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Original Research Article

Development of a novel RP-HPLC method for the estimation of empagliflozin

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Abstract

Background: Empagliflozin is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor used for the treatment of type 2 diabetes mellitus, offering both glycemic control and cardiovascular benefits. A reliable and validated analytical method is essential to ensure the quality and safety of pharmaceutical dosage forms containing this drug.
Aims and Objective: The present study aimed to develop and validate a simple, rapid, precise, and cost-effective Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the estimation of Empagliflozin in bulk and tablet formulations.
Materials and Methods: Chromatographic separation was achieved using a YMC C18 column (4.6 × 150 mm, 5 µm) with a mobile phase of methanol: water (80:20 v/v), delivered iso-cratically at a flow rate of 0.6 mL/min. Detection was carried out at 320 nm, with an injection volume of 20 µL and a total runtime of 6 min. The method was validated according to ICH Q2(R1) guidelines.
Results: Empagliflozin showed a sharp and symmetrical peak at 2.425 min. Theoretical plates (4159) and tailing factor (1.5) satisfied system suitability requirements. The method demonstrated excellent linearity (5–50 µg/mL, $R^2 \geq 0.999$), accuracy (98–102%), precision (%RSD < 2%), robustness, and high sensitivity (LOD ~0.05 µg/mL, LOQ ~0.15µg/mL). Assay of marketed tablets revealed 98.94% purity.
Conclusion: The developed RP-HPLC method is validated, economical, and highly suitable for routine quality control of empagliflozin in pharmaceutical dosage forms.

Keywords: Empagliflozin, RP-HPLC, Method development, Validation, ICH Q2(R1), Quality control.

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1. Introduction

Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide, characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1,2] The condition is associated with long-term complications such as cardiovascular disease, nephropathy, retinopathy, and neuropathy, making effective glycemic control essential for reducing morbidity and mortality. In recent years, sodium-glucose co-transporter 2 (SGLT2) inhibitors have emerged as a novel class of oral anti- diabetic agents that improve glycemic control by reducing renal glucose reabsorption, thereby promoting urinary glucose excretion.[3,4]

Empagliflozin is a selective SGLT2 inhibitor approved for the management of type 2 diabetes mellitus. Beyond glycaemic control, empagliflozin has demonstrated significant cardiovascular and renal protective effects, leading to its wide therapeutic adoption. Given its clinical relevance, reliable analytical methods are necessary for routine quality control of empagliflozin in bulk and pharmaceutical dosage forms to ensure product safety and efficacy.[5-8]

High-Performance Liquid Chromatography (HPLC) remains the most widely employed analytical tool in pharmaceutical analysis due to its high resolution, reproducibility, and applicability for complex matrices. Although several analytical methods for empagliflozin have been reported, the majority focus on combination formulations (e.g., with linagliptin or metformin) or bio-analytical assays involving plasma samples, frequently utilizing advanced techniques such as LC–MS/MS. Reports on simple, robust, and economical single-drug RP-HPLC

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methods for empagliflozin in pharmaceutical dosage forms are limited.[9-14]

Therefore, the present study was undertaken with the objective of developing and validating a novel, simple, rapid, and cost-effective RP-HPLC method for the estimation of empagliflozin in bulk drug and tablet formulations.[15-20] The method was systematically optimized and validated in accordance with ICH Q2(R1) guidelines, ensuring specificity, linearity, precision, accuracy, robustness, and sensitivity. The developed method is expected to be highly suitable for routine quality control applications in pharmaceutical industries and regulatory laboratories.

2. Materials and Methods

2.1. Chemicals and reagents

Empagliflozin working standard and marketed tablet formulation were procured from certified sources. Methanol (HPLC grade), acetonitrile, and phosphate buffer reagents were purchased from Merck (India). Milli-Q water was used throughout the study.

2.2. Instrumentation and chromatographic conditions

The analysis was performed on an RP-HPLC system equipped with a quaternary pump, autosampler, and UV detector. Chromatographic separation was achieved on a YMC C18 column (4.6 × 150 mm, 5 µm) maintained at ambient temperature. The optimized mobile phase consisted of methanol: water (80:20 v/v), delivered isocratically at a flow rate of 0.6 mL/min. The detection wavelength was set at 320 nm, and the injection volume was 20 µL. The total runtime was 6 min, with a retention time of 2.497 min for Empagliflozin.

