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Development and Validation of a Robust UPLC Method for the Concurrent Determination of Metformin and Canagliflozin in Combined Tablet Dosage Forms

Karicharla SreeYamini Kalpana[1]; Peram Divya[2]; Sanagam Shambhavi[3]; Dandra Niveditha[4]; Dr. Srinivas Chinta[5*]

[5]Assistant Professor
[1;2;3;5]Department of Pharmaceutical Analysis, JNTUH UCPSC-Sulthanpur, Sanga Reddy, TG.
[4]Department of Pharmaceutical Analysis, Vaagdevi Pharmacy College, Bollikunta, Warangal, TG.

Corresponding Author: Dr. Srinivas Chinta[5*]

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Abstract:

- ***Background:***

Because of the complimentary ways in which Metformin and Canagliflozin work together, fixed-dose combination pills are frequently used to treat type 2 diabetes mellitus. For routine quality control of such formulations, a rapid, reliable, and sensitive analytical method is required.

- ***Aim and Objective:***

In compliance with ICH recommendations, the current work sought to create and validate a straightforward, accurate, precise, and robust Ultra Performance Liquid Chromatography (UPLC) method for the simultaneous estimation of metformin and canagliflozin in tablet dose form.

- ***Methods:***

Using a mobile phase of phosphate buffer (pH 3.0) and acetonitrile (60:40, v/v) at a flow rate of 0.3 mL/min, chromatographic separation was accomplished on a C18 column. UV detection was carried out at 230 nm for metformin and 264 nm for cnagliflozin. The method was validated for specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness.

- ***Results and Discussion:***

Metformin and Canagliflozin were well separated with retention times of approximately 2.10 min and 3.53 min, respectively, and a resolution greater than 3.0. The method showed excellent linearity in the concentration ranges of 10–100 µg/mL for Metformin and 1–20 µg/mL for Canagliflozin with correlation coefficients (r^2) of 0.999 and 0.999, respectively. Precision studies showed %RSD values below 1%, indicating good repeatability. Accuracy studies yielded recoveries in the range of 99.0–100.8% for Metformin and 99.0–100.6% for Canagliflozin. The LOD and LOQ were found to be 0.12 and 0.36 µg/mL for Metformin and 0.2 and 0.73 µg/mL for Canagliflozin, respectively, demonstrating good sensitivity. Assay of the marketed tablet formulation showed drug content of 99.7% for Metformin and 101.2% for Canagliflozin.

- ***Conclusion and Outcome:***

The developed UPLC method is simple, rapid, accurate, precise, and sensitive, and is suitable for routine quality control analysis of Metformin and Canagliflozin in combined tablet dosage forms.

Keywords: *UP-LC, Metformin, Canagliflozin, Simultaneous Estimation, Validation, Tablet Dosage form.*

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I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. Metformin hydrochloride (MET) is widely recommended as a first-line oral antidiabetic agent due to its ability to reduce hepatic glucose production and improve peripheral insulin sensitivity [1]. Canagliflozin (CANA) belongs to the class of sodium–glucose co-transporter-2 (SGLT2) inhibitors, which lower blood glucose levels by promoting urinary glucose excretion through inhibition of renal glucose reabsorption [2].

Because MET and CANA work through complimentary pathways, they enhance glycemic control and provide other benefits like weight loss and a lower risk of hypoglycemia, making their combination therapeutically useful [3,4]. As more people are being prescribed fixed-dose combination tablets with MET and CANA to treat type 2 diabetes, accurate and quick analytical techniques are crucial for ensuring the pharmaceutical formulations' quality [5].

Numerous analytical techniques, primarily utilizing reversed-phase high-performance liquid chromatography (RP-HPLC), have been documented for the simultaneous measurement of metformin and canagliflozin in bulk and dose forms [6–8]. These techniques frequently require longer run durations and higher solvent consumption, despite offering respectable accuracy and precision. Compared to traditional HPLC, Ultra-Performance Liquid Chromatography (UPLC) has several benefits, such as improved resolution, quicker analysis times, and less solvent use[9].

LC-MS/MS techniques for the simultaneous measurement of metformin and canagliflozin have also been reported in a few publications, primarily for pharmacokinetic and bioanalytical purposes [10]. Nevertheless, these methods are costly and not necessarily appropriate for standard quality control labs. Therefore, for the simultaneous assessment of MET and CANA in tablet dosage forms, a straightforward, quick, economical, and reliable UPLC approach is required.

