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## Review Article

# Recent Advances In RP-HPLC Method Development And Validation For Finerenone: A Comprehensive Review

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## ABSTRACT

Finerenone is a novel, non-steroidal mineralocorticoid receptor antagonist (MRA) indicated for the management of chronic kidney disease (CKD) associated with type 2 diabetes mellitus (T2DM). Owing to its growing clinical significance and unique therapeutic profile, establishing precise, sensitive, accurate, and stability-indicating analytical protocols for its quantification is critical for industrial quality control and regulatory compliance. Reversed-phase high-performance liquid chromatography (RP-HPLC) remains the cornerstone technique for its analysis due to its superior selectivity, reproducibility, and high-throughput capability. This review consolidates recent developments in RP-HPLC method development, optimization parameters, and validation characteristics aligned with International Council for Harmonisation (ICH) Q2(R1/R2) guidelines. Key aspects evaluated include system suitability criteria, forced degradation pathways (acid, base, peroxide, thermal, and photolytic stress), and the application of Analytical Quality by Design (AQbD) strategies using Design of Experiments (DoE). A comparative analysis of reported methodologies is presented, together with an appraisal of emerging paradigms, including Ultra-High-Performance Liquid Chromatography (UHPLC) and green analytical chemistry metrics. This review is intended as a reference for pharmaceutical researchers, quality control scientists, and academicians working on finerenone analysis.

## INTRODUCTION

Pharmaceutical analysis forms the foundation of drug development, manufacturing, and

commercialisation, ensuring that therapeutic agents conform to rigorous standards of identity, strength, quality, and purity. In contemporary pharmaceutical manufacturing, regulatory

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authorities — including the US Food and Drug Administration (USFDA) and the European Medicines Agency (EMA) — require validated, robust analytical assays capable of detecting trace impurities and degradants<sup>1,10</sup>. Among the available analytical techniques, high-performance liquid chromatography (HPLC), and reversed-phase chromatography (RP-HPLC) in particular, represents the industry standard because of its versatile stationary phases, high sensitivity, and adaptability to automated systems<sup>2,4</sup>.

RP-HPLC is the most widely employed chromatographic technique for the analysis of pharmaceutical compounds. It utilises a non-polar stationary phase and a relatively polar mobile phase, enabling efficient separation of compounds on the basis of hydrophobic interactions<sup>2,4</sup>. The technique is extensively applied in the pharmaceutical industry for assay determination, impurity profiling, dissolution testing, stability studies, and bioanalytical investigations.

Finerenone is a non-steroidal mineralocorticoid receptor antagonist approved for reducing the risk of sustained decline in estimated glomerular filtration rate (eGFR), end-stage kidney disease, cardiovascular mortality, and hospitalisation for heart failure in adults with CKD and T2DM. Unlike conventional steroidal MRAs such as spironolactone and eplerenone, finerenone possesses a bulky dihydronaphthyridine core that binds with higher selectivity to the mineralocorticoid receptor, thereby reducing the incidence of adverse effects such as hyperkalaemia and gynaecomastia. Given its increasing clinical use, the development of validated analytical methods for assay determination, dissolution monitoring, and impurity profiling is essential. This review provides a critical appraisal of the chromatographic strategies reported for the quantification of finerenone.

## 2. DRUG PROFILE OF FINERENONE

Finerenone is a synthetic, non-steroidal dihydronaphthyridine derivative. Its principal physicochemical characteristics, consistent with compendial descriptions<sup>8,9</sup>, are summarised below:

- Chemical name: (4S)-4-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide
- Molecular formula: C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>3</sub>
- Molecular weight: 378.43 g/mol
- Mechanism of action: highly selective antagonist of the mineralocorticoid receptor, counteracting inflammation and fibrosis driven by pathogenic aldosterone overactivation
- Chromatographic character: an aromatic core with distinct chromophores, yielding a UV absorbance profile typically monitored between 219 nm and 252 nm; its hydrophobic structure imparts adequate retention on alkyl-bonded silica phases (C18 or C8)<sup>5,6,7</sup>

## 3. PRINCIPLE OF RP-HPLC

RP-HPLC operates on the principle of differential partitioning of analytes between a non-polar stationary phase and a polar mobile phase. The stationary phase typically consists of spherical silica particles surface-functionalised with hydrophobic hydrocarbon chains, most commonly octadecylsilane (C18). The mobile phase comprises a binary or ternary mixture of polar solvents (e.g., water, aqueous buffers) and organic modifiers (e.g., acetonitrile, methanol)<sup>2,4</sup>.

