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Research Paper

A Validated RP-HPLC Method for Simultaneous Estimation of Empagliflozin and Linagliptin in Pharmaceutical Tablet Dosage Form

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ABSTRACT

A simple, rapid, precise, and validated Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed and validated for the simultaneous estimation of Empagliflozin (EMPA) and Linagliptin (LINA) in combined pharmaceutical tablet dosage form. Chromatographic separation was achieved on a Shimadzu Shim-pack CLC-ODS C18 column (250 × 4.6 mm, 5 µm) using Acetonitrile:0.1% Orthophosphoric acid (60:40 v/v, pH 3.0) as the isocratic mobile phase at a flow rate of 1.0 mL/min. Detection was carried out at 254 nm with an injection volume of 10 µL. Empagliflozin and Linagliptin eluted at retention times of approximately 2.1 and 3.5 minutes, respectively, within a total run time of 10 minutes.The method was validated as per ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision (intraday and inter-day), limit of detection (LOD), limit of quantification (LOQ), and robustness. Linearity was established over 25–150 µg/mL (r^2 = 0.9998) for EMPA and 12.5–75 µg/mL (r^2 = 0.9996) for LINA. LOD values were 0.245 µg/mL (EMPA) and 0.126 µg/mL (LINA); LOQ values were 0.743 µg/mL (EMPA) and 0.383 µg/mL (LINA). Precision (%RSD < 1%) and accuracy (mean recovery 97.7–101.4%) conformed to ICH acceptance criteria. The method was successfully applied for the assay of the marketed formulation Glyxambi® (Empagliflozin 10 mg / Linagliptin 5 mg), yielding 101.4% and 97.7% for EMPA and LINA, respectively. The method is suitable for routine quality control analysis of the fixed-dose combination.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is one of the most prevalent chronic metabolic disorders globally, with the International Diabetes

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Federation (IDF) estimating approximately 537 million adults living with diabetes in 2021 — a figure projected to reach 783 million by 2045. The progressive nature of T2DM necessitates multi-drug combination therapy to achieve and sustain optimal glycemic control, driving the development of fixed-dose combinations (FDCs) with complementary mechanisms of action.

Empagliflozin (EMPA), a selective Sodium-Glucose Co-Transporter 2 (SGLT-2) inhibitor, and Linagliptin (LINA), a potent Dipeptidyl Peptidase-4 (DPP-4) inhibitor, represent two mechanistically distinct and complementary antidiabetic drug classes. Their combination in a single tablet (Glyxambi®, Boehringer Ingelheim/Eli Lilly; EMPA 10 mg + LINA 5 mg and EMPA 25 mg + LINA 5 mg) received US FDA approval in February 2015 as the first SGLT-2/DPP-4 inhibitor FDC. SGLT-2 inhibition lowers blood glucose by promoting urinary glucose excretion in an insulin-independent manner, while DPP-4 inhibition attenuates incretin degradation, enhancing postprandial insulin secretion and suppressing the compensatory glucagon elevation induced by SGLT-2 inhibitors.

Accurate and reliable analytical methods for the simultaneous determination of both APIs in the finished dosage form are indispensable for batch release testing, in-process quality control, stability studies, and regulatory compliance. Despite the clinical significance of the Empagliflozin-Linagliptin FDC, the published literature on simultaneous RP-HPLC methods for this combination remains limited. The few available methods exhibit limitations including long run times (>12 min), incomplete robustness evaluation, or lack of comprehensive forced degradation studies.

The present work describes the development and comprehensive ICH Q2(R1)-compliant validation of a simple, rapid, and precise RP-HPLC method for the simultaneous estimation of Empagliflozin and Linagliptin in the combined tablet dosage form, applicable for routine pharmaceutical quality control.

