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Concurrent validation of an active therapeutic molecule for Type II diabetes by Reverse Phase- High Performance Liquid Chromatography: A Quality Assessment Approach

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Abstract

Background: Canagliflozin is an important therapeutic agent used in the management of Type II diabetes mellitus. Although several analytical methods have been reported for its simultaneous estimation with other drugs, dedicated methods for its individual quantitative assessment remain limited.
Objective: The present study aimed to develop and concurrently validate a robust, precise, and stability-indicating RP-HPLC method for the quantitative estimation of Canagliflozin.
Methods: Chromatographic separation was performed using a C18 column (4.6 mm × 250 mm, 5 µm) with a mobile phase consisting of formic acid and acetonitrile (45:55, v/v) under isocratic conditions. The flow rate was maintained at 1.1 mL/min, and detection was carried out at 290 nm. The developed method was validated according to standard analytical parameters, including specificity, linearity, precision, accuracy, robustness, and forced degradation studies.
Results: The developed method exhibited a retention time of 5.8 min and demonstrated excellent linearity over the concentration range of 1–30 µg/mL with a correlation coefficient (R^2) of 0.9988. The limits of detection (LOD) and quantification (LOQ) were determined to be 0.177 µg/mL and 0.536 µg/mL, respectively. Validation results confirmed the method's specificity, precision, accuracy, robustness, and stability-indicating capability
Conclusion: The validated RP-HPLC method was found to be reliable, sensitive, and suitable for routine quality control analysis of Canagliflozin. Furthermore, the method can be extended to future bioanalytical and pharmacokinetic studies involving Canagliflozin in pharmaceutical and biological matrices.

INTRODUCTION

Type II diabetes mellitus (T2DM) is a chronic and progressive metabolic condition characterized by insulin resistance and impaired glucose homeostasis. It currently affects over 400 million individuals worldwide, and its prevalence continues to rise owing to sedentary lifestyles and changing dietary patterns. This growing global health challenge underscores the urgent need for effective therapeutic interventions that not only regulate blood glucose levels but also provide ancillary benefits, such as cardiovascular and renal protection [1-2].

Canagliflozin, a selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, has emerged as a valuable treatment for T2DM.

By inhibiting SGLT2 in the proximal renal tubules, canagliflozin reduces glucose reabsorption in the kidneys, promoting urinary glucose excretion (glycosuria), and consequently lowering blood glucose levels via an insulin-independent mechanism. This unique mode of action contributes to its growing clinical significance [3, 4].

Chemically, Canagliflozin hemihydrate is typically administered in a tablet form as a hemihydrate salt. It is a white to off-white powder, sparingly soluble in water but freely soluble in several organic solvents such as ethanol, methanol, tetrahydrofuran, and acetone [5]. The molecular structure of canagliflozin is shown in Figure 1.

Marketed under the brand name *Invokana*, Canagliflozin is approved for the oral treatment of type II diabetes and is used in conjunction with diet and exercise. However, this is not indicated in patients with type I diabetes. Similar to other SGLT2 inhibitors, common adverse effects include an increased risk of urinary tract infections, genital mycotic infections, polyuria, thirst, and transient hypotension [6, 7]. The pharmacokinetics of canagliflozin is not significantly affected by food; however, to optimize glycemic control, it is generally recommended to administer the drug before the first meal of the day [8].

For pharmaceutical quality control, accurate and reproducible quantification of canagliflozin in both bulk drug substances and tablet dosage forms is essential. Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) is widely regarded as a reliable technique for this purpose, offering advantages in terms of resolution, sensitivity, and reproducibility [9-10]. Although several analytical methods have been reported for the simultaneous estimation of canagliflozin and other drugs in fixed-dose combinations, limited methods focus exclusively on individual quantification in single-ingredient formulations.

Moreover, concurrent method validation, conducted alongside routine production, has gained importance in aligning current regulatory expectations and ensuring consistent product quality. This approach supports real-time quality assurance and continuous process verification in accordance with current good manufacturing practices (cGMP) and guidelines established by the International Council for Harmonisation (ICH) [11-13].

This study aimed to develop and validate a simple, precise, and robust isocratic RP-HPLC method for the quantitative estimation of canagliflozin in its bulk and tablet forms. The method was designed to serve as an efficient quality control tool,

offering accuracy, cost-effectiveness, and regulatory compliance for routine analytical applications. Therefore, we report a rapid, accurate, and cost-effective quality control tool and a reliable method for the estimation of canagliflozin.

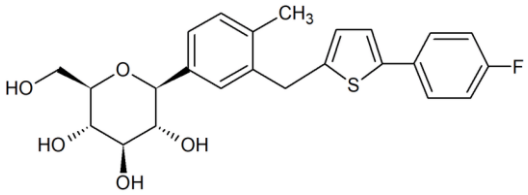

Figure 1: Chemical structure of Canagliflozin

Table 1: Comparison of present method with the published methods

S.No	Method	Work done	Wavelength	Linearity	Flow rate	Ref.
1.	RP - HPLC	INERTSIL column, C18 (150x4.6)5µm and water and acetonitrile as mobile phase (70:30), Run time:6min	264nm	100-500µg/mL	1ml/min	14
2.	RP - HPLC	C18 column (100 mm x 4.6 mm 5 µm) and Acetonitrile: Water (pH-2.5 adjusted with ortho phosphoric acid) as mobile phase 50: 50 v/v, Run time- 2min	260nm	10 - 200 µg/mL	1.0mL/min	15
3.	HPTLC	Toluene: Methanol: Triethyl amine: Glacial Acetic acid (7:2.6:0.2:0.2, v/v/v/v) as a mobile phase	254nm	75-750 ng/band	-	16
4.	LC-MS	Inertsil ODS 5 µm C18, 50 × 4.6 mm 10 mM ammonium acetate: methanol as an isocratic mobile phase (30:70)	-	5-600ng/mL	0.8 ml/min	17
5.	RP – HPLC	Shimadzu C18 column (250 mm x 4.6 mm 250mm) and Formic acid:	290 nm	1-30 µg/mL	1.1 mL/min	Present work

(Present)	Acetonitrile as mobile phase 45:55, Runtime- 10 min

MATERIALS AND METHODS

Chemical Requirements

The pure drug (Canagliflozin Hemihydrate) sample was gifted by Yarrow Chem Products, Mumbai. Methanol HPLC (Gradient Grade) was provided by Thermo Fisher Scientific India Pvt. Ltd. (Mumbai, India). Acetonitrile for HPLC was used in this research work by SD Fine Chem Limited, Mumbai (India), and Water (HPLC grade) was procured by Loba Chemie Pvt. Ltd., Mumbai. Formic acid was used as an analytical reagent.