During elution, separation is governed by hydrophobic interactions: molecules with higher lipophilicity are retained more strongly on the stationary phase, whereas more polar analytes elute earlier<sup>2,4</sup>. The principal advantages that



justify the use of RP-HPLC for finerenone analysis include:

- High resolution — capable of resolving the parent drug from structurally related synthetic intermediates or degradation products<sup>5,6,7</sup>
- Linearity and sensitivity — high precision when coupled with UV/visible or photodiode array (PDA) detectors across a wide concentration range<sup>1</sup>
- Matrix compatibility — efficient handling of excipient matrices in solid oral dosage forms or complex biological fluids

#### 4. INSTRUMENTATION OF HPLC

The instrumentation used for finerenone analysis comprises several high-pressure modules optimised for continuous, reproducible operation<sup>2,4</sup>:

##### 4.1 SOLVENT RESERVOIR

Holds HPLC-grade solvents, typically acetonitrile, methanol, or aqueous buffers such as 0.1% orthophosphoric acid.

##### 4.2 DEGASSING SYSTEM

An online vacuum degasser removes dissolved oxygen and micro-bubbles, preventing baseline noise and pressure fluctuations.

##### 4.3 High-Pressure Pump

Isocratic or gradient quaternary/binary pumps deliver a precise solvent stream at pressures up to 400–600 bar (or up to 1000 bar for UHPLC configurations).

##### 4.4 Sample Injector

Automated loop injectors (autosamplers) deliver precise injection volumes, typically 10–20 µL.

##### 4.5 Chromatographic Column

The stationary-phase housing (e.g., 150 mm or 250 mm × 4.6 mm; 5 µm particle size) is temperature-controlled within a column oven to ensure retention-time reproducibility.

##### 4.6 Detector

UV-visible or PDA detectors are configured to target the primary absorption maxima of finerenone.

##### 4.7 Data Processing System

Chromatography data software (CDS; e.g., Empower, ChemStation) enables electronic integration of peaks, peak-purity profiling, and system suitability calculations.

#### 5. System Suitability Parameters

Before any analytical run intended for regulatory batches is executed, system suitability test (SST) parameters must be assessed to confirm that the instrument configuration is operating within validated limits<sup>1,8</sup>. Key indicators include:

##### 5.1 Retention Time (Rt)

The time elapsed between injection and maximum peak response, monitored to verify system equilibration and flow consistency.

##### 5.2 Theoretical Plates (N)

A measure of column efficiency, calculated using the standard equations:

$$N = 16 (t_R / W)^2 \text{ or } N = 5.54 (t_R / W_{0.5})^2$$



Pharmacopoeial specifications require N to exceed 2000<sup>8,9</sup>.

### 5.3 Tailing Factor (T)

Quantifies peak asymmetry and is calculated at 5% of peak height:

$$T = W_{0.05} / 2f$$

An ideal Gaussian peak has  $T = 1.0$ . The regulatory-acceptable value for finerenone is  $T \leq 2.0$ <sup>1,8</sup>.

### 5.4 Resolution (Rs)

Evaluates the separation between the analyte peak and adjacent peaks (impurities or degradants):

$$R_s = 2(t_{R2} - t_{R1}) / (W_1 + W_2)$$

Complete baseline resolution requires  $R_s \geq 1.5$ <sup>1,8</sup>.

### 5.5 Percentage Relative Standard Deviation (%RSD)

Evaluates injection precision across  $n = 5$  or 6 replicate standard injections; the %RSD of peak areas must not exceed 2.0%<sup>1</sup>.

## 6. Method Development for Finerenone

Method development involves systematically optimising mobile-phase chemistry and stationary-phase architecture to achieve an optimal chromatographic run.

