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REVIEW ARTICLE
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A SYSTEMATIC REVIEW ON ANALYTICAL METHOD FOR THE ESTIMATION OF FINERENONE AND EMPAGLIFLOZIN

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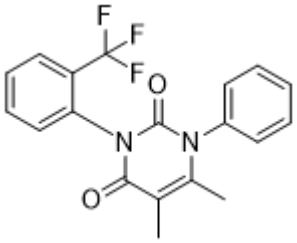
Abstract:
This review focuses on analytical methods for finerenone, a non-steroidal mineralocorticoid receptor antagonist, and empagliflozin, a selective SGLT2 inhibitor, used in the management of type 2 diabetes mellitus with chronic kidney disease. Accurate estimation of these drugs is essential for pharmacokinetics, clinical monitoring, and quality control. Finerenone is mainly analyzed using chromatographic techniques such as HPLC, UHPLC, and LC–MS/MS, while empagliflozin is frequently estimated by spectrophotometric, chromatographic, and hyphenated methods, with LC–MS/MS being the most sensitive in biological matrices. This review critically evaluates reported analytical approaches, comparing their applicability, advantages, and limitations, and highlights the future scope for developing robust, cost-effective, and regulatory-compliant methods.

Keywords: Finerenone, Empagliflozin, T2DM, CKD, Analytical method, HPLC, LC – MS/MS, Spectrophotometry, Bioanalytical method.

Introduction: Chronic kidney disease (CKD) affects more than 850 million people worldwide and is strongly associated with increased morbidity, premature mortality, and a reduced quality of life [1,2]. People suffering from both chronic kidney disease and type 2 diabetes (T2D) experience higher mortality rates than those diagnosed with T2D alone [3]. Chronic kidney disease is diagnosed when there is a sustained reduction in eGFR to less than 60 mL/min/1.73 m², or when UACR is 30 mg/g or above, for a period of at least three months [4].
The American Diabetes Association and the 2024 kidney disease: Improving Global Outcomes (KDIGO) guideline recommend a number of medications to lower the risk of chronic kidney disease (CKD). These medications include sodium-glucose cotransporter 2 inhibitors (SGLT2is), renin angiotensin system inhibitors, finerenone, a selective nonsteroidal mineralocorticoid receptor antagonist, and a glucagon-like peptide-1 receptor agonist (GLP-1 R62A), which has been shown to be beneficial in CKD and T2D populations [5,6]. Empagliflozin successfully reduces the incidence of primary composite cardiorenal events, which are defined as the development of kidney disease or cardiovascular mortality, in chronic kidney disease, according to evidence from many large placebo-controlled outcome trials [7.8]. The potential additive benefits of combining finerenone with sodium–glucose cotransporter-2 inhibitors (SGLT2is) on renal and cardiovascular outcomes in patients with

type 2 diabetes are supported by their distinct yet complementary mechanisms of action.[9] This concept is further reinforced by findings from the FIDELITY analysis, a pooled pharmacokinetic–pharmacodynamic evaluation of the FIGARO-DKD and FIDELIO-DKD trials, where Eissing et al. demonstrated that the combination of finerenone and an SGLT2i was associated with enhanced reductions in urinary albumin-to-creatinine ratio (UACR) and attenuation of chronic eGFR decline compared to either treatment alone.[10] Evidence from the **EMPA-REG OUTCOME trial** highlighted that empagliflozin reduced major cardiovascular events, delayed CKD progression, and lowered renal event rates in patients with T2D and elevated CV risk. Complementing this, the **EMPEROR-Reduced trial** confirmed its efficacy in reducing cardiovascular death and heart failure hospitalization, as well as in mitigating kidney function decline in HFrEF patients independent of diabetes status. [11,12,13]. We predicted that a combination of finerenone, and empagliflozin would lower the urine albumin-to-creatinine ratio more than either medication alone in persons with type 2 diabetes and chronic kidney disease. Additionally, we monitored adverse events and measured blood pressure, serum potassium levels, and estimated glomerular filtration rate (eGFR) at regular predetermined intervals to assess the safety of starting these two medications at the same time. [14,15]