2.3. Method development strategy

Several chromatographic trials were performed to optimize method parameters. Initial attempts using methanol–buffer and acetonitrile–water systems resulted in inadequate separation and peak tailing. Replacement of RP18 and RP-C8 columns failed to improve peak characteristics. Optimal resolution, retention, and symmetry were finally achieved with a YMC C18 column and methanol: water (80:20 v/v) as the mobile phase under isocratic conditions.

2.4. Preparation of standard and sample solutions

Standard solution: Accurately weighed 10 mg empagliflozin was transferred into a 10 mL volumetric flask, dissolved in ~2 mL of mobile phase, sonicated, and diluted to volume (stock solution). A working standard of 10 µg/mL was prepared by suitable dilution.

Sample solution: Tablet powder equivalent to 10 mg empagliflozin was dissolved in mobile phase, sonicated, and diluted to 10 mL (stock solution). An aliquot of this solution was further diluted to yield a working concentration of 10 µg/mL.

2.5. Chromatographic procedure

Blank, standard, and sample solutions (10 µL each) were injected in triplicate. Peak areas were recorded, and assay values were calculated using the equation:

$$\textbf{\textit{Assay}}\ \% = \frac{sample\ area}{standard\ area} x \frac{dilution\ sample}{dilution\ of\ std} x \frac{P}{100} x \frac{Avg.wt}{Lc} x100$$

Where:

AAA = Peak area,
DDD = Dilution factor,
PPP = Purity of working standard,
Avg. Wt. = Average tablet weight,
LC = Label claim of Empagliflozin.

2.6. System suitability

System suitability parameters were evaluated using the standard solution. Acceptance criteria were:

Tailing factor: ≤ 1.5
Theoretical plates: ≥ 2000

2.7. Method development

The present investigation aimed to develop and validate a novel RP-HPLC method for the estimation of Empagliflozin in pharmaceutical dosage forms. Literature review revealed a lack of single-drug RP-HPLC methods for Empagliflozin, with most existing methods focused on combination formulations (e.g., with linagliptin, metformin) or bioanalytical studies using LC–MS/MS. This justified the need for a cost-effective, simple, and robust assay for routine quality control.

The detection wavelength was determined by scanning a 10 µg/mL solution of Empagliflozin in the UV range of 200–400 nm, with λmax observed at 320 nm (**Figure 1**).

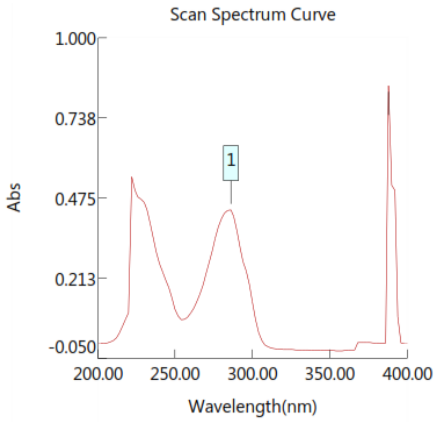

Figure 1: Wavelength was determined by scanning

Several chromatographic trials were conducted by varying stationary phases (C18, C8), mobile phase compositions (methanol–buffer, acetonitrile–water, phosphate buffer–organic solvent), and flow rates. Many of these trials resulted in peak tailing or inadequate resolution.

The final optimized conditions were as follows:

1. Column: YMC C18 (4.6 × 150 mm, 5 µm)
2. Mobile phase: Methanol: Water (80:20 v/v)
3. Flow rate: 0.6 mL/min
4. Detection wavelength: 320 nm
5. Injection volume: 20 µL
6. Column temperature: Ambient
7. Run time: 6.0 min
8. Retention time: ~2.425 min

These conditions produced sharp, symmetrical peaks with good baseline separation and acceptable run time. Importantly, the absence of buffers and gradient elution makes the method more economical and environmentally sustainable.