### 6.1 Column Selection

The non-steroidal structure of finerenone favours separation on hydrophobic bonded-silica phases. Silanol end-capping is preferred to minimise peak tailing arising from secondary interactions with residual acidic silanol sites. Columns reported in the literature include<sup>5,6,7</sup>:

- Phenomenex Luna C18 / Phenomenex C18 ( $250 \times 4.6$  mm, 5  $\mu$ m) — provides good steric selectivity and carbon loading<sup>5,7</sup>
- Waters Sunfire C18 ( $250 \times 4.6$  mm, 5  $\mu$ m) — delivers symmetrical peak shapes for basic compounds under acidic conditions<sup>6</sup>
- Symmetry ODS C18 — offers reproducible retention profiles

### 6.2 Mobile Phase Optimisation

The choice of mobile phase affects ionisation state, retention time, and peak resolution<sup>5,6,7</sup>:

- Aqueous modifiers — water or weak acidic/basic buffers (e.g., 0.1% v/v orthophosphoric acid, or triethylamine-adjusted neutral pH) maintain ionisation consistency and reduce tailing<sup>6,7</sup>
- Organic modifiers — acetonitrile (ACN) is generally preferred over methanol owing to its lower viscosity and lower UV cut-off, giving sharper peaks and shorter run times<sup>2,4</sup>

### 6.3 Detection Wavelength

Spectrophotometric profiling of finerenone shows maximum absorption at defined UV wavelengths. PDA screening allows selection of the optimal monitoring wavelength<sup>6,7</sup>:

- 219 nm — optimised for maximum sensitivity in bulk drug and simple dosage-form assays<sup>6</sup>
- 252 nm — suited to stability-indicating assays, minimising baseline interference from degradation products or excipient bands<sup>7</sup>

### 6.4 Flow Rate Optimisation

Flow rates are generally optimised between 0.8 and 1.0 mL/min to balance column back-pressure against resolution and total run time<sup>5,6,7</sup>.

## 7. Validation According to ICH Guidelines



To demonstrate suitability for regulatory and quality-control use, an RP-HPLC method must be validated in accordance with ICH Q2(R1/R2)<sup>1,10</sup>.

### 7.1 Specificity

The method must demonstrate that finerenone is clearly resolved from excipients, synthesis impurities, and forced-degradation products, with no co-elution at the target retention time. PDA peak-purity profiling confirms that the analyte peak represents a single, homogeneous component<sup>1,7</sup>.

### 7.2 Linearity

Linearity is established by analysing a series of standard solutions across a predefined working range (e.g., 8–30 µg/mL). The correlation coefficient ( $r^2$ ) should consistently be  $\geq 0.999$ , confirming a directly proportional relationship between concentration and peak area<sup>1,5,6,7</sup>.

### 7.3 Accuracy

Accuracy is evaluated by recovery studies using the standard-addition method at three levels (50%, 100%, and 150%). Mean percentage recovery should fall within the accepted range of 98.0–102.0%<sup>1,5,6,7</sup>.

### 7.4 Precision

- Repeatability (system/method precision) — six successive injections of a uniform test sample should give a %RSD of peak areas  $\leq 2.0\%$ <sup>1</sup>
- Intermediate precision — assessed through intra-laboratory variation, such as different days, instruments, or analysts<sup>1</sup>

### 7.5 Robustness

Robustness measures an assay's tolerance to small, deliberate variations in operating parameters, confirming its reliability during routine use. Parameters typically examined include flow rate ( $\pm 0.1$  mL/min), mobile-phase organic ratio ( $\pm 2\%$ ), and column-oven temperature ( $\pm 3$  °C)<sup>1,5,6,7</sup>.

### 7.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ define the minimum sensitivity of the method and are derived from the slope (S) of the calibration curve and the standard deviation of the blank response ( $\sigma$ ):

$$\text{LOD} = 3.3\sigma / S \quad \text{and} \quad \text{LOQ} = 10\sigma / S$$

These parameters are estimated in accordance with ICH Q2(R1/R2) recommendations<sup>1</sup>.

## 8. Stability-Indicating Methods (SIM)

A stability-indicating method (SIM) is an analytical assay capable of accurately quantifying the active pharmaceutical ingredient (API) without interference from degradation products, process impurities, or excipients. To validate a SIM for finerenone, forced-degradation studies are performed under defined stress conditions to generate representative degradation products<sup>1,6,7</sup>:

- Acid hydrolysis — exposure to 0.1–1.0 M HCl at elevated temperature<sup>6,7</sup>
- Alkaline hydrolysis — exposure to 0.1–1.0 M NaOH<sup>6,7</sup>
- Oxidative degradation — treatment with 3–30% H<sub>2</sub>O<sub>2</sub> solution<sup>6,7</sup>
- Thermal stress — dry heat exposure of the drug substance or formulation matrix (60–105 °C)<sup>6,7</sup>
- Photolytic degradation — exposure to UV and visible light in accordance with ICH Q1B<sup>1,7</sup>



The resulting chromatograms should demonstrate that all degradation products resolve cleanly from the intact finerenone peak, confirming the assay's suitability for shelf-life evaluation and stability-batch analysis<sup>6,7</sup>.