1. Finerenone:



Chemical profile:

- (4-(3-(Cyclopentylmethyl)-7-(5-methylpyrazole-1-yl)-3H-pyrrolo[3,2-c] pyridin-4-yl)-5-methylthiazol-2-yl) carbonitrile
- **Molecular Formula:** C21H22N6OS
- **Molecular Weight:** 400.51 g/mol
- **Chemical Class:** Non-steroidal mineralocorticoid receptor antagonist (MRA)
- **Structure Description:** Finerenone is a **non-steroidal dihydropyridine derivative** featuring a pyrrolopyridine backbone and thiazole ring, designed to selectively bind and block mineralocorticoid receptors with high potency.[16]
- **Physicochemical Properties:**

○ Appearance: White to off-white crystalline powder
○ Solubility: Slightly soluble in aqueous solutions; soluble in organic solvents like DMSO and ethanol [17]
○ LogP: ~3.3 (moderate lipophilicity)

- **Pharmacological Profile**
- **Mechanism of Action:** Finerenone selectively antagonizes **mineralocorticoid receptors (MRs)**, inhibiting MR-mediated transcription of pro-inflammatory and pro-fibrotic genes in renal and cardiovascular tissues. Its **non-steroidal structure** allows greater receptor selectivity and balanced distribution between kidney and heart tissue compared to steroidal MRAs like spironolactone. [18]
- **Pharmacodynamics:**

○ Reduces inflammation and fibrosis in renal and cardiovascular tissues.
○ Lowers urinary albumin-to-creatinine ratio (UACR), slowing CKD progression.
○ Exhibits lower risk of hyperkalemia compared to traditional MRAs.[19]

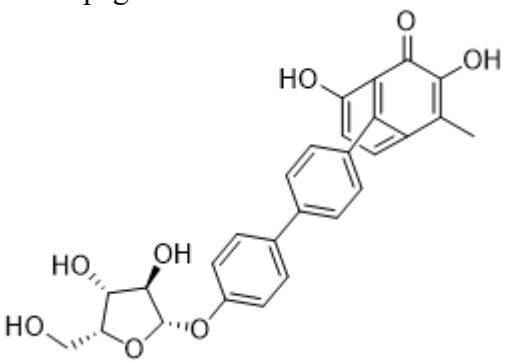
- **Pharmacokinetics:**

○ **Absorption:** Oral bioavailability ~43%; Tmax ~0.5–1.25 h.
○ **Distribution:** ~90% plasma protein bound; Vd ~52 L.
○ **Metabolism:** Extensively metabolized by **CYP3A4**; minimal renal excretion.

- **Elimination half-life:** ~2 h (parent), but pharmacodynamic effects persist longer.
- **Excretion:** ~80% via feces, ~20% via urine (mostly as metabolites).[20]

- **Therapeutic Indications:** Approved for reducing the risk of sustained eGFR decline kidney failure, CV death, nonfatal MI, and hospitalization for heart failure in CKD associated with T2D. [21]

2. Empagliflozin:



Chemical Profile

- **IUPAC Name:**

(2S,3R,4R,5S,6R)-2-[4-Chloro-3-[(4-ethoxybenzyl)oxy]phenyl]-6(hydroxymethyl)oxane-3,4,5-triol

- **Molecular Formula:** C23H27ClO7
- **Molecular Weight:** 450.91 g/mol
- **Chemical Class:** Sodium-glucose co-transporter-2 (SGLT-2) inhibitor
- **Structure Description:** Empagliflozin is a **C-glucoside derivative** with high selectivity for SGLT-2 transporters over SGLT-1, making it highly effective in reducing renal glucose reabsorption.[22]
- **Physicochemical Properties:**
  - Appearance: White to off-white crystalline powder
  - Solubility: Freely soluble in water, ethanol, methanol
  - LogP: ~1.7
  - pKa: ~12.6 (weak acid) [23]