2. DRUG PROFILES

2.1 Empagliflozin (EMPA)

IUPAC Name: D-Glucitol, 1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3-furanyl]oxy]phenyl]methyl]phenyl]
Molecular Formula: $C_{23}H_{27}ClO_7$
Molecular Weight: 450.91 g/mol
Melting Point: 145°C–160°C
Solubility: Very slightly soluble in water; slightly soluble in acetonitrile and ethanol; sparingly soluble in methanol.
Half-Life: ~13.1 hours (once-daily dosing)
Approved Dose: 10 mg and 25 mg once daily

Empagliflozin selectively and potently inhibits SGLT-2 in the proximal renal tubules, which mediates approximately 90% of renal glucose reabsorption. Inhibition of SGLT-2 increases urinary glucose excretion in an insulin-independent manner, reducing plasma glucose levels. Beyond glycemic control, Empagliflozin has demonstrated significant cardiovascular and renoprotective benefits. It is commercially available as Jardiance® (10 mg and 25 mg tablets).

2.2 Linagliptin (LINA)

IUPAC Name: 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione
Molecular Formula: $C_{25}H_{28}N_8O_2$
Molecular Weight: 472.54 g/mol
Melting Point: 190–196°C
Solubility: Soluble in methanol; sparingly soluble in ethanol; very slightly soluble in isopropanol.
Half-Life: ~131 hours (effective DPP-4 inhibition half-life ~12 hours)
Approved Dose: 5 mg once daily (no renal dose adjustment required)

Linagliptin is a xanthine-based DPP-4 inhibitor with picomolar potency (IC_{50} ~1 nM). DPP-4 inhibition prevents rapid deactivation of incretin hormones GIP and GLP-1, enhancing glucose-dependent insulin secretion and suppressing glucagon. Linagliptin is unique among DPP-4 inhibitors in that it undergoes primarily biliary/fecal elimination (~80%) without significant renal excretion, making it safe across all stages of renal impairment without dose modification. It is marketed as Tradjenta® (5 mg tablets).

3. MATERIALS AND METHODS

3.1 Wavelength Selection

UV absorption spectra of Empagliflozin and Linagliptin (10 µg/mL each in ACN:Water, 50:50 v/v) were recorded between 200 and 400 nm using the Shimadzu UV-1800 spectrophotometer. Empagliflozin exhibited maximum absorbance (λmax) at 227 nm, attributable to the π→π* transition of its aromatic 4-chlorophenyl-phenyl glucoside chromophore system, with a secondary shoulder at approximately 280 nm. Linagliptin displayed strong maximum absorbance at 254 nm due to its highly conjugated xanthine-purine ring system combined with the quinazoline substituent. A detection wavelength of 254 nm was selected for simultaneous detection: it provides maximum sensitivity for Linagliptin (λmax) while affording adequate, quantifiable absorbance for Empagliflozin (~35% of its λmax response). The wavelength is also a standard UV-HPLC wavelength with minimal mobile phase background noise.

3.2 Mobile Phase Optimization

Systematic mobile phase optimization was performed through five sequential trials varying the organic modifier type, buffer composition, and pH:

Table 2: Mobile Phase Optimization Trials

Trial	Mobile Phase	Observations
Trial 1	Methanol:Water (60:40 v/v)	Poor peak symmetry for Linagliptin; high backpressure
Trial 2	ACN:Water (60:40 v/v)	Sharp peaks but baseline drift; no pH control
Trial 3	ACN:0.02M KH_2PO_4 pH 3.5 (55:45 v/v)	Good separation (Rs=6.2) but high UV absorbance <250 nm
Trial 4	ACN:Ammonium acetate pH 4.5 (60:40 v/v)	Good peaks but salt precipitation on standing overnight
Trial 5 (Optimized)	ACN:0.1% OPA pH 3.0 (60:40 v/v)	Best peak symmetry (Tf<1.2); Rs>2; run time 10 min

The optimized mobile phase Acetonitrile:0.1% Orthophosphoric acid (60:40 v/v, pH 3.0) provided the best peak symmetry and resolution within a 10-minute run time. The low pH (3.0) effectively suppresses silanol activity on the C18 surface and maintains both analytes in their neutral/protonated forms, ensuring reproducible retention. Acetonitrile was preferred over methanol for its lower UV background below 250 nm and lower viscosity, reducing column backpressure.

3.3 Flow Rate and Column Temperature Optimization

Flow rates of 0.8, 1.0, and 1.2 mL/min were evaluated. At 0.8 mL/min, run time exceeded 13 minutes with decreased peak areas. At 1.2 mL/min, backpressure approached column limits and peaks broadened slightly. A flow rate of 1.0 mL/min yielded optimal peak shape, resolution, and a 10-minute run time within acceptable backpressure (<200 bar). Column temperatures of 25°C, 30°C, and 35°C were also evaluated; 30°C was optimal, providing best peak shapes without excessive temperature-induced changes in selectivity.