In accordance with ICH Q2(R1) guidelines, the current study intends to develop and validate a UPLC method for the simultaneous determination of metformin and canagliflozin in mixed tablet dosage forms and to show that it is applicable for routine quality control analysis[11].

II. MATERIALS AND METHODS

➢ *Chemicals and Reagents*

During this inquiry, high purity (more than 99%) reference standards for metformin hydrochloride and canagliflozin were used. The mobile phase and sample solutions were prepared using organic solvents such as HPLC-grade acetonitrile and methanol. The buffer solution was prepared and its pH was adjusted using potassium dihydrogen phosphate and orthophosphoric acid. All aqueous solutions were prepared using water that had been purified using a Milli-Q purification system in order to ensure exceptional purity and avoid interference during chromatographic analysis.

➢ *Instrumentation*

An Ultra Performance Liquid Chromatography (UPLC) system with a column oven, autosampler, binary solvent supply pump, and UV detector was used to perform the chromatographic study. A 50 × 2.1 mm Acquity BEH C18 column with a particle size of 1.7 µm was used to achieve separation. Empower software or a similar chromatographic data handling system was used for data collecting and processing.

➢ *Determination of Maximum Wavelength (λmax)*

The UV absorption spectra of Metformin and Canagliflozin were recorded in the range of 200–400 nm using a UV–Visible spectrophotometer. Each drug was scanned separately in a suitable solvent. UV spectral analysis of Metformin and Canagliflozin showed maximum absorbance at 230 nm and 264 nm, respectively.

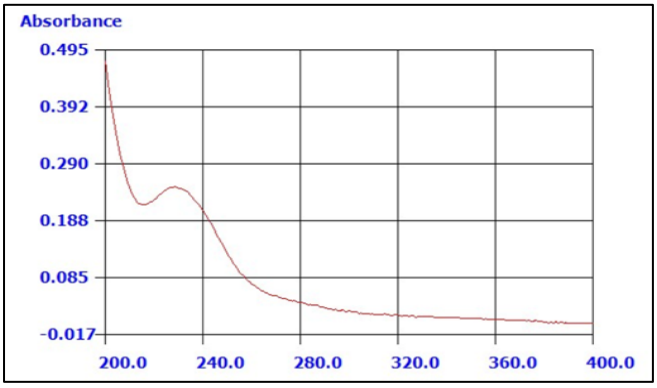

Fig 1 UV Maximum Absorbance of Metformin

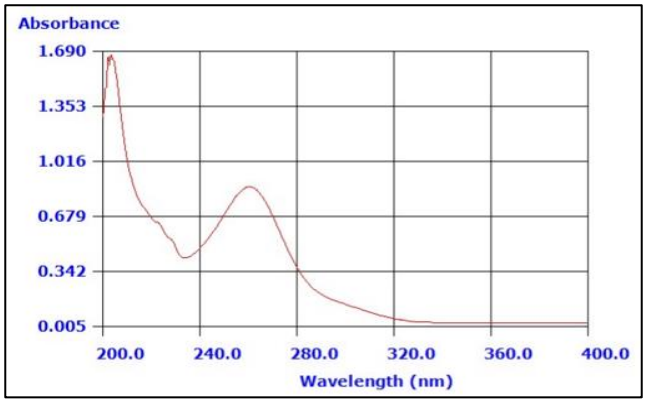

Fig 2 Maximum absorbance of Canagliflozin

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Table 1 Chromatographic Conditions for Metformin

Parameter	Condition
Mobile phase	Phosphate buffer (pH 3.0): Acetonitrile (60:40 v/v)
Column	C18 (50 × 2.1 mm, 1.7 µm)
Flow rate	0.3 mL/min
Detection wavelength	230 nm
Volume of Injection	2 µL
Temperature of the column	30 ℃

Table 2 Chromatographic Conditions for Canagliflozin

Parameter	Condition
Mobile phase	Phosphate buffer (pH 3.0): Acetonitrile (60:40 v/v)
Column	C18 (50 × 2.1 mm, 1.7 µm)
Flow rate	0.3 mL/min
Detection wavelength	264 nm
Injection volume	2 µL
Column temperature	30 ℃

➢ *Preparation of Standard Solutions*

Accurately weighed quantities of Metformin (100 mg) and Canagliflozin (10 mg) were separately transferred into suitable volumetric flasks. The drugs were dissolved in the mobile phase and the volumes were made up to the mark with the same solvent to obtain stock solutions having concentrations of 1000 µg/mL for Metformin and 100 µg/mL for Canagliflozin.