Pharmacological Profile

- **Mechanism of Action:** Empagliflozin **selectively inhibits SGLT-2** in the proximal renal tubules, blocking glucose reabsorption and increasing urinary glucose excretion, thereby lowering plasma glucose levels. It also exerts cardiovascular and renal benefits independent of glycemic control, likely through improved hemodynamics, natriuresis, and reduced oxidative stress.[24]
- **Pharmacodynamics:**
  - Reduces fasting and postprandial glucose.
  - Lowers HbA1c, systolic blood pressure, and body weight.
  - Provides cardio-renal protection through hemodynamic effects.[25].
- **Pharmacokinetics:**
  - **Absorption:** Oral bioavailability ~78%; Tmax ~1.5 h.
  - **Distribution:** Plasma protein binding ~86%.
  - **Metabolism:** Mainly via **UGT2B7, UGT1A9** (minor CYP involvement).
  - **Elimination half-life:** ~12 h.
  - **Excretion:** ~54% in urine, ~41% in feces (as metabolites and unchanged drug).[26]
- **Therapeutic Indications:** Used in type 2 diabetes for glycemic control, reduction of major adverse cardiovascular events, and to slow progression of CKD.[27]

Detailed Overview of Analytical Techniques for Finerenone and Empagliflozin

Accurate estimation of Finerenone and Empagliflozin in pharmaceutical formulations and biological matrices is critical for drug development, quality control, pharmacokinetic studies, and therapeutic drug monitoring. Due to their distinct physicochemical properties—Finerenone's moderate lipophilicity and Empagliflozin's hydrophilic glucose moiety—different analytical approaches have been developed. These techniques can be broadly classified into spectrophotometric, chromatographic, and hyphenated analytical methods, each with unique advantages and limitations. [28,29]

1. **UV-Visible Spectrophotometry**

- **Principle:** Drug molecules with chromophoric groups absorb UV or visible light at specific wavelengths.[30]
- **Applications:**
  - Widely used in the preliminary estimation of Empagliflozin in bulk drugs and formulations.
  - Involves methods like zero-order, first-derivative, and ratio derivative spectrophotometry to improve accuracy.
  - Finerenone's aromatic structure and nitrogen heterocycles also make it amenable to UV detection. [31,32]
- **Advantages: Simple, inexpensive, requires minimal sample preparation.**
- Limitations: Low sensitivity (LOD typically >0.5 µg/mL), poor selectivity in biological matrices; not suitable for pharmacokinetic studies.[33]

2. **High-Performance Liquid Chromatography (HPLC)**

- **Principle:** Separation based on polarity and hydrophobic interactions between analytes and stationary phases.[34]
- Column & Mobile Phases:
  - RP-HPLC with C18 columns is most common.
  - Mobile phases often consist of acetonitrile or methanol with water/buffer; pH adjustments enhance peak resolution.[34]
- Detection:
  - UV/Photodiode Array (PDA) detectors are widely used.
  - Detection wavelengths: ~244–290 nm for Empagliflozin; ~260–280 nm for Finerenone.[34,35]
- Applications:
  - Quantification in raw materials, tablets, and stability studies.
  - Simultaneous estimation in fixed-dose combinations (FDCs) with other antidiabetic agents (e.g., Metformin, Linagliptin).[36]
- **Advantages:** High reproducibility, validated methods compliant with **ICH Q2(R1)**.
- **Limitations:** Lower sensitivity than LC–MS/MS; time- and solvent-intensive. [36]