Mobile Phase Composition

Using a 500 mL freshly washed and dried flask 0.5 mL of formic acid dissolved in 500 mL of water that had been graded for HPLC to formulate 0.1 % formic acid buffer solution. The mixture was stirred using an ultrasonicator for 30 minutes (for complete dissolution of the salt). It was then filtered through a 0.45 µ nylon filter.

The mobile phase was prepared by mixing 0.1% formic acid and ACN at a ratio of 45:55. It was filtered through a 0.45µ µm membrane filter to remove impurities that may interfere with the final chromatogram.

Determination of working wavelength (λmax)

The λmax (wavelength of maximum absorption) of Canagliflozin was determined by scanning a 20 µg/ml solution of the drug (methanol used as diluent) using a UV-visible spectrophotometer within the wavelength region of 200 to 400 nm against ethanol as blank. The absorption curve showed characteristic absorption maxima at 290 nm for Canagliflozin.

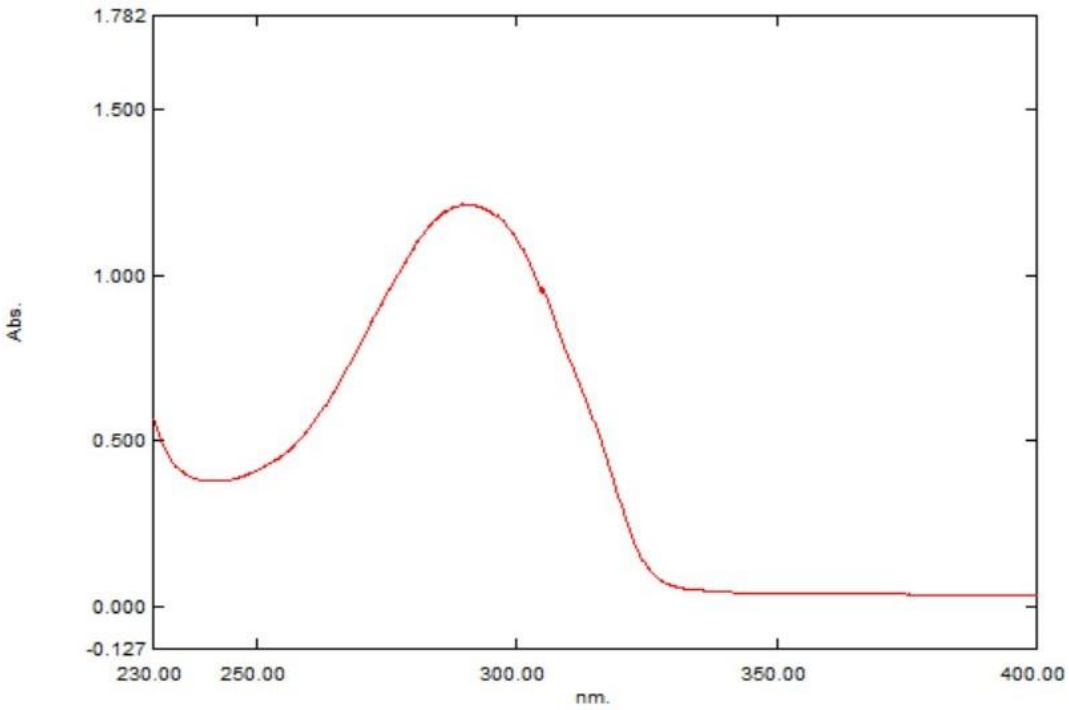

Figure 2: Spectrum of canagliflozin in UV region (200-400 nm) at 20 µg/mL

Figure 2 illustrates the UV absorption spectrum of Canagliflozin (20 µg/mL), scanned in the 200–400 nm range. Based on the spectrum, no significant absorbance was observed at other wavelengths, confirming the selectivity of 290 nm for Canagliflozin without interference from solvents or degradation products. Accordingly, this wavelength was selected for the development of a UV spectrophotometric method, ensuring maximum sensitivity and accuracy in estimating Canagliflozin concentration. The method development further considered parameters such as linearity, precision, and reproducibility at this wavelength, thereby establishing a robust and reliable analytical approach for the determination of Canagliflozin in pharmaceutical formulations.

Solution preparations

Preparation of phosphate buffer (pH 6.8)

For the preparation of phosphate buffer (pH 6.8), 11.45 g of sodium dihydrogen phosphate and 28.80 g of disodium hydrogen phosphate was dissolved in 100 mL of water.

Preparation of standard stock solution

Then, 10 mg of canagliflozin working standard was accurately weighed and transferred into a 10 mL clean, dry volumetric flask. The diluent was added, sonicated to dissolve it entirely, and made

up to the mark with the same solvent. (Stock solution)

Further, pipette 5 ml of the above stock solutions into a 50 ml volumetric flask and dilute up to the mark with diluent. (100 µg/mL of Canagliflozin)

Sample Solution Preparation

Accurately weighed and transferred equivalent to 100 mg of canagliflozin. The sample was placed into a 100 mL clean, dry volumetric flask, diluted with diluent, sonicated for up to 30 min to dissolve, centrifuged for 30 min to dissolve it completely, and made up to the mark with the same solvent. Then, it was filtered through a 0.45-micron injection filter. (stock solution).

Further, 5 mL of the above stock solutions was pipetted into a 50 mL volumetric flask and diluted to the mark with diluent (100 µg/mL of canagliflozin) as a working solution to obtain a final concentration of 15 µg/mL. The working sample was repeatedly injected with 20 µl solution at a flow rate of 1.0 mL/min. The optimal detection wavelength was 290 nm.