From these stock solutions, appropriate aliquots were further diluted with the mobile phase to prepare working standard solutions of the required concentrations for construction of the calibration curves.

➢ *Preparation of Sample Solutions*

After weighing and powdering twenty tablets, 500 mg of metformin and 50 mg of canagliflozin were added to a 100 mL volumetric flask, sonicated with mobile phase, and filtered. In order to produce final concentrations for examination, the filtrate was suitably diluted.

III. METHOD VALIDATION

The appropriateness of the devised UPLC approach for the simultaneous measurement of metformin and canagliflozin was demonstrated by validation in compliance with recognized analytical parameters. Specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and resilience were among the validation parameters.

➢ *Specificity*

The specificity of the method was evaluated by analyzing blank, placebo, and sample solutions. To confirm that there were no interfering peaks at the retention times associated with metformin and canagliflozin, the resultant chromatograms were closely inspected. This study proved that the approach is specific for both medications by confirming that the excipients and other ingredients in the formulation did not obstruct the analytes' detection.

➢ *Linearity*

For each medication, calibration standards were prepared at five distinct concentration levels in order to evaluate the method's linearity. The concentration ranges for canagliflozin and metformin were 1–20 µg/mL and 10–100 µg/mL, respectively. Following the injection of each solution into the UPLC system, the associated peak areas were noted. Peak area was plotted versus concentration to create calibration curves, and to evaluate the linear relationship, linear regression analysis was employed. Based on the response consistency and correlation coefficient, the approach was deemed linear over the examined ranges.

➢ *Accuracy (Recovery)*

The accuracy of the method was evaluated using recovery assessments using the standard addition approach. Previously examined samples were supplemented with known concentrations of metformin and canagliflozin standards at three different levels: 80%, 100%, and 120% of the nominal concentration. Following analysis of the tampered samples, the % recovery of each substance was determined. The relative standard deviation (%RSD) and percent recovery were used to express the method's accuracy, demonstrating how closely the measured values matched the genuine values.

➢ *Precision*

Repeatability (intra-day precision) and intermediate precision (inter-day precision) were used to assess the method's precision. Six replicate injections of the identical sample solution were examined in a single day to ensure repeatability. The analysis was conducted again on different days for intermediate precision. Low %RSD readings showed that the procedure is accurate and consistently yields results under typical operating conditions. The %RSD was calculated using the peak areas obtained.

➢ *Limits of Quantification (LOQ) and Detection (LOD)*

The sensitivity of the approach was assessed by determining the LOD and LOQ for both drugs. These parameters were obtained using the signal-to-noise method.The LOD is equivalent to a signal-to-noise ratio of

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roughly 3:1, while the LOQ is equivalent to a ratio of roughly 10:1. These numbers represent the lowest levels of canagliflozin and metformin that the suggested approach can accurately detect and measure.

➢ *Robustness*

Small, intentional changes to the chromatographic conditions—such as adjustments to the flow rate, mobile phase composition, and detection wavelength—were used to test the method's robustness. We looked at how these differences affected assay findings and system performance. When retention duration, peak shape, or assay values did not significantly vary, the method was deemed robust, indicating that it is still dependable even when experimental conditions somewhat alter.

