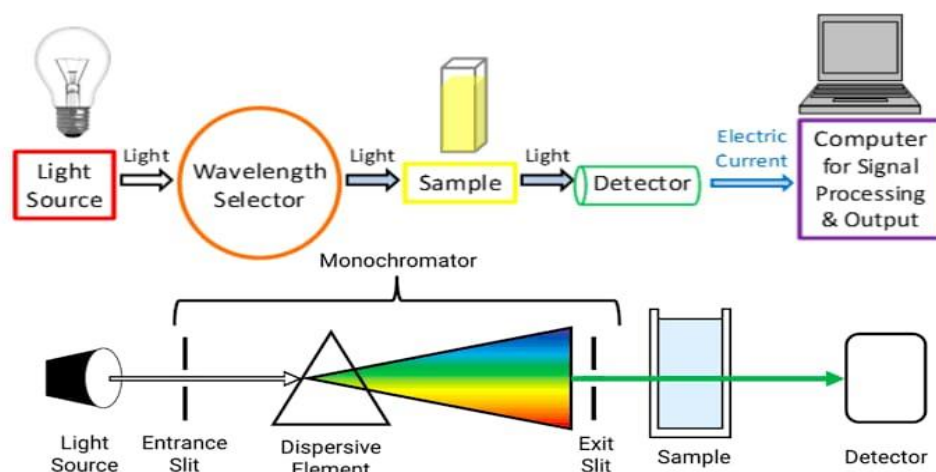


Fig. 5: UV Spectrophotometer Instrumentation.

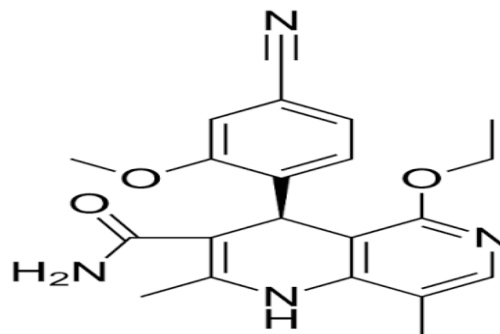
DRUG PROFILE

NAME: Finerenone

IUPAC Name: (4S)-4-(4-cyano-2-methoxyphenyl)-5-ethoxy-1,4-dihydro-2,8-dimethyl-1,6-naphthyridine-3-carboxamide.

CHEMICAL FORMULA: C₂₁H₂₂N₄O₃

MOLECULAR MASS: 378.432 g/mol



STRUCTURE

Fig.6: Structure of Finerenone.

PHARMACODYNAMICS

Finerenone is administered orally once daily, with or without food. It selectively blocks the mineralocorticoid receptor (MR), thereby reducing aldosterone-mediated inflammation and fibrosis in the kidneys and cardiovascular system. Finerenone is primarily used to reduce the risk of kidney and cardiovascular complications in adults with chronic kidney disease associated with type 2 diabetes. Its use requires monitoring of serum potassium and renal function, as it can cause hyperkalaemia and changes in eGFR.

MECHANISM OF ACTION

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.

Finerenone's binding to the MR prevents binding of MR coactivators, which in turn prevents pro-inflammatory and pro-fibrotic gene transcription. 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 $\leq 35\%$. Patients taking finerenone developed new onset atrial fibrillation or flutter (AFF) with a hazard ratio of 0.71. Finerenone lowered the risk of first onset of kidney failure, a sustained eGFR decrease of $\geq 40\%$, or death from a renal cause to a hazard ratio of 0.82. Cardiovascular outcomes including cardiovascular death, nonfatal heart attacks, nonfatal strokes, and hospitalization for heart failure in patients taking finerenone had a hazard ratio of 0.86 in patients with a history of AFF and 0.85 in patients without a history of AFF.

