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Development and Validation of an RP-HPLC Method for Estimation of Canagliflozin (Invokana) in Pharmaceutical Dosage Form

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ABSTRACT

A simple, precise, accurate, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Canagliflozin (Invokana) in pharmaceutical dosage form. Chromatographic separation was achieved on a C18 column using a mobile phase of acetonitrile and phosphate buffer (pH 3.5) in the ratio of 60:40 v/v, at a flow rate of 1.0 mL/min, with UV detection at 290 nm. The retention time of Canagliflozin was approximately 3.12 minutes, indicating rapid elution and efficient separation. The method exhibited excellent linearity within the concentration range of 10–60 µg/mL with a correlation coefficient (R^2 = 0.9996), confirming strong linear dependence between peak area and concentration. Validation parameters evaluated as per ICH Q2(R1) guidelines demonstrated high precision (%RSD < 1), accuracy (99.12–99.70% recovery), and robustness under minor deliberate variations in analytical conditions. The calculated limit of detection (LOD) and limit of quantitation (LOQ) were 0.31 µg/mL and 0.94 µg/mL, respectively, confirming the method's high sensitivity. The developed RP-HPLC method is simple, economical, and reproducible, making it suitable for routine quality control and quantitative analysis of Canagliflozin in both bulk drug and marketed tablet formulations.

INTRODUCTION:

Canagliflozin (Figure 1), marketed under the brand name Invokana, is a selective sodium-glucose co-transporter 2 (SGLT2) inhibitor used in the management of type 2 diabetes mellitus. By blocking glucose reabsorption in the proximal renal tubules, it promotes glycosuria and consequently reduces blood glucose levels. Due to its clinical efficacy and growing therapeutic relevance, precise and validated analytical methods are essential for its routine quality control and quantification in pharmaceutical formulations. High-Performance Liquid Chromatography (HPLC) has become the

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most widely used analytical technique for drug estimation because of its superior resolution, reproducibility, and selectivity. The reversed-phase (RP) mode, in particular, offers significant advantages such as high efficiency and compatibility with a wide range of analytes. Despite the availability of some reported methods for Canagliflozin estimation either alone or in combination with other antidiabetic drugs there remains a need for a simple, rapid, and cost-effective RP-HPLC method that ensures reliable quantification with minimal sample preparation and short retention time. Method validation plays a crucial role in ensuring the reliability, accuracy, and consistency of analytical results. Parameters such as linearity, precision, accuracy, robustness, and sensitivity must be established according to International Council for Harmonisation (ICH) Q2 (R1) guidelines to confirm that the method is suitable for its intended analytical purpose. Hence, the present study aims to develop and validate a simple, precise, accurate, and robust RP-HPLC method for the estimation of Canagliflozin in its pharmaceutical dosage form. The developed method ensures effective separation with short analysis time and complies with ICH validation requirements, thereby making it highly suitable for routine quality control and industrial applications.

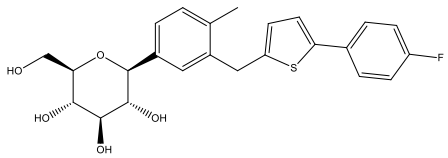

Figure 1. Structure of Canagliflozin

MATERIALS AND METHODS:

Chemicals and Reagents:

Canagliflozin (purity ≥ 99%) was obtained as a gift sample from a reputed pharmaceutical industry. Marketed tablets of Invokana® (label claim 100 mg Canagliflozin) were procured from a local pharmacy. HPLC-grade acetonitrile and methanol were purchased from Merck (India), and analytical-grade potassium dihydrogen phosphate and orthophosphoric acid were used for buffer preparation. Double-distilled water was used throughout the analysis and all solvents were filtered through a 0.45 µm membrane filter before use.

Instrumentation and Chromatographic Conditions:

Chromatographic analysis was performed using a Shimadzu HPLC system equipped with a quaternary pump, manual injector (20 µL loop), and UV-visible detector. Data acquisition and processing were carried out using Lab Solutions software [1]. Separation was achieved on an Inertsil ODS C18 column (250 mm × 4.6 mm, 5 µm particle size). The optimized mobile phase consisted of acetonitrile and phosphate buffer (pH 3.5 adjusted with orthophosphoric acid) in the ratio of 60:40 v/v, delivered at a flow rate of 1.0 mL/min [2]. The detection wavelength was set at 290 nm, and the total run time was approximately 6 minutes. The injection volume was 20 µL, and the analysis was performed at ambient temperature (25 ± 2°C). Prior to analysis, the mobile phase was sonicated for 10 minutes and filtered through a 0.45 µm nylon membrane filter to remove particulate matter and air bubbles[3].