3.4 Optimized Chromatographic Conditions

Table 3: Optimized Chromatographic Conditions

Parameter	Condition
Column	Shimadzu Shim-pack CLC-ODS C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	Acetonitrile : 0.1% Orthophosphoric acid (pH 3.0) = 60:40 v/v
Flow Rate	1.0 mL/min (isocratic)
Detection Wavelength	254 nm
Column Temperature	30°C
Injection Volume	10 µL
Diluent	Acetonitrile : Water (50:50 v/v)
Run Time	10 minutes
Retention Time – EMPA	~2.1 min
Retention Time – LINA	~3.5 min

4. METHOD VALIDATION

The developed method was validated in accordance with ICH Q2(R1) guidelines for the following parameters: system suitability, linearity, precision (intraday and interday), accuracy, LOD, LOQ, and robustness.

4.1 System Suitability

System suitability was evaluated by injecting the working standard solution (EMPA 100 µg/mL; LINA 50 µg/mL) six consecutive times under the optimized conditions. Peak areas, %RSD, USP plate count, and tailing factor were recorded.

Table 4: System Suitability Parameters

Parameter	Empagliflozin	Linagliptin	Acceptance Criterion
Retention Time (min)	~2.1	~3.5	—
Average Peak Area	1,661,360	978,068	—
SD	6,645	4,317	—
%RSD	0.4%	0.4%	≤ 2.0%
Resolution (Rs)	>2	>2	≥ 2.0

4.2 Linearity

Linearity was established over six concentration levels: 25–150 µg/mL for Empagliflozin and 12.5–75 µg/mL for Linagliptin (n=3 at each level). Peak areas were plotted against concentrations and linear regression analysis was performed.

Table 5: Linearity Summary

Drug	Linearity Range	Slope	Intercept	R^2
Empagliflozin	25–150 µg/mL	16,792	~0	0.9998
Linagliptin	12.5–75 µg/mL	19,048	~0	0.9996

Both drugs demonstrated excellent linearity with correlation coefficients ≥ 0.9996, well above the ICH acceptance criterion of $R^2 \geq 0.999$, confirming proportional detector response across the entire analytical range relevant to pharmaceutical dosage form analysis.

4.3 Precision

7.3.1 Intraday Precision (Repeatability)

Table 6: Intraday Precision Results (n=6)

S.No	EMPA (%Assay)	LINA (%Assay)
1	101.9	97.9
2	101.2	97.0
3	101.8	97.6
4	101.4	98.2
5	101.5	97.8
6	101.4	97.3
Mean	101.5	97.6
SD	0.27	0.43
%RSD	0.26%	0.44%

4.3.2 Interday Precision (Intermediate Precision)

Table 7: Interday Precision Results (n=6)

S.No	EMPA (%Assay)	LINA (%Assay)
1	101.3	97.5
2	101.7	97.9
3	101.1	97.2

4	101.6	98.1
5	101.4	97.8
6	101.5	97.6
Mean	101.4	97.7
SD	0.22	0.30
%RSD	0.22%	0.31%

%RSD values for both intraday (EMPA: 0.26%; LINA: 0.44%) and interday precision (EMPA: 0.22%; LINA: 0.31%) were well within the ICH acceptance limit of ≤ 2%, demonstrating excellent method reproducibility.

4.4 Accuracy (Recovery Studies)

Accuracy was evaluated at three spiking levels (50%, 100%, 150% of the nominal concentration; n=3 per level) for each drug.

Table 8: Accuracy Studies — Recovery Data

Drug	Level (%)	Amount Added (mg)	Amount Found (mg)	Mean Recovery (%)
Empagliflozin	50	4.97	4.96	99.8
Empagliflozin	100	9.94	9.97	100.3
Empagliflozin	150	14.91	14.92	100.1
Linagliptin	50	2.55	2.55	99.9
Linagliptin	100	5.10	5.11	100.2
Linagliptin	150	7.65	7.68	100.5

Mean recovery values ranged from 99.8% to 100.5% for both drugs, confirming the accuracy of the developed method within the ICH acceptance range of 98–102%.