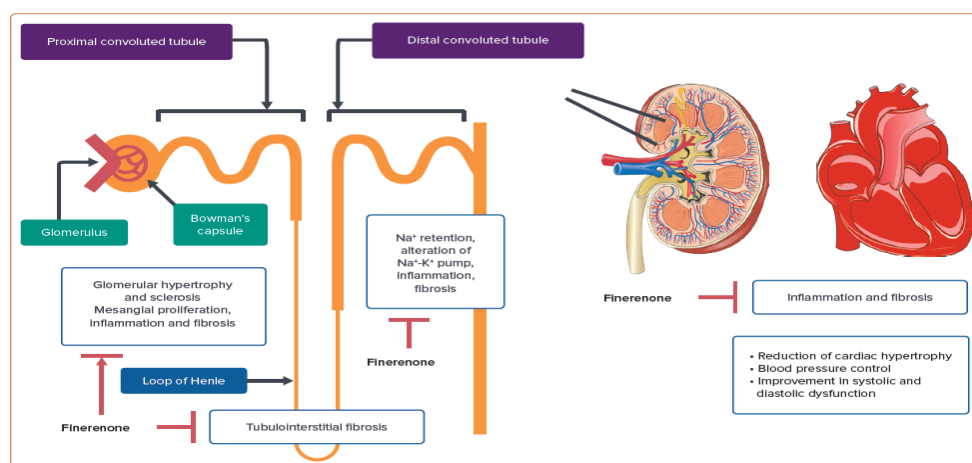


Fig. 7: Finerenone mechanism of action.

VOLUME OF DISTRIBUTION

The mean apparent volume of distribution (V_d) of finerenone is approximately 52 L.

PROTEIN BINDING

Finerenone is highly bound to plasma proteins, approximately 92%, mainly to albumin.

METABOLISM

Finerenone is extensively metabolized in the liver, mainly by CYP3A4 and to a lesser extent by CYP2C8. It forms several metabolites with minimal pharmacological activity. The drug is

eliminated mainly through the feces, while a smaller proportion is eliminated through the urine.

ROUTE OF ELIMINATION

Following oral administration of Finerenone is eliminated mainly through feces (approximately 80%), primarily as metabolites. A smaller amount is eliminated through urine (approximately 20%). The unchanged drug accounts for only a small fraction of the total elimination.

HALF-LIFE: The mean effective half-life of finerenone is 2–3 hours.

Table No: 1- Different analytical techniques of Finerenone.

S.NO	DRUG NAME	ANALYTICAL TECHNIQUE	DESCRIPTION OF TECHNIQUE	AUTHOR & YEAR
1	Finerenone	RP-HPLC	Column: C18 reversed phase column (250 x 4.6mm, 5µm) Mobile phase: Methanol: water: trifluoroacetic acid (68:32:0.05 v/v/v) Flow rate: 1.0 ml/min LOD: 0.03 µg/ml LOQ: 0.09 µg/ml Wavelength: 245 nm	Ms. Aditi Ashutosh Mhasatkar.et.al 2025
2	Finerenone	RP-HPLC	Column: Symmetry C18, (4.6mm x 250 mm, 5 µm) Mobile phase: Acetonitrile : Methanol (80:20 v/v) Flow rate: 1 ml/min LOQ: 1.811 µg/ml Linearity Equation: 10-50 ppm	Tamilselvi N.et.al 2025
3	Finerenone	RP-HPLC	Column: Sunfire C18, (4.6mm x 250mm, 5 µm) Mobile phase: 0.1% OPA : Methanol (60:40) Flow rate: 0.1 ml/min LOD: 0.08 µg/ml LOQ: 0.24 µg/ml Linearity Equation: 23931x + 2701.6 Wavelength: 219nm	Ruhi Rukhsar.et.al 2025
4	Finerenone	RP-HPLC	System: Shimadzu Prominence-iSeries Model: LC-2030 C 3D Column: Phenomenex C18, (4.6 x 250mm, 5µm) Flow rate: 0.8 ml/min	Aya A. Marie.et.al 2025