3. **Ultra-Performance Liquid Chromatography (UPLC)**

- **Principle:** Similar to HPLC but uses smaller particle-size columns (sub-2 µm), enabling higher efficiency and shorter run times.[37]
- Applications:
  - Simultaneous quantification of Finerenone and Empagliflozin in formulations with enhanced throughput.
  - Suitable for stability-indicating studies under forced degradation conditions (acid/base hydrolysis, oxidation, photolysis). [37,38]
- **Advantages:** High resolution, shorter analysis time, and reduced solvent consumption.
- **Limitations:** Higher cost of instrumentation. [38]

4. Liquid Chromatography–Mass Spectrometry (LC–MS/MS)

- **Principle:** Combines chromatographic separation with mass-based detection for superior sensitivity and selectivity.[39]
- **Applications:**

○ Pharmacokinetic (PK) and bioequivalence (BE) studies in plasma and urine samples.
○ Detection limits in the ng/mL range (LOD ~0.1–1 ng/mL), crucial for Finerenone due to its lower plasma concentrations.
○ Empagliflozin quantified for therapeutic drug monitoring and drug- drug interaction studies. [39]

- **Advantages:** High sensitivity, specificity, and capability to identify metabolites and impurities.
- **Limitations:** Requires sophisticated instrumentation and expertise; cost- intensive. [39,40]

5. Stability-Indicating Assays (SIAs)

- Developed to differentiate intact drugs from degradation products, crucial for regulatory submissions.
- **ICH Q1A(R2)**-guided stress studies for Finerenone and Empagliflozin often include:

○ Acidic/Alkaline hydrolysis (acid/base degradation).
○ Oxidative degradation (H2O2).[40]
○ Photolytic and thermal degradation studies.

HPLC and UPLC are most widely employed for SIAs; degradation peaks are resolved with gradient elution methods. [40,41]

Simultaneous estimation ready to use methods and key precedents

1) Finerenone + Empagliflozin (RP-HPLC, tablets/synthetic mix) practical, QC- friendly

- **Technique/Detector:** RP-HPLC / UV (245 nm)
- **Column:** Hypersil BDS C18, 250 × 4.6 mm, 5 µm
- **Mobile phase (isocratic):** Methanol: Water: TFA (68:32:0.05, v/v)
- **Flow / Temp:** 1.0 mL min^{-1} / ambient
- **tR (min):** ~3.2 (finerenone), ~4.6 (empagliflozin)

Performance (validation per ICH): $r^2 \approx 0.99999$ (FINE), 0.99995 (EMPA); LOD/LOQ (µg mL^{-1}): FINE 0.03/0.09; EMPA 0.27/0.81; assay ~97–101% on lab- prepared tablets; system suitability within limits. Suitable for routine QC and assay.[42]

2) Finerenone + Empagliflozin (UV spectrophotometry, synthetic mixture) low- cost screening

- **Technique:** UV (zero-order/derivative approach reported)
- **Use case:** Rapid simultaneous estimation in **synthetic mixtures** with acceptable accuracy (≈98–101%) and ICH-compliant validation (linearity/precision/robustness). Good as a **pre-HPLC screen** or where resources are limited.[43]

3) Empagliflozin + Metformin (UPLC-DAD, stability-indicating, tablets) fast, robust

- **Technique/Detector:** RP-UPLC / PDA
- **Column:** C18, 2.1 × 50 mm, 1.8 µm
- **Mobile phase:** 40% phosphate buffer pH 3: 60% acetonitrile
- **Flow:** 0.6 mL min^{-1}; **λ:** 248 nm[44]

4) Empagliflozin + Metformin (RP-HPLC, tablets) — widely used alternative

- **Typical conditions from multiple reports:** C18 (150–250 mm), 0.1% OPA buffer: ACN ≈ 50:50, ~1.0 mL min^{-1}, λ 210–260 nm; validated for linearity, precision, robustness; stability-indicating variants available. **Good transferability** across labs. [44;45]

5) Empagliflozin + Linagliptin (RP-HPLC, tablets) — fixed-dose combo precedent

- **Technique:** RP-HPLC / UV or DAD
- **Use case:** Routine assay of the marketed Empa/Lina FDC; several validated procedures exist (tablets). Handy when building ternary **methods** that add metformin.[46]