System suitability

Approximately 10 mg of canagliflozin was weighed and transferred to a 10 mL volumetric flask. Initially, 5 mL of the mobile phase was added for dissolution, followed by sonication in an ultrasonic cleaner for 5 min. The volume was made up to 10 mL with the mobile phase and mixed well to get a 1000 µg/mL concentration. From the above stock solution, 1 mL was pipetted and made up to a volume of 10 mL in a volumetric flask with a diluent, that is, formic acid: acetonitrile (45:55, *v/v*), to obtain a concentration of 100 µg/mL. Chromatograms were recorded and the peak responses were measured.

Method validation

The developed method was validated according to ICH guidelines (ICH Q2R1) for linearity, specificity, precision, accuracy, robustness, limit of detection, and quantification [18-19].

Linearity

Linearity is an analytical technique that yields test results that are proportional to the analyte concentration in the test sample. A series of solutions were prepared for the standard calibration curve between 1 and 30 µg/mL to determine linearity.

Intra-day Precision

Six successive injections of 15 µL of the working standard mixture solutions were injected separately on the same day, and chromatograms were recorded. The % relative standard deviation was calculated for the concentration of the drug in replicates.

Inter-day Precision

On three consecutive days, six consecutive subsequent injections of 15 µL of working standard combination solutions were administered, and the chromatograms were recorded. The % RSD was calculated as the concentration of the drug in the replicates.

Accuracy

The accuracy of the analytical procedure expresses the closeness of the agreement between the value accepted as either a conventional true value or an accepted reference value and the value found. For the recovery study, 10 mL of diluent was prepared by dissolving 10 mg of canagliflozin in the excipients required for the drug formulation. A dilution of 15µL was prepared and injected into the column. Percent recovery of 50%,100%, and 150% was determined with concentrations of 7.5µg/mL, 15µg/mL, and 22.5 µg/mL, respectively. The resulting peaks were compared to those of the reference standard.

Specificity

A specificity study was performed by injecting one blank and one placebo injection, and the results were reported as the observation of peaks.

Robustness

The analysis was performed under different conditions to determine the variability in the test results. The conditions, such as flow rate and mobile phase, were checked for variation of the results.

Analysis of commercial dosage form

About 18.25 mg of the canagliflozin sample was weighed and placed in a 100 mL volumetric flask, dissolved in methanol, and made up to the mark with the same solvent. The solution was then filtered using Whitman filter paper No.40. From this filtrate, a dilute 0.15 mL solution was prepared in a 10 mL volumetric flask with water to obtain the desired concentration (15 µg/mL). These solutions were analyzed by HPLC, and the results are indicated by the% assay. The formula for Assay:

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where: AT = average area counts of test (sample) preparation.

AS = average area counts of the standard preparation.

WS = Weight of working standard taken in mg.

DS = Dilution of working standard in ml.

DT = Dilution of test (sample) in ml.

WT = Weight of test (sample) taken in mg.

P = Percentage purity of working standard

LC = Label Claim mg/ml.

Stress degradation studies [20]

Acid degradation studies

About 0.15 mL of the stock solution was placed in a 10 mL volumetric flask, and 3 mL of 0.5 N HCl was added. Then, the volumetric flask was kept at 60 °C for 1 h, neutralized with 1 N NaOH, and made up to 10 mL with diluent. The solution was filtered with 0.22 micron syringe filters, injected into the HPLC, and analyzed.

Alkali degradation

About 0.15 ml of the stock solution was placed in a 10 ml volumetric flask, and 3 3 ml of NaOH (0.5 N) was added. Then, the volumetric flask was kept at 60 °C for 1 h, neutralized with 1N HCl and made up to 10 mL with diluent. The solution was filtered through 0.45-micron syringe filters, injected into the HPLC, and analyzed.

Peroxide degradation

About 0.15 ml of stock solution was placed in a 10 ml volumetric flask, 3 ml of 3% hydrogen peroxide was added to the 10 ml volumetric flask, and the volume was made up to the mark with diluent. The volumetric flask was then maintained at room temperature for 15 min. The solution was filtered with 0.45-micron syringe filters and injected into the HPLC and analysed.

Photolytic degradation

The canagliflozin sample was placed under sunlight for 24 h. The sample was then diluted with diluent, injected into the HPLC system, and analyzed.

RESULTS AND DISCUSSION

UV spectrophotometric method

To optimise the solvent system for the spectrophotometric method, various solvent ratios were tested to obtain a linear response. The wavelength of maximum absorption of the solution of canagliflozin in a mixture of Formic acid: Acetonitrile (45:55) was selected within the wavelength region of 200-400 nm. The UV spectrum shown in Figure 3 indicated that the compound absorbed maximum at 290 nm. This wavelength can be used to develop and validate a UV-spectrophotometric method for the determination of canagliflozin. The proposed method for canagliflozin utilizes the spectrum mode of analysis of a spectrophotometer and can be employed for routine analysis. Linearity was obtained in

the concentration range of 1-25 µg/mL, with a regression of 0.996, an intercept of- 0.2587, and a slope of- 0.0573 for canagliflozin (Table 2). The summary of the Optical parameters was given in Table 3.

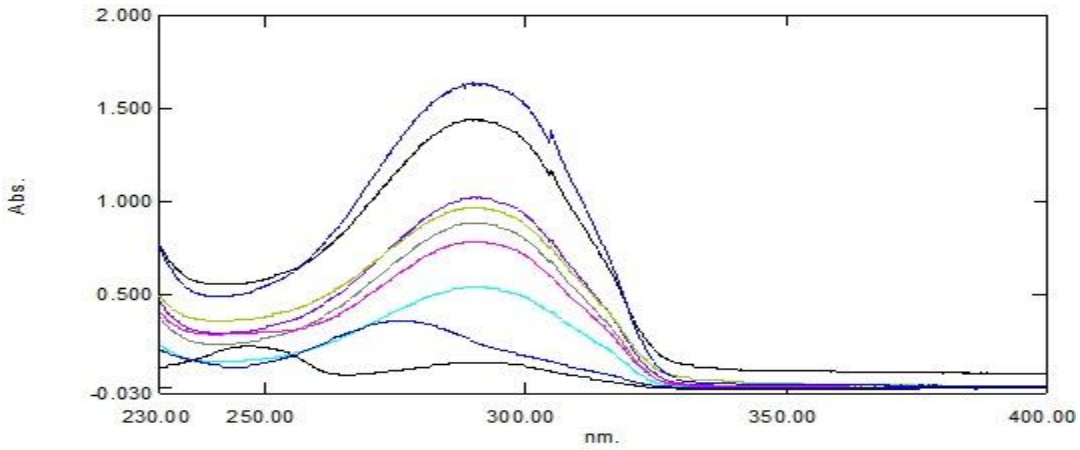

Figure 3: Overlay spectrum of Canagliflozin at 1 - 25µg/mL

Figure 3 presents the overlay UV absorption spectra of Canagliflozin at various concentrations (1–25 µg/mL). All spectra show a consistent absorption maximum at 290 nm, confirming the maximum wavelength and linear absorbance behavior across the tested range.