5	Finerenone	RP-HPLC	Mobile Phase: Water: Acetonitrile: Triethylamine (450:550:10ml) Linearity : 8-30 µg/ml Wavelength: 252 nm Injection volume:10 µl System: HPLC with UV-Visible detector Model: Waters HPLC with Empower 2 software, isocratic mode Column: Symmetry ODS C18,(4.6 × 250 mm, 5 µm) Mobile phase: Methanol: Phosphate buffer (35:65% v/v) Flow rate: 1.0 mL/min Run time: 8 min Linearity: 6–14 µg/mL Correlation coefficient: r = 0.999 (abstract reports R² = 0.9996) LOQ: 3.6 µg/mL LOD: 1.2 µg/mL Wavelength: 235 nm Injection volume: 10 µL Retention time:3.006 min	Santhosh Illendula.et.al2024
6	Finerenone	RP-HPLC	Mobile phase: Potassium dihydrogen orthophosphate &acetonitrile [15:85v/v]. Flow rate: 1ml/min. LOQ:0.06 µg/ml LOD:0.02 µgm/ml Linearity:8-12 µg/ml Column: Isocratic C18 Analytical column (250 x4.4mm,5 µm) Wavelength: 284nm	Mugdha Manoj Varade.et.al 2024
7	Finerenone	RP-HPLC	Column: Symmetry ODS C18 Mobile phase: Methanol: Phosphate (35:65v/v) Flow rate: 0.1ml/min Wavelength: 235nm	Vijay Birappa Metkari.et.al 2024
8	Finerenone	RP-HPLC	Column: Inertsil ODS-3V C18 (250 x4.6mm,5µm) Mobile phase:0.03M Potassium dihydrogen orthophosphate buffer:Acetonitrile (70:30v/v) Flow rate: 1ml/min Run time: 9.11min	Mohammed Arsalan.et.al 2023

9	Finerenone	RP-HPLC	LOD: 0.5 µg/ml LOQ: 0.15 µg/ml Linearity: 40-120 µg/ml Wavelength: 309nm Injection volume:10 µl Column: Symmetry ODS C18, (4.6 x 250nm ,5µm) Mobile phase: Acetonitrile & Methanol (80:20v/v) Flow rate:1.0ml/min LOD:1.1 µg/ml LOQ: 3.2 µg/ml Linearity: 10-50 µg/ml Wavelength:272nm	Jyothi Mirjapuram .et.al 2023
10	Finerenone	Spectrophotometry	System: UV-Visible double beam spectrophotometer Model: Shimadzu 1700S Mobile phase: Ethanol LOD:1.220 µg/ml LOQ:5.220 µg/ml Wavelength: 239.8nm Linearity:2-14 µg/ml	Husnain Fathima .et.al 2023

Table No 2: Brand Names of Finerenone.

S.NO	BRAND NAME	MANUFACTURING COMPANY
1	Kerendia®	Bayer
2	Lyvelsa	Sun Pharma
3	Fincor	ACI Limited
4	Finen	General Pharmaceuticals
5	Finera	Opsonin Pharma
6	Fineron	Everest Pharmaceuticals
7	Finmax	Delta Pharma
8	Finorex	Ziska Pharmaceuticals
9	Firendia	Beacon Pharmaceuticals
10	Kanopas	Incepta Pharmaceuticals

CONCLUSION

A structured and updated literature review of analytical methods reported for finerenone. A comparative table of HPLC/RP-HPLC and other relevant analytical techniques, including major chromatographic and validation parameters. Identification of commonly used stationary phases, mobile-phase systems, detection wavelengths and analytical ranges. A clear summary of validation characteristics such as specificity, linearity, accuracy, precision, LOD, LOQ and robustness. Identification of stability-indicating approaches and the degradation conditions used in published studies. Understanding of the advantages and

limitations of conventional HPLC, QbD-based methods and bioanalytical approaches. Identification of literature gaps and potential areas for development of rapid, sensitive, robust and environmentally considerate analytical methods. A scientifically organized basis for selecting or designing an analytical method suitable for future laboratory work on finerenone.

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CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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