4.5 LOD and LOQ

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated using the ICH Q2(R1) formula based on calibration curve residual standard deviation (σ) and slope (S): LOD = 3.3σ/S; LOQ = 10σ/S.

Table 9: LOD and LOQ Summary

Analyte	Calibration Range (µg/mL)	Slope (S)	σ	R^2	LOD (µg/mL)	LOQ (µg/mL)
Empagliflozin	5–30	24,850	1,847	0.99998	0.245	0.743
Linagliptin	2.5–15	31,420	1,203	0.99997	0.126	0.383

The low LOD and LOQ values confirm the high sensitivity of the method, making it suitable for detection of trace levels of both analytes including potential degradation products.

4.6 Robustness

Robustness was evaluated by deliberately varying chromatographic parameters within small ranges: flow rate (±0.1 mL/min), mobile phase organic composition (±2%), detection wavelength (±2 nm), and buffer pH (±0.2 units).

Table 10: Robustness Study Results

Parameter	Condition	RT EMPA (min)	RT LINA (min)	%RSD	Rs	Inference
Flow Rate	0.9 mL/min	2.21	3.68	0.72	2.45	Acceptable
Flow Rate	1.0 mL/min (Opt.)	2.05	3.41	0.40	2.60	Optimized
Flow Rate	1.1 mL/min	1.92	3.20	0.68	2.38	Acceptable
Mobile Phase	58:42	2.15	3.55	0.75	2.50	Acceptable
Mobile Phase	60:40 (Opt.)	2.05	3.41	0.40	2.60	Optimized
Mobile Phase	62:38	1.95	3.28	0.70	2.42	Acceptable
Wavelength	252 nm	2.06	3.42	0.65	2.58	Acceptable
Wavelength	254 nm (Opt.)	2.05	3.41	0.40	2.60	Optimized
Wavelength	256 nm	2.05	3.40	0.69	2.57	Acceptable
pH	2.8	2.12	3.48	0.78	2.44	Acceptable
pH	3.0 (Opt.)	2.05	3.41	0.40	2.60	Optimized
pH	3.2	2.00	3.36	0.74	2.48	Acceptable

Under all varied conditions, %RSD values remained below 2%, resolution between EMPA and LINA was consistently maintained above 2.0, and tailing factors were within acceptable limits. These results confirm the robustness of the developed method to small, deliberate changes in chromatographic parameters.

5. ASSAY OF MARKETED FORMULATION

The validated method was applied for the assay of Empagliflozin and Linagliptin in commercially available Glyxambi® tablets (label claim: EMPA 10 mg / LINA 5 mg per tablet). Twenty tablets were weighed and powdered; a quantity equivalent to one tablet weight was extracted with diluent (ACN:Water, 50:50 v/v) by sonication for 15–20 minutes, filtered through 0.45 µm nylon membrane, and appropriately diluted to obtain working concentrations of 100 µg/mL (EMPA) and 50 µg/mL (LINA).

Table 11: Assay of Marketed Formulation (Glyxambi®)

Drug	Label Claim (mg)	Standard Area	Sample Area	% Assay
Empagliflozin	10 mg	1,661,360	1,684,500	101.4%
Linagliptin	5 mg	978,068	955,500	97.7%

Assay values of 101.4% (EMPA) and 97.7% (LINA) are within the pharmacopoeial acceptance range of 95–105% of labelled claim. The results confirm that the tablet formulation contains the drugs at their stated strengths and that the method is suitable for accurate quantification of both APIs in the presence of tablet excipients.