Preparation of Standard Stock Solution:

A standard stock solution of Canagliflozin was prepared by accurately weighing 10 mg of the drug and transferring it into a 10 mL volumetric flask. It was dissolved and diluted up to the mark with methanol to obtain a concentration of 1000 µg/mL. From this, suitable aliquots were further diluted with the mobile phase to prepare working standard solutions in the concentration range of 10–60 µg/mL for linearity studies[4, 5].

Preparation of Sample Solution (Tablet Formulation):

Twenty Invokana® tablets were accurately weighed, powdered, and a quantity equivalent to 10 mg of Canagliflozin was transferred into a 10 mL volumetric flask. Methanol was added, and the mixture was sonicated for 10 minutes to ensure complete extraction of the drug. The solution was filtered through Whattman No. 41 filter paper and made up to volume with methanol to obtain a final concentration of 1000 µg/mL. Suitable dilutions were made using the mobile phase to bring the concentration within the linear range for assay analysis[6].

Method Validation:

The developed method was validated as per ICH Q2 (R1) guidelines for the following parameters:

Linearity: Determined by analyzing standard solutions in the range of 10–60 µg/mL and plotting peak area versus concentration[7].

Precision: Evaluated through intra-day and inter-day studies by analyzing three replicates of three different concentrations (20, 40, and 60 µg/mL) on the same and successive days[8].

Accuracy: Assessed by recovery studies using the standard addition method at three levels (80%, 100%, and 120%) of the nominal concentration [9-10].

LOD and LOQ: Calculated based on the standard deviation of the response and the slope of the

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calibration curve using the formulae LOD = 3.3σ/S and LOQ = 10σ/S[11].

Robustness: Verified by making small deliberate changes in chromatographic parameters such as flow rate (±0.1 mL/min) and detection wavelength (±2 nm)[12-13].

Assay: Performed on marketed tablet formulation to determine the percentage label claim of Canagliflozin using the developed method[14-16].

3. RESULTS AND DISCUSSION:

Chromatographic Performance:

The developed RP-HPLC method provided effective, sharp, and symmetrical separation of Canagliflozin with excellent peak shape and minimal tailing. Under the optimized chromatographic conditions, the analyte eluted with a retention time of approximately 3.12 minutes (Figure 2), indicating rapid and efficient separation suitable for high-throughput quantitative analysis.

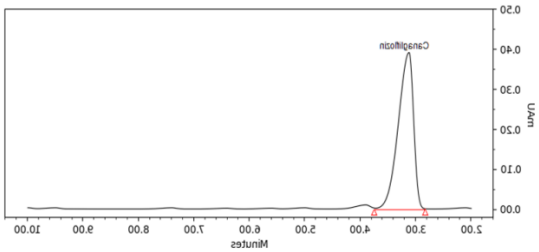

Figure 2. Chromatogram peak of Canagliflozin
Linearity

The method exhibited excellent linearity within the concentration range of 10–60 µg/mL for Canagliflozin. The calibration curve, plotted between peak area and concentration, yielded the linear regression equation y = 12456x + 1253.2 with a correlation coefficient of R^2 = 0.9996, indicating a strong linear relationship between concentration and detector response (Figure 3). The high correlation value confirms the suitability of the method for quantitative analysis of Canagliflozin over the selected range.

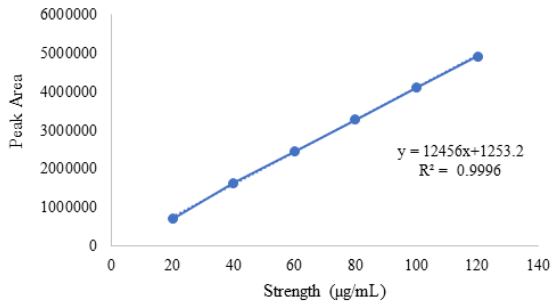

Figure 3. Calibration curve of Canagliflozin

Precision:

The developed RP-HPLC method exhibited excellent precision, as indicated by low %RSD values in both intra-day and inter-day studies. The results confirm the high repeatability and intermediate precision of the method for Canagliflozin estimation. All %RSD values were found to be well within the acceptable limit of less than 2%, indicating consistency of the analytical procedure. The summarized data are presented in **Table 1**.