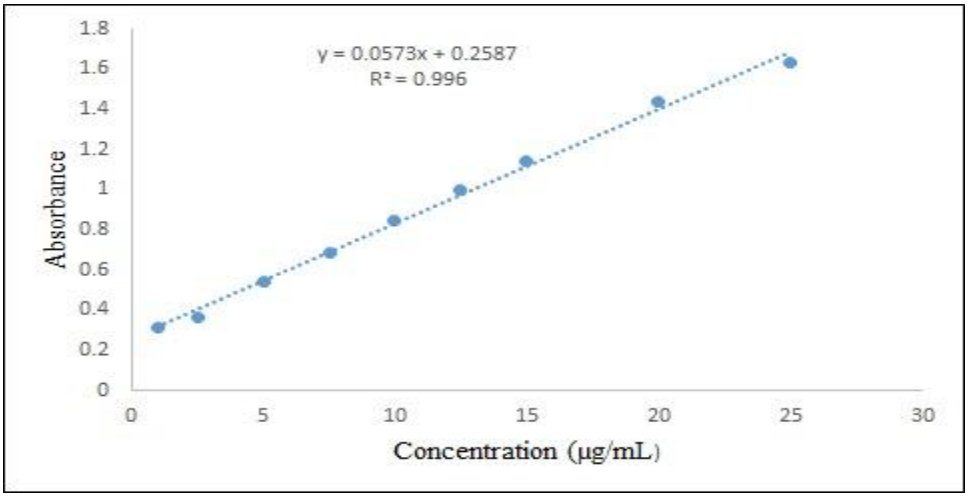

Figure 4: Calibration curve of Canagliflozin

Figure 4 shows the calibration curve illustrating the linear relationship between absorbance and the concentration of Canagliflozin in the range of 1–25 µg/mL. The regression equation ($y = 0.0573x – 0.2587$) and correlation coefficient ($R^2 = 0.996$) confirm excellent linearity, making it suitable for quantitative analysis.

Table 2: Table for Linearity of Canagliflozin

Concentration (µg/mL)	Absorbance
1	0.314
2.5	0.369
5	0.539
7.5	0.687
10	0.845
12.5	1.003
15	1.139
20	1.44
25	1.636

Table 3: Optical parameters for Canagliflozin

Parameters	Method
Reagent	Formic acid: Acetonitrile (45:55)
Linearity range (µg/mL)	1-25µg/mL
Wavelength (nm)	290nm
Molar Extinction coefficient (litre/mole/cm)	1.47×10^4
Sandell's sensitivity (µg/cm$^{2/}$0.001 A.U$^{\cdot}$)	0.31
Slope	0.0573
Intercept	0.2587
Correlation coefficient	0.996
Precision	1.32

HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Validation of the developed method

The method developed was validated according to the ICH Guidelines as follows:

- **System suitability**: System suitability was performed initially by injecting six successive injections, and the results are reported as %RSD.

Table 4: System suitability parameters for Canagliflozin

S.No	Parameter	Canagliflozin
1	Retention time	5.65
2	Plate count	8692.7
3	Tailing factor	1.169
4	Resolution	---
5	%RSD	0.986

This method was confirmed by low %RSD values of peak area for all components and reported in table 4. The % RSD values were within 2, and the method was found to be suitable.

Linearity

The linearity of the proposed method was tested by injecting six concentrations of the drug, and the results were reported as the correlation coefficient (Table 5). The obtained slope was y = 20495x-3535.3. The range was calculated from the calibration graph constructed using the concentration versus the peak area.

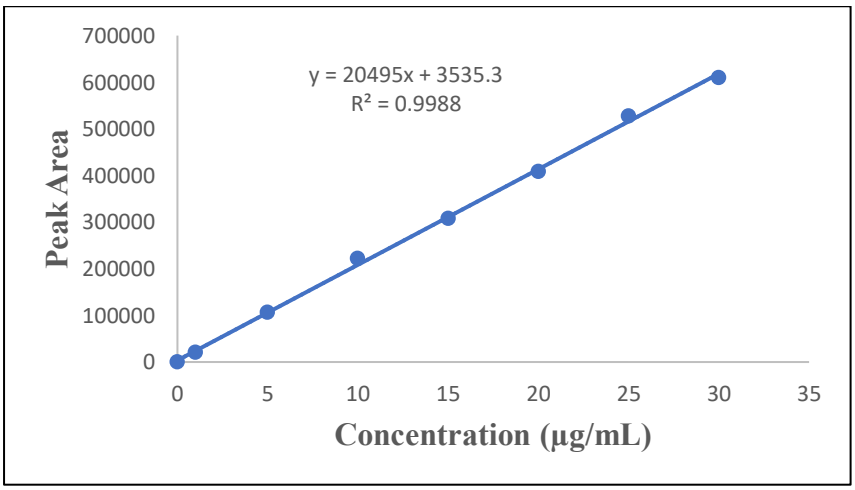

Figure 5: Calibration graph for Canagliflozin

Figure 5 represents the plot illustrating the linear response of Canagliflozin peak area versus concentration (1–30 µg/mL) as analyzed by RP-HPLC. The correlation coefficient (R^2 =

0.9988) demonstrates strong linearity, validating the method's suitability for accurate quantification.