3. Results

3.1. Assay calculation for empagliflozin

The assay of empagliflozin was carried out by injecting three replicates each of the sample and standard solutions into the HPLC system. The representative chromatograms are shown in **Figure 2**, while the corresponding peak areas are presented in **Table 1**.

Table 1: Sample peak area results

S. No.	Name	Area
1	Empagliflozin	698,557
2	Empagliflozin	699,824
3	Empagliflozin	693,170
Mean		697,184
Std. Dev.		3,533.2
% RSD		0.51

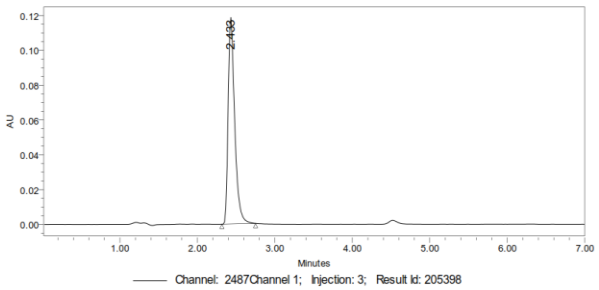

Figure 2: Sample chromatograms

The mean peak area for the standard injections was 694,667 with a %RSD of 0.07 (data not shown). The retention time of Empagliflozin was consistently observed at 2.425 minutes. Theoretical plate count and tailing factor were found to be 4159 and 1.5, respectively, meeting the system suitability requirements. Based on the assay calculation, the percentage purity of Empagliflozin in the pharmaceutical dosage form was 98.94%, which complies with pharmacopeial specifications.

6.2. Validation report (ICH Q2(R1))

6.2.1. Specificity

The specificity of the method was confirmed by injecting a blank (mobile phase). No peaks were observed at the retention time of Empagliflozin, indicating that excipients or impurities did not interfere with the analysis. The chromatogram of the sample solution exhibited a sharp, symmetrical peak at approximately 2.425 minutes.

6.2.2. Linearity

The method exhibited excellent linearity across the concentration range of 20–100 µg/mL. The regression analysis produced a correlation coefficient (r^2) of 0.999, confirming the linear relationship between concentration and peak area. The results are presented in **Table 2**.

Table 2: Linearity results for empagliflozin

Concentration (µg/mL)	Area
20	264,840
40	491,415
60	677,620
80	873,311
100	1,048,958
Correlation coefficient (r^2)	0.999

6.2.3. Accuracy

Accuracy was evaluated by recovery studies at three levels: 50%, 100%, and 150% of the target concentration.(**Figure 3**) Each level was analyzed in triplicate, and results are summarized in **Table 3**. The recoveries were found to be within the acceptance criteria of 98–102%.

Table 3: Accuracy results for empagliflozin

Level	Average area	Amount added (mg)	Amount found (mg)	% Recovery
50%	728,287	5	4.96	99.91
100%	1,378,200	10	9.98	99.18
150%	2,115,480	15	15.02	99.60
Mean recovery				99.95

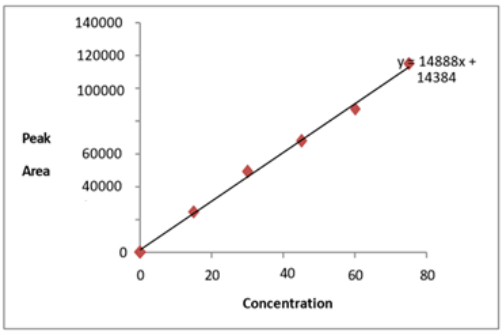

Figure 3: Showing calibration graph for Empagliflozin

6.2.4. Precision

The precision of the method was established through repeatability and intermediate precision studies. Five replicate injections of the standard solution yielded a mean peak area of 695,468 with a %RSD of 0.24. For intermediate precision, five replicate injections on a different day resulted in a mean area of 694,335 with a %RSD of 0.15. Both results complied with the acceptance criterion of %RSD not more than 2%, confirming the reproducibility of the method.

6.2.5. Detection and quantitation limits

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ) using the standard deviation of the response and slope of the calibration curve. The LOD and LOQ values were calculated as 0.36 µg/mL and 1.10 µg/mL, respectively, indicating that the method is capable of detecting and quantifying very low concentrations of Empagliflozin.