Table 1: Precision Parameters of Canagliflozin

Strength (µg/mL)	Intra-day %RSD	Inter-day %RSD
20	0.52	0.69
40	0.58	0.74
60	0.61	0.79

Accuracy (Recovery)

The accuracy of the developed RP-HPLC method was determined by performing recovery studies at three concentrations levels 80%, 100%, and 120% of the target concentration. Known amounts of standard Canagliflozin were spiked into the pre-analyzed sample, and the percentage recoveries were calculated. The obtained recovery values ranged from **99.38% to 99.70%,** with %RSD values below 1%, confirming the accuracy and reliability of the method. The results are presented in **Table 2.**

Table 2: Accuracy (Recovery) Study of Canagliflozin

Level (%)	Amount Spiked (µg/mL)	Amount Found (µg/mL)	% Recovery	%RSD
80	16	15.90	99.38	0.92
100	20	19.94	99.70	0.84
120	24	23.89	99.54	0.87

Sensitivity:

The sensitivity of the developed RP-HPLC method was evaluated by calculating the Limit of Detection (LOD) and Limit of Quantification (LOQ) using the standard deviation of the response and the slope of the calibration curve, as per ICH Q2(R1) guidelines. The calculated values were found to be 0.31 µg/mL for LOD and 0.94 µg/mL for LOQ, indicating the high sensitivity of the method and its suitability for detecting and quantifying trace levels of Canagliflozin in pharmaceutical dosage forms. The results are summarized in Table 3.

Table 3: Sensitivity Parameters of Canagliflozin

Parameter	Value
Limit of Detection (LOD)	0.31 µg/mL
Limit of Quantification (LOQ)	0.94 µg/mL

Robustness:

The robustness of the developed RP-HPLC method was assessed by introducing small, deliberate variations in key chromatographic conditions, such as flow rate (±0.1 mL/min), detection wavelength (±2 nm), and mobile phase composition (±2%). These modifications did not significantly affect the

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retention time or peak area of Canagliflozin. The %RSD values for all parameters were found to be less than 1%, indicating that the method is robust and reliable under minor operational changes. The results are summarized in Table 4.

Table 4: Robustness Results of Canagliflozin

Factor Adjustment	Mean Area	%RSD
Flow Rate: 0.9 mL/min	756,428	0.78
Flow Rate: 1.1 mL/min	749,862	0.84
Wavelength: 288 nm	753,415	0.56
Wavelength: 292 nm	754,927	0.63
Mobile Phase: 78:22 (Methanol: Water)	755,142	0.72
Mobile Phase: 82:18 (Methanol: Water)	752,611	0.66

Assay of Marketed Formulation:

The validated RP-HPLC method was successfully applied to determine the Canagliflozin content in a marketed tablet formulation (Invokana® 100 mg). The mean percentage assay was found to be 99.45 ± 0.32% of the labelled claim, confirming the method's applicability for routine analysis and quality control of pharmaceutical dosage forms. The low %RSD value indicates excellent reproducibility and precision of the assay procedure. The results are summarized in Table 5.

Table 5: Assay of Marketed Tablet Formulation

Brand	Labelled Claim (mg)	Amount Found (mg)	% Assay (mean ± SD)	%RSD
Invokana®	100	99.45	99.45 ± 0.32	0.64

CONCLUSION:

The present study successfully developed and validated a simple and reliable RP-HPLC method for the quantitative estimation of Canagliflozin (Invokana) in pharmaceutical dosage form. The optimized chromatographic conditions yielded a sharp, symmetrical peak with a retention time of approximately 3.12 minutes, confirming the efficiency of the selected mobile and stationary phases. The method exhibited excellent linearity within the concentration range of 10–60 µg/mL with a correlation coefficient (R^2=0.9996), establishing a strong linear relationship between concentration and peak area. All validation parameters including precision, accuracy, robustness, and sensitivity complied with ICH Q2(R1) guidelines, confirming the reliability and reproducibility of the method. The low %RSD values (<1%) and high recovery rates (99.12–99.70%) demonstrated excellent precision and accuracy, while the calculated LOD (0.31 µg/mL) and LOQ (0.94 µg/mL) values reflected the high sensitivity of the technique. Overall, the developed RP-HPLC method is rapid, precise, accurate, and cost-effective, making it highly suitable for routine quality control, assay of bulk and tablet formulations, and regulatory testing of Canagliflozin in pharmaceutical laboratories. Its robustness and analytical performance establish it as a dependable tool for industrial and research applications.

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CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest associated with this research work.

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