Table 5: Linearity results for Canagliflozin

S.No	Conc.(µg/mL)	Peak area	Back cal conc.	% Accuracy
1	1	19956	0.801	80.121
2	5	106558	5.027	100.534
3	10	221489	10.634	106.345
4	15	307317	14.822	98.815
5	20	408146	19.742	98.71
6	25	527444	25.563	102.251
7	30	609805	29.581	98.604

The calibration curves were linear in the range1 to 30 µg /mL for Canagliflozin. The correlation coefficient ('r') value was found to be 0.9988 for Canagliflozin & the results are presented in

Limit of detection (LOD) and limit of quantification (LOQ):

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per International Conference Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

LOD for Canagliflozin was found to be 0.177 µg/mL and LOQ was found to be 0.536 µg/mL.

PRECISION

Intra-precision studies were performed by injecting a 15 µg /mL sample of Canagliflozin. Six were done in same day and results are reported in % relative standard deviation (% RSD) (Table 6 and 7).

Table 6: Intraday precision

No of injections	Retention time	Area	Tailing factor	NTP
1	5.921	300999	0.96	6550
2	5.923	297862	0.968	6554

3	5.919	285851	0.965	6622
4	5.916	297688	0.971	6642
5	5.913	299980	0.97	6662
6	5.911	305582	0.972	6678
Mean	5.917	297993.667	0.968	6618.000
S. D	0.005	6604.778	0.005	54.494
%RSD	0.079	2.216	0.465	0.823

Table 7: Inter day precision

Day 1

No. of Injections	Retention Time	Area	Assay (%)
1	5.885	305390	98.188
2	5.881	309068	99.384
3	5.887	301600	96.955
4	5.886	310699	99.915
5	5.884	311702	100.241
6	5.881	305712	98.293
Mean	5.884	307361.8333	**98.829**
S. D	0.003	3812.966	1.240
%RSD	0.043	1.241	1.255

Day 2

No. of Injections	Retention Time	Area	Assay (%)
1	5.822	306777	98.639
2	5.826	307850	98.988
3	5.835	309588	99.554
4	5.842	306123	98.427
5	5.846	311602	100.209
6	5.852	312522	100.508
Mean	5.837	309077	99.387
S.D	0.012	2608.694	0.849
%RSD	0.200	0.844	0.854

Day 3

No. of Injections	Retention Time	Area	Assay (%)

1	5.888	310606	102.3661
2	5.886	307555	101.3738
3	5.883	312600	103.0146
4	5.881	302414	99.70179
5	5.881	309900	102.1365
6	5.885	310328	102.2757
Mean	5.884	308900.5	101.8114
S.D	0.003	3564.178	1.159196
%RSD	0.04807	1.154	1.138571

ACCURACY: Accuracy study was performed by injecting three different levels i.e., 50%, 100%, 150 %. The results are reported in % mean recovery.

Table 8: Accuracy results for Canagliflozin (n = 3)

Level (%)	Conc added (µg/mL)	Peak area	Conc found (µg/mL)	Recovery (%)	SD	RSD (%)
50	7.5	159688.67	7.62	101.59	0.679	0.68
100	15	306103.00	14.76	98.41	0.560	1.05
150	22.5	456845.66	22.11	98.30	0.231	1.21

An accuracy study was performed by injecting three different levels, i.e., 50%,100%, and 150 %. Three injections of each level were done for % accuracy & the results are reported in % mean recovery in table 8.

SPECIFICITY

A specific study was performed by injecting one blank and one placebo injection were done & the results are reported in the observation of peaks as shown below in the figure

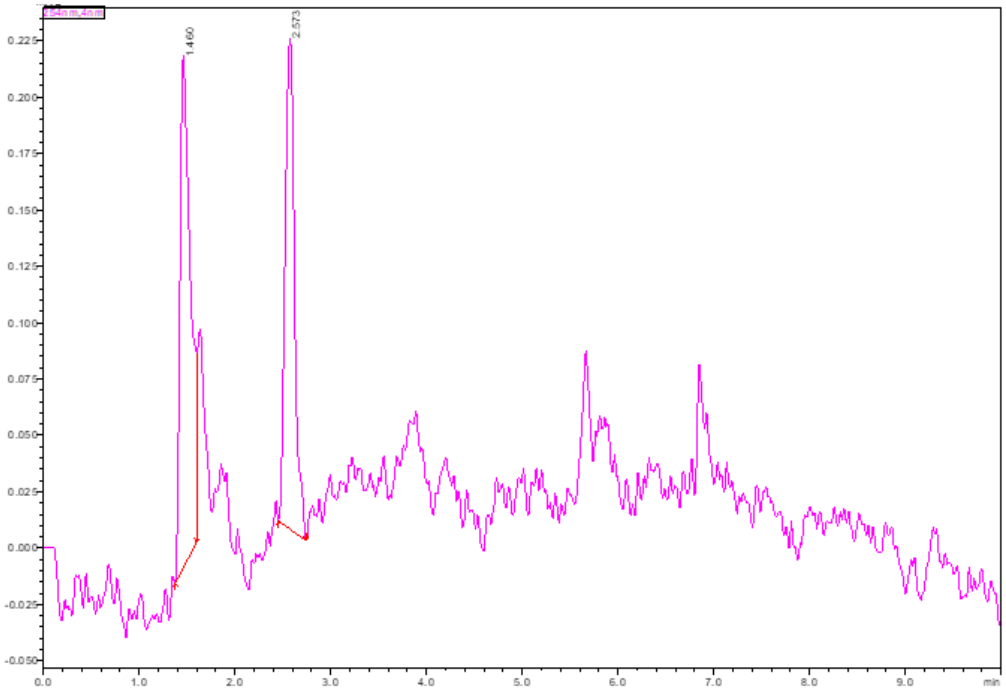

Figure 6(a): Chromatogram for Blank (Mobile phase)