6) Empagliflozin + Linagliptin (LC-MS/MS, human/rat plasma) bioanalytical PK/BE

- **Technique:** LC–MS/MS (ESI+, deuterated IS)
- **Example conditions:** C8 column; ACN–ammonium chloride mobile phase (≈55:45); linear over ~1.5–500 ng mL^{-1} (EMPA) and 0.05–7 ng mL^{-1} (LINA); CV < ~3.7%. Fit for **BE/PK** and TDM-style work. [47,48]

7) . Empagliflozin + Linagliptin + Metformin (RP-UPLC, stability-indicating, tablets) ternary benchmark

- **Technique/Detector:** RP-UPLC / PDA
- **Column:** C18, 2.1 × 50 mm, 1.8 µm
- **Mobile phase:** 40% phosphate buffer pH 3: 60% ACN; **λ:** 248 nm
- **Quantitation ranges:** ~50–150 µg mL^{-1} (MET), 5–15 (LINA), 10–30 (EMPA).
- **Notes:** Full ICH validation and forced degradation; excellent platform to model **resolution/retention** behavior of glucoside + biguanide + DPP-4 inhibitor systems. [44,49,50]

Combination	Matrix	Technique	Core conditions (summary)	Description
Finerenone + Empagliflozin	Tablets	**RP-HPLC/UV**	C18 250×4.6 mm; MeOH:Water:TFA 68:32:0.05; 1.0 mL min^{-1}; **245 nm**; tR ≈ 3.2/4.6 min	r^2≈0.99999/0.99995; LOD/LOQ (µg mL^{-1}) FINE 0.03/0.09, EMPA 0.27/0.81; assay ≈97–101%; ICH compliant. [42]
Finerenone+Empagliflozin	Synthetic mix	**UV SPECTRO**	Zero-ordre /derivative based; dual-drug calibration	Accuracy ≈98101%; ICH parameters acceptable; economical screen. [43]
Empagliflozin + Metformin	Tablets	**RP-UPLC/PDA**	C18 2.1×50 mm; buffer pH 3: ACN 40:60; 0.6 mL min^{-1}; **248 nm**	Rapid run time; robust linear ranges; low LOQs. [44]
Empagliflozin+ Metformin	Tablets	**RP-HPLC/UV**	C18 150–250 mm; 0.1% OPA: ACN ≈ 50:50; ~1.0 mL min^{-1}; 210–260 nm	Multiple validated reports; good robustness & transferability. [44,45]
Empagliflozin + linagliptin	Tablets	**RP-HPLC/UV**	Standard C18; isocratic ACN/buffer; λ 225– 250 nm range	FDC assay methods; routine QC usage. [46]
Empagliflozin + linagliptin	Human /Rat plasma	**LC-MS/MS**	C8; ACN–ammonium salt mobile phase; ESI+; deuterated IS	Linear ~1.5–500 ng mL^{-1} (EMPA), 0.05–7 ng mL^{-1} (LINA); PK/BE-Ready. [47,48]
Empagliflozin + linagliptin +Metformin	Tablets	**RP-UPLC/PDA**	C18 2.1×50 mm; buffer pH 3: ACN 40:60; **248 nm**	Full ICH validation; ternary separation template. [49]
Finerenone	Bulk drug (API)	**RP-HPLC**	C18; Methanol: phosphate buffer **220-225nm**	tR(min)~4.43 robust linear ranges; low LOQs. [50]

Finerenone	Tablet	**RP-HPLC**	C18(250mm × 4.6mm); ACN: Water **225nm**	tR(min)~9-10 Multiple validated reports; good robustness. [51]
Empagliflozin	Plasma	**LC-MS/MS**	C18 +MS Detector Volatile buffer: ACN MS(MRM)	Linear ~1.5–500 ng mL^{-1} (EMPA), 0.05–7 ng mL^{-1}. [52]
Empagliflozin	Tablets	**RP-HPLC**	C18 ACN: Water 222nm	tR(min) ~6 outline assay of the marketed Empa FDC; several validated procedures exist (tablets).[53]