## 9. Analytical Quality by Design (AQbD)

Analytical Quality by Design (AQbD) is a modern, risk-based approach to method development that moves away from traditional trial-and-error optimisation. AQbD aligns with the ICH Q14 framework for analytical procedure development and establishes a systematic, multi-dimensional understanding of method performance.

Key phases of AQbD application:

- Analytical Target Profile (ATP) definition — establishing predefined requirements, such as a finerenone retention time within 5 minutes and a tailing factor < 1.5

- Critical Analytical Attributes (CAAs) — selecting core response measures, including resolution (Rs), theoretical plates (N), and tailing factor (T)
- Risk assessment — using Ishikawa (fishbone) diagrams and Failure Mode and Effects Analysis (FMEA) to identify high-risk operational inputs such as mobile-phase pH, organic-modifier ratio, and flow rate
- Design of Experiments (DoE) — applying statistical designs (e.g., Central Composite Design or Box–Behnken Design) to build mathematical models of the operational space
- Method Design Space (Method Operable Design Region, MODR) — defining the multi-dimensional zone of operating parameters within which method performance is statistically assured, providing regulatory flexibility for minor post-approval changes

## 10. Comparative Review of Reported Methods

**Table 1 summarises the RP-HPLC methods reported for finerenone to date.**

| Author (Reference)             | Stationary Phase                        | Mobile Phase                                 | Wavelength | Retention Time (Rt) |
|--------------------------------|-----------------------------------------|----------------------------------------------|------------|---------------------|
| Nachimuthu et al. <sup>5</sup> | Phenomenex Luna C18                     | Water : Acetonitrile                         | PDA        | 7.3 min             |
| Rukhsar <sup>6</sup>           | Waters Sunfire C18 (250 × 4.6 mm, 5 µm) | 0.1% OPA : Methanol (60:40 v/v)              | 219 nm     | 2.26 min            |
| Marie et al. <sup>7</sup>      | Phenomenex C18 (250 × 4.6 mm, 5 µm)     | Water : ACN : TEA (450:550:10 v/v/v), pH 7.0 | 252 nm     | 4.43 min            |
| Santhosh et al. <sup>†</sup>   | Waters Symmetry ODS C18                 | Methanol : Ammonium acetate buffer           | 235 nm     | 3.0 min             |

Table 1: Comparative summary of reported RP-HPLC methods for finerenone. †Full citation for this entry could not be matched to the supplied reference list — please provide the source so it can be added as a numbered reference (see note at end of References).

Most reported methods demonstrate good sensitivity, acceptable peak symmetry, short retention times, stability-indicating capability, and high reproducibility<sup>5,6,7</sup>.

## 11. Future Perspectives



The analytical lifecycle of finerenone is evolving toward greater efficiency and environmental sustainability:

- Ultra-High-Performance Liquid Chromatography (UHPLC/UPLC) — transitioning to sub-2  $\mu\text{m}$  stationary phases to reduce run times to under two minutes, lowering solvent consumption and increasing throughput<sup>2,4</sup>
- Green Analytical Chemistry (GAC) — replacing hazardous organic modifiers with eco-friendly alternatives (e.g., ethanol or biodegradable surfactants) and maximising Analytical Greenness (AGREE) scores
- Hyphenated LC-MS/MS techniques — coupling liquid chromatography with tandem mass spectrometry for ultra-trace identification of degradation pathways and bioanalytical/pharmacokinetic mapping
- Automation and AI-driven software — predicting retention behaviour and systematically defining the Method Operable Design Region (MODR)

## 12. CONCLUSION

Finerenone represents a significant advance in the management of cardio-renal complications associated with type 2 diabetes mellitus. Ensuring its analytical quality through validated testing protocols is therefore essential. This review highlights that RP-HPLC continues to serve as the benchmark methodology for finerenone analysis in both industrial quality-control laboratories and research settings<sup>1,5,6,7</sup>.

The available literature indicates that current methods successfully balance resolution, sensitivity, and run-time while complying with standard validation criteria. Going forward, the integration of modern stability-indicating assays, Analytical Quality by Design (AQbD)

frameworks, and green chromatography metrics is expected to further improve the regulatory robustness and sustainability of these methods.

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12. [Optional] ICH Q14: Analytical Procedure Development — cited conceptually in Section 9 (AQbD) but not present in the original

reference list; add if you wish to formally cite the guideline.

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