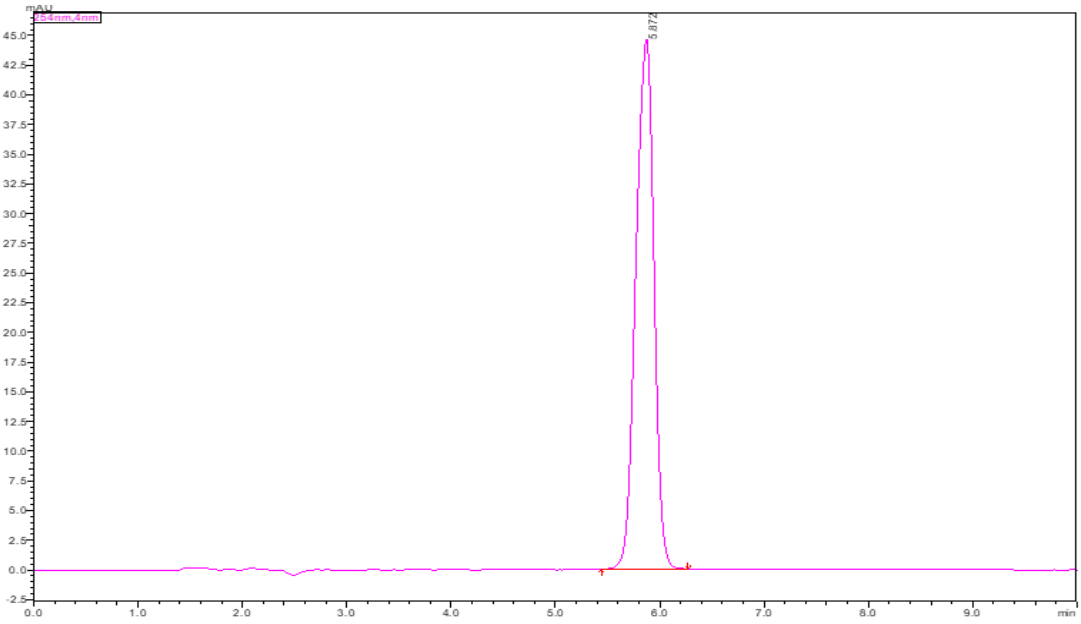

Figure 6 (b): Chromatogram for the Standard solution.

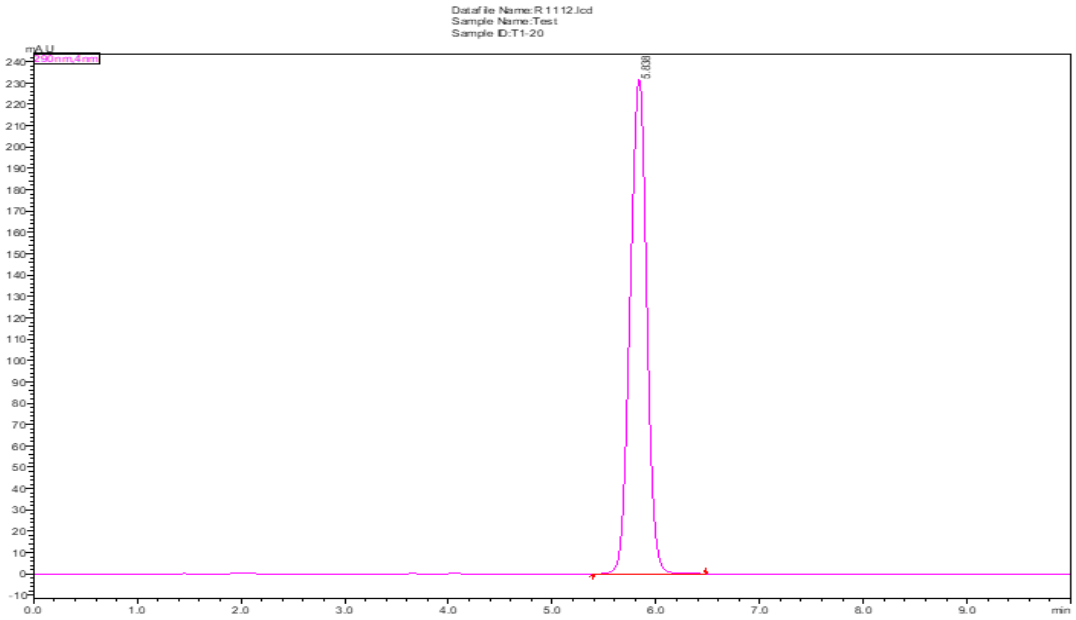

Figure 6(c): Chromatogram for Tablet Sample

Figure 6 demonstrates the specificity of the developed RP-HPLC method through comparative chromatographic analysis: **(a)** The chromatogram of the blank (mobile phase) shows no detectable peaks, confirming the absence of interfering substances in the mobile phase. **(b)** The chromatogram of the standard solution exhibits a sharp and symmetrical peak at the retention time corresponding to Canagliflozin, indicating precise detection of the analyte. **(c)** The chromatogram of the tablet formulation reveals a well-defined Canagliflozin peak at the same retention time as the standard, with no interference from excipients or formulation components. These results confirm the specificity of the method, ensuring that the analyte peak is not affected by any endogenous or formulation-related substances, in compliance with ICH validation guidelines.

ROBUSTNESS

Robustness was performed by flow rate variation and mobile phase composition variation. Two injections each were made & the acceptance criteria for the tailing factor & plate count were determined (Table 9).

Table 9: Robustness Table for Canagliflozin

Variations	Retention time	Area	Average	SD	%RSD
Flow rate(1.0mL/min)	6.341	312650	310986.500	2352.544	0.756
	6.341	309323			
Flow rate(1.1mL/min)	5.806	305618	305313.500	430.628	0.141

	5.885	305009			
Flow rate(1.2mL/min)	5.451	304898	308415.000	4973.789	1.613
	5.454	311932			
Mobile phase composition Formic acid: Acetonitrile (40:60, v/v)	4.726	342071	346192.500	5828.681	1.684
	4.72	350314			
Mobile phase composition Formic acid: Acetonitrile (50:50, v/v)	7.949	298073	301510.500	4861.359	1.612
	7.962	304948			
Mobile phase composition Formic acid: Acetonitrile (45:55, v/v)	5.951	312583	310149.000	3442.196	1.110
	5.858	307715			

STRESS DEGRADATION STUDIES [20]

In these studies, the canagliflozin drug product was subjected to stress degradation under acidic, basic, oxidative, thermal, and photolytic conditions, and the peak obtained was the separation of the degradation product from the pure active ingredient. When the drug interacts with acid, acid degradation produces primary degradation in the desirable range. HCL or H2SO4 (0.5N-1N) is widely used for acid analysis. In basic degradation, when the drug interacts with the base, it produces primary degradation in the desirable range. NaOH or KOH (0.5–1 N) is widely used for base analysis. Hydrogen peroxide is widely used for oxidative degradation. The drug structure will allow selecting the concentration and condition of the oxidizing agent. In photolytic degradation, the canagliflozin was open to direct sunlight to calculate degradation. The drug was used at different intervals and injected into a system to determine the degradation of the drug. In thermal degradation, according to ICH Q1A accelerated testing conditions, it should be carried out in dry heat or wet heat. The study may be conducted at high temperatures for a short period. The result was tabulated in Table 10.