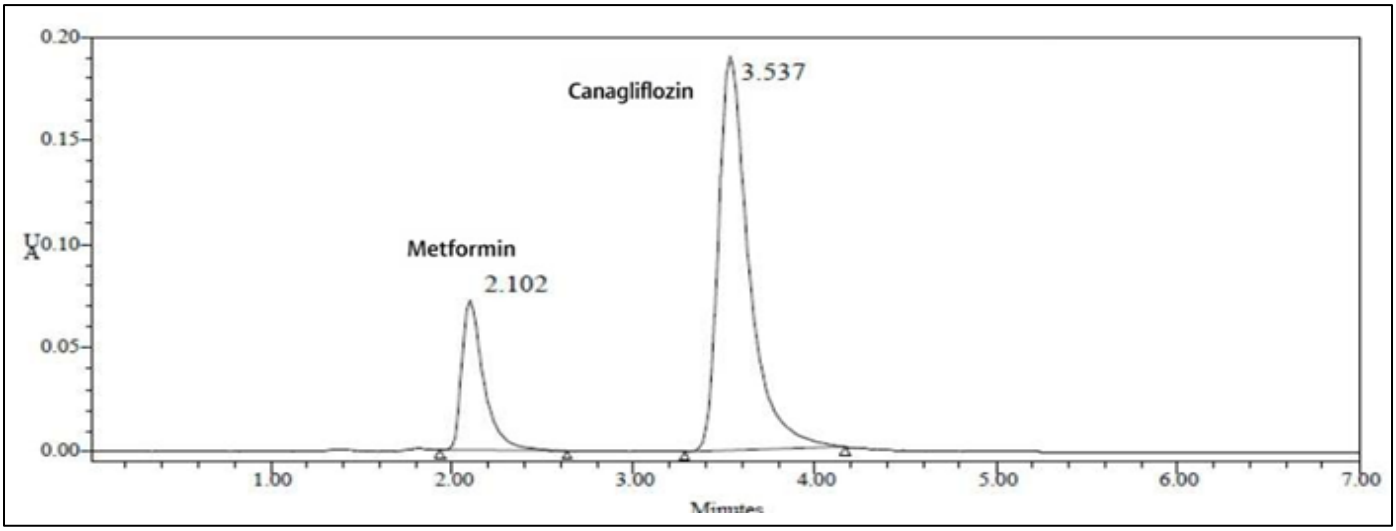
IV. RESULTS AND DISCUSSION

➢ *System Suitability Studies*

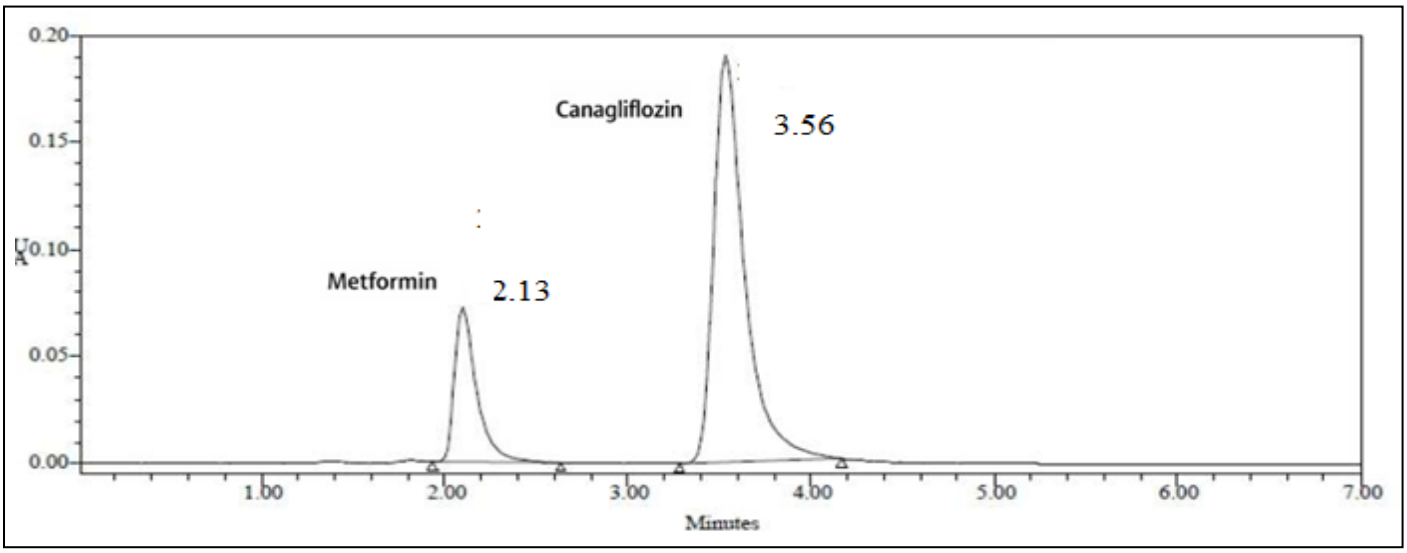
The system suitability properties were evaluated using six injections of the standard solution. Parameters such as retention time, peak area, theoretical plates, tailing factor, and resolution were calculated to ensure the chromatographic system was suitable.

Table 3 Studies on System Suitability Parameters

Parameter	Metformin	Canagliflozin
Retention time (min)	2.102	3.53
Theoretical plates (N)	5200	6100
Tailing factor	1.12	1.09
Resolution	—	3.5
%RSD of peak area	0.62	0.58



a)



b)

Fig 3 UPLC Chromatograms for Metformin and Canagliflozin Standard Solution (a) and Tablet Solution (b)

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Peak areas' %RSD values were less than 2%, which suggests that the system is highly precise. Good column efficiency and peak symmetry were confirmed by theoretical plates and tailing factors that fell within allowable bounds. There was sufficient separation between the canagliflozin and metformin peaks, as indicated by a resolution of more than 2.