Discussion:

The increasing clinical use of finerenone, a novel non-steroidal mineralocorticoid receptor antagonist, in combination with empagliflozin, a widely used SGLT2 inhibitor, has highlighted the need for robust, sensitive, and validated analytical methods for their quantification in both pharmaceutical formulations and biological matrices. This systematic review indicates that chromatographic techniques primarily RP-HPLC, UPLC, and LC–MS/MS remain the gold standard for estimation due to their high sensitivity, reproducibility, and specificity. [44,50,54]

RP-HPLC and UPLC methods have demonstrated excellent linearity, precision, and accuracy in the assay of finerenone and empagliflozin, whether in bulk drug, tablet dosage forms, or multi-drug combinations. Stability-indicating methods are especially critical in identifying degradation products under stress conditions (acidic, basic, oxidative, thermal, photolytic), ensuring the safety, efficacy, and shelf-life of pharmaceutical formulations. Moreover, simultaneous estimation methods allow accurate quantification of multiple APIs in combination therapies, which is increasingly relevant given the emerging trend of fixed-dose combinations (FDCs) in managing CKD and T2D. [55,56,11]

LC–MS/MS methods, particularly bioanalytical techniques, provide the highest sensitivity for plasma and urine quantification, supporting pharmacokinetic studies, therapeutic drug monitoring, and bioequivalence assessments. These methods are indispensable for personalized medicine applications, especially in populations with renal impairment, where dose optimization is critical. The integration of high-resolution and hyphenated techniques (LC–HRMS, LC–NMR) has further enhanced the ability to detect low-level impurities, metabolites, and degradation products, meeting stringent regulatory requirements. [39,54]

Despite these advancements, several gaps remain. Current research emphasizes single-drug assays or binary combinations, whereas real-world clinical regimens often involve ternary or quaternary combinations (e.g., finerenone + empagliflozin + metformin). Future work should focus on high-throughput**, eco-friendly, and multi-analyte analytical methods** capable of resolving complex mixtures while adhering to ICH and pharmacopeial guidelines. Additionally, integration of automation, artificial intelligence, and point-of-care analytical platforms could revolutionize therapeutic drug monitoring and personalized therapy. [57,58,59]

In terms of clinical translation, validated analytical methods are critical not only for drug development and quality control but also for monitoring patient adherence, correlating plasma drug levels with biomarkers (e.g., UACR, eGFR), and evaluating the efficacy and safety of combination therapies. As regulatory expectations evolve, harmonized global standards and robust method validation will be essential for facilitating the approval of new formulations and fixed-dose combinations. [56,59,60]

Conclusion:

Analytical methods for finerenone and empagliflozin play a pivotal role across the drug development and clinical continuum from quality control and stability studies to pharmacokinetic analysis and therapeutic monitoring. RP-HPLC, UPLC, UV spectrophotometry, and LC–MS/MS techniques have been successfully applied for single-drug and combination assays, providing high sensitivity, specificity, and reproducibility.[56]

Simultaneous estimation methods are particularly valuable in the context of multi-drug therapies, enabling efficient quality control and regulatory compliance. Furthermore, advances in high-

resolution, automated, and environmentally sustainable analytical techniques hold promise for future-proofing drug monitoring, supporting personalized medicine, and facilitating the development of fixed-dose combinations.

Overall, the systematic development and validation of analytical methods for finerenone and empagliflozin ensure drug safety, efficacy, and quality, while supporting the ongoing evolution of therapeutic strategies for T2D and CKD. Future research should continue to emphasize multi- analyte assays, green analytical chemistry, and translational applications, ultimately improving patient outcomes and optimizing clinical practice. [61,62,63]

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