Table 10: Table for Stress degradation studies of Canagliflozin

Type	Condition	Retention time	Peak Area	Assay (%)	Degradation (%)
Control	-	5.856	308246	100	-
Acid	0.5N HCL at 60°C for 1 hr	5.876	229969	74.606	25.394
Base	0.5N NaOH at 60°C for 1 hr	5.8	158602	51.453	48.547

Peroxide	3% H_2O_2 at 60° for 1 hr	2.323 5.812	244173 294713	79.214 95.610	20.786 4.390
Thermal	At 60°C for 1 hr	5.81	294651	95.590	4.410
Photolytic	In sunlight for 3 hr	5.812	281702	91.389	8.611

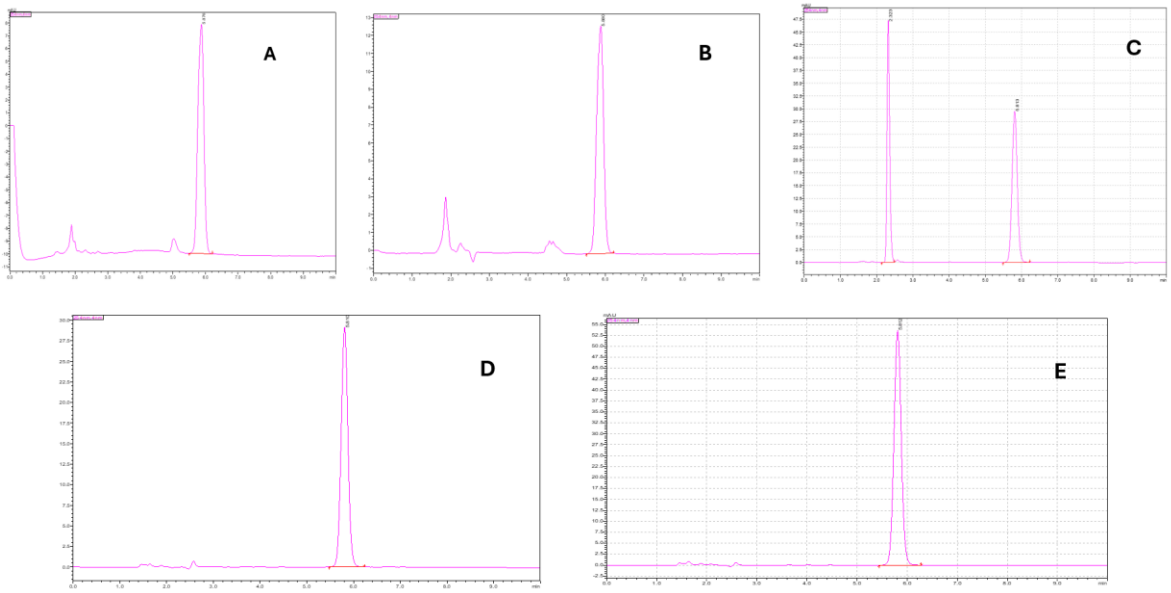

Figure 7. HPLC chromatograms for stressed samples of canagliflozin. (A) 0.5N HCl at 60°C for 1 hr (B) 0.5N NaOH at 60°C for 1 hr, (C) 3 % H_2O_2 at 60°C for 1 hr, (D) Thermal stress at 60°C for 1 hr, (E) Photolytic stress under sunlight for 3 hr.

Figure 7 presents the chromatographic profiles of Canagliflozin subjected to various stress degradation conditions in accordance with ICH guidelines to evaluate the stability-indicating capability of the developed RP-HPLC method Exposure to 0.5N HCl at 60°C for 1 hour resulted in significant degradation, with a 25.39% decrease in assay value, indicating susceptibility to acidic conditions. Treatment with 0.5N NaOH at 60°C for 1 hour led to the highest level of degradation (48.55%), confirming that Canagliflozin is highly unstable under basic conditions. Incubation with 3% hydrogen peroxide at 60°C for 1 hour produced two peaks, one of which corresponds to the degraded product (20.79% degradation), indicating moderate sensitivity to oxidative stress. Heating at 60°C for 1 hour caused minimal degradation (4.41%), suggesting that Canagliflozin is relatively stable under thermal stress. Exposure to sunlight for 3 hours resulted in 8.61% degradation, showing some sensitivity to light-induced stress. Appearance of additional peaks and reduced assay percentages across conditions demonstrates the method's ability to separate the parent compound from its degradation products effectively.

These results confirm that the developed RP-HPLC method is stability-indicating and suitable for detecting Canagliflozin degradation under various stress environments.

❖ **Assay of Tablets**

The validated method was applied to determine Canagliflozin in commercially available Suliseni 100 mg, and its percentage assay for the drug was determined to be between 98.63 and 102.27. The tablet analysis results are given Table 11.

Table 11 : Assay of Canagliflozin

Drug	Labelled claim (mg)	Found (mg)	Purity (%)	RSD (%)
Canagliflozin	100 mg	99.22	99.22	1.15

Discussion

The present study aimed to develop and validate a simple, cost-effective, and reliable RP-HPLC method for the quantitative estimation of canagliflozin in bulk and tablet formulations. The findings revealed several favorable aspects that highlight the efficacy of the method developed. The method showed excellent linearity in the concentration range of 1–30 µg/mL, with a correlation coefficient (r^2) of 0.9988, indicating a strong linear response. The precision of the method, both intra-day and inter-day, yielded %RSD values below 2.2%, demonstrating high reproducibility. Moreover, the assay values of the marketed formulation were found to be between 98.63% and 102.27%, confirming the accuracy and suitability of the method for routine quality control.