6.2.6. Robustness

The robustness of the method was examined by making small deliberate variations in flow rate (0.8, 1.0, and 1.2 mL/min) and in the organic phase composition of the mobile phase (±5%). System suitability parameters under these conditions are shown in **Table 4**.

Table 4: Robustness: effect of flow rate variation and organic change

	USP plate count	**USP tailing**
Flow Rate (mL/min)		
0.8	4187	1.5
1.0	4512	1.4
1.2	4084	1.4
Organic Change		
−5%	4194	1.5
Actual	4524	1.5
+5%	3097	1.4

In both variations, system suitability parameters remained within the acceptance criteria (plate count ≥2000, tailing factor ≤1.5). A slight decrease in efficiency was observed at a higher flow rate (1.2 mL/min) and with +5% organic phase; however, results remained acceptable, demonstrating that the method is robust to minor changes in analytical conditions.

4. Discussion

The present study successfully established a novel, simple, and robust RP-HPLC method for the estimation of Empagliflozin in bulk and tablet dosage forms. The choice of a methanol–water (80:20 v/v) mobile phase under isocratic conditions provided sharp and symmetrical peaks with a retention time of approximately 2.425 minutes. This demonstrates that the method is rapid compared to several previously reported assays, which often rely on gradient elution systems or buffer-containing mobile phases. The absence of buffers not only simplifies sample preparation but also improves column longevity and minimizes environmental burden.[12]

The method validation, performed according to ICH Q2(R1) guidelines, confirmed the suitability of the developed procedure for routine analysis. Specificity was demonstrated as no interfering peaks were detected in the blank chromatograms at the retention time of Empagliflozin, confirming that excipients or impurities did not affect quantification. The linearity results indicated excellent correlation ($r^2 = 0.999$) over the tested concentration range of 20–100 µg/mL, which is sufficient to cover the dosage strengths typically encountered in marketed formulations.

Accuracy studies yielded recoveries between 99.18% and 99.91% with a mean recovery of 99.95%, showing that the method is highly reliable for content uniformity and assay testing. Precision studies further reinforced this reliability, with %RSD values well below the acceptance limit of 2%, both under repeatability and intermediate precision conditions. Such low variability highlights the reproducibility of the method across different days and experimental conditions.[17,18]

The sensitivity of the method was evident from the calculated LOD (0.36 µg/mL) and LOQ (1.10 µg/mL), which allow for the detection and quantification of Empagliflozin even at trace levels. This is particularly valuable for stability studies and impurity profiling. Robustness testing confirmed that small, deliberate variations in flow rate and mobile phase composition did not significantly impact system suitability parameters, further underscoring the method's reliability in routine laboratory settings.[19]

Collectively, these findings demonstrate that the developed method not only meets regulatory requirements but also provides an economical, rapid, and environmentally sustainable alternative to more complex methods reported in

the literature, such as LC–MS/MS or multi-drug HPLC assays.

5. Conclusion

A novel RP-HPLC method was developed and validated for the estimation of Empagliflozin in bulk drug and tablet formulations. The method employs a simple mobile phase of methanol and water (80:20 v/v) under isocratic conditions, achieving a short run time of 6 minutes with a retention time of approximately 2.425 minutes. Validation studies confirmed that the method is specific, linear, accurate, precise, sensitive, and robust, fulfilling all ICH Q2(R1) criteria.

The assay value of 98.94% and mean recovery of 99.95% demonstrate the accuracy and reliability of the procedure. The low LOD and LOQ values highlight its sensitivity, while robustness testing ensures its applicability under routine variations in analytical conditions.

Given its simplicity, cost-effectiveness, and compliance with international guidelines, this method is highly suitable for routine quality control of Empagliflozin in pharmaceutical industries and regulatory laboratories. It can serve as a reliable tool for batch release testing, stability studies, and post-marketing surveillance, thereby contributing to the assurance of safety and efficacy of Empagliflozin-containing products.

6. Conflict of Interest

None.

7. Source of Funding

None.

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