RESULTS SUMMARY

Table 12: Consolidated Validation Results Summary

Validation Parameter	Empagliflozin	Linagliptin	ICH Criterion
Linearity Range (µg/mL)	25–150	12.5–75	Min. 5 levels
Correlation Coefficient (R^2)	0.9998	0.9996	≥ 0.999
Intraday Precision (%RSD)	0.26%	0.44%	≤ 2.0%
Interday Precision (%RSD)	0.22%	0.31%	≤ 2.0%
Mean Accuracy (% Recovery)	99.8–100.3%	99.9–100.5%	98–102%
LOD (µg/mL)	0.245	0.126	S/N ≥ 3:1
LOQ (µg/mL)	0.743	0.383	S/N ≥ 10:1
System Suitability (%RSD)	0.4%	0.4%	≤ 2.0%
Assay of Glyxambi®	101.4%	97.7%	95–105%

CONCLUSION

A validated RP-HPLC method has been successfully developed for the simultaneous determination of Empagliflozin and Linagliptin in their combined pharmaceutical tablet dosage form. The method employs a Shimadzu Shim-pack CLC-ODS C18 column (250 × 4.6 mm, 5 µm) with Acetonitrile:0.1% Orthophosphoric acid (pH 3.0) in a ratio of 60:40 v/v as the isocratic mobile phase at a flow rate of 1.0 mL/min, UV detection at 254 nm, and a total run time of only 10 minutes.

Both drugs were baseline-resolved with retention times of approximately 2.1 min (EMPA) and 3.5 min (LINA) and resolution >2. Comprehensive validation per ICH Q2(R1) demonstrated excellent linearity (R^2 ≥ 0.999), high precision (%RSD <1%), accurate recovery (98–102%), and sensitivity (LOD: 0.126–0.245 µg/mL; LOQ: 0.383–0.743 µg/mL). Robustness studies confirmed method stability under deliberate parameter variations, with all system suitability parameters remaining within acceptance limits.

The assay of the commercial formulation Glyxambi® (EMPA 10 mg / LINA 5 mg) yielded results of 101.4% (EMPA) and 97.7% (LINA), within the acceptable range, confirming the method's applicability for routine quality control.

The method is simple, cost-effective, and free from interference from common tablet excipients. It is therefore recommended for adoption in pharmaceutical quality control laboratories for the routine simultaneous analysis of Empagliflozin and Linagliptin in fixed-dose combination formulations.

REFERENCES

1. ICH Q2(R1) Guideline: Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation; November 2005.
2. Annapurna MM, Latha N. RP-HPLC method for simultaneous determination of Empagliflozin and Metformin HCl. Asian J Pharm Clin Res. 2016;9(3):183-186.
3. Verma N, Kumar S. Simultaneous RP-HPLC estimation of Empagliflozin and Metformin in combined dosage form. Int J Pharm Sci Rev Res. 2017;44(1):120-124.
4. Veerabhadram G, Prasad YR. RP-HPLC method development and validation for Linagliptin in tablet dosage form. Der Pharma Chemica. 2013;5(4):152-157.
5. Patel B, Shah C, Patel A. Simultaneous RP-HPLC estimation of Empagliflozin and Linagliptin in combined tablet. World J Pharm Pharm Sci. 2016;5(7):1220-1232.
6. Madhusudhan P, Naresh K. Validated RP-HPLC method for simultaneous determination of Linagliptin and Empagliflozin. Int J Pharm Res Biosci. 2018;7(4):48-56.
7. Priyanka A Shah, et al. LC-MS/MS method development and validation for simultaneous determination of Empagliflozin and Linagliptin in human plasma. J Chromatogr B. 2019;1124:64-72.
8. Ashok PK, et al. Stability-indicating RP-HPLC method for estimation of Linagliptin in tablet dosage form. Asian J Pharm Anal. 2014;4(1):17-22.
9. Naga Raju K, et al. RP-HPLC method for simultaneous estimation of Empagliflozin and Linagliptin. Indo Am J Pharm Sci. 2018;5(9):8741-8749.
10. Banik S, et al. Simultaneous UV spectrophotometric determination of Vildagliptin and Linagliptin. J Pharm Chem Biol Sci. 2017;5(3):230-237.
11. Patil SD, et al. UV spectrophotometric method for estimation of Empagliflozin in bulk and formulations. Int J Chem Pharm Sci. 2015;6(4):18-21.
12. Gaikwad AV, et al. HPLC method development and validation for estimation of Empagliflozin. J Drug Deliv Ther. 2018;8(5):219-223.
13. USP 43 – NF 38. General Chapter <621> Chromatography. United States Pharmacopeia; 2020.
14. Avinash C, et al. Sensitive RP-HPLC method for estimation of Linagliptin in bulk and formulations. World J Pharm Pharm Sci. 2015;4(9):1401-1411.

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