Compared to previously reported methods, the present approach offers a better detection range and improved sensitivity. For instance, Swapna et al. (2021) reported a linearity range of 100–500 µg/mL, which is significantly higher and less suited for low-dose analysis. Similarly, the method by Maddu et al. (2014) using RP-HPLC covered a range of 10–200 µg/mL. In contrast, the present study effectively captures lower concentrations (1–30 µg/mL), which is more appropriate for analytical applications involving trace analysis or pharmacokinetic studies. The UV-spectrophotometric method developed alongside the RP-HPLC analysis also

showed good linearity (1–25 µg/mL, r^2 = 0.996) and could serve as an alternative or preliminary screening tool. However, compared to RP-HPLC, the spectrophotometric method lacked robustness in complex matrices and offered lower specificity. In terms of method validation, the LOD and LOQ were found to be 0.177 µg/mL and 0.536 µg/mL, respectively, which are markedly lower than values reported in earlier literature, especially when compared to methods based on HPTLC or UV detection that generally have higher detection thresholds. This makes the current method highly suitable for detecting trace levels of the drug.

The specificity studies showed no interference from excipients or degradation products, further reinforcing the method's applicability for stability testing and formulation analysis. Additionally, the robustness of the method was confirmed under deliberate variations in flow rate and mobile phase composition, with %RSD values remaining below 2%, affirming its consistency under slight procedural changes. On the less favorable side, the total runtime of 10 minutes is longer than some other reported methods (e.g., Maddu et al.'s 6-minute runtime and Darshan Bhatt et al.'s LC-MS method with high sensitivity and faster run times). However, this trade-off ensures better resolution and peak symmetry, particularly important in complex matrix evaluations.

Stress degradation studies confirmed the stability-indicating nature of the method. The drug showed significant degradation under acidic and basic conditions, with degradation percentages of 25.39% and 48.54%, respectively. The method effectively separated the degradation products, unlike some earlier studies that did not incorporate forced degradation assessments. This reinforces its utility for stability studies as per ICH Q1A(R2) guidelines.

In summary, the developed RP-HPLC method demonstrates strong analytical performance and aligns well with or surpasses several previously established methods. While the slightly extended runtime could be a limitation in high-throughput environments, its advantages in terms of sensitivity, specificity, robustness, and regulatory compliance outweigh this drawback. It can thus be recommended for routine use in pharmaceutical quality control and bioanalytical applications involving canagliflozin.

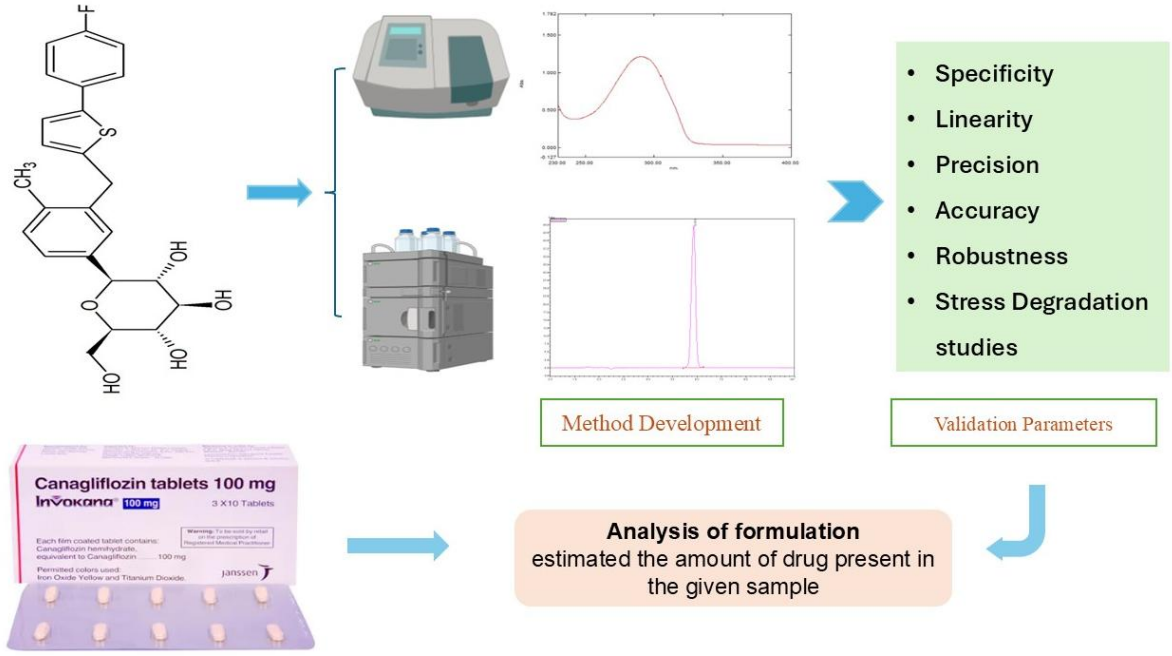

Figure 8: Graphical Representation of the analytical workflow for the development and validation of an RP-HPLC method for canagliflozin

Figure 8 illustrates the overall approach used in the study, starting with the chemical structure of Canagliflozin, followed by UV and RP-HPLC-based method development. The developed method was validated as per ICH guidelines for parameters including specificity, linearity, precision, accuracy, robustness, and stress degradation studies. Finally, the validated method was applied to estimate the drug content in marketed tablet formulations.

CONCLUSION

This study focused on developing and validating an RP-HPLC method for estimating Canagliflozin in API and its pharmaceutical dosage form. The experimental data obtained concluded that the chromatographic method developed for canagliflozin estimation was simple, precise, accurate, and sensitive, with good reproducibility and recovery. All parameters were validated according to ICH guidelines and were found to be within the acceptance criteria. Therefore, the developed method may be recommended for the analysis of quality control departments in industry to determine Canagliflozin in API and its pharmaceutical dosage form.

AUTHOR CONTRIBUTIONS

G. Ramya Priya: Investigation, methodology, validation. Nalanda Revu: Conceptualization, Writing-original draft, review and editing, supervision.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY The data supporting the findings of this study are available within the article. No external datasets were generated or analysed during the current study.

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