➢ *Linearity*

By creating standard solutions of metformin and canagliflozin at five distinct concentration levels within their respective operating ranges, the linearity of the UPLC method was evaluated. The concentration range chosen was 10–100 µg/mL for metformin and 1–20 µg/mL for canagliflozin.

Each concentration level was analyzed under the optimized chromatographic conditions, and the corresponding peak areas were recorded. Calibration curves were constructed by plotting peak area against the respective concentrations of each drug. The linear relationship between concentration and detector response was evaluated using regression analysis, demonstrating that the method provides a proportional and consistent response over the selected concentration ranges.

Table 4 Calibration Data for Metformin

Concentration (µg/mL)	Peak Area
10	152340
25	381200
50	758900
75	1122000
100	1504200

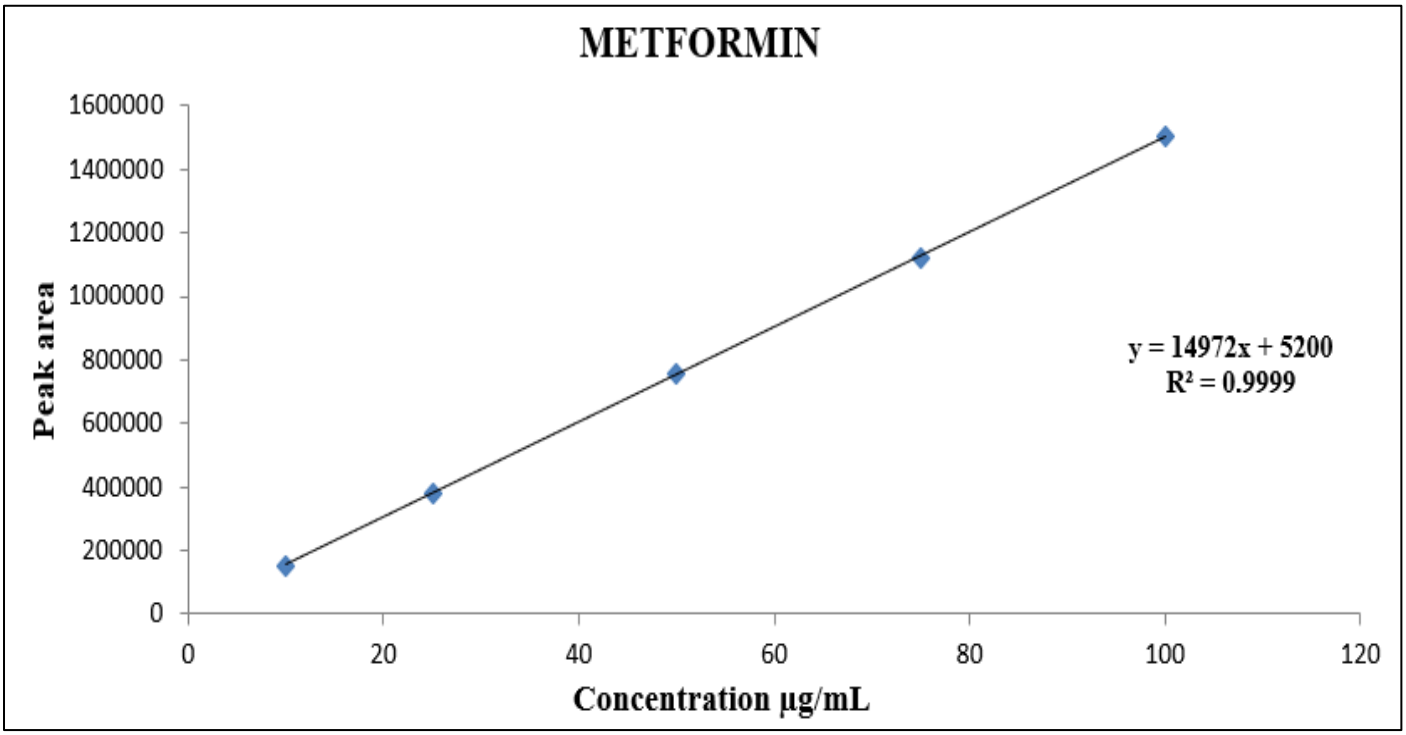

Fig 4 Standard Graph for Metformin

Table 5 Calibration Data for Cana Gliflozin

Concentration (µg/mL)	Peak Area
1	84560
5	412300
10	825600
15	1199200
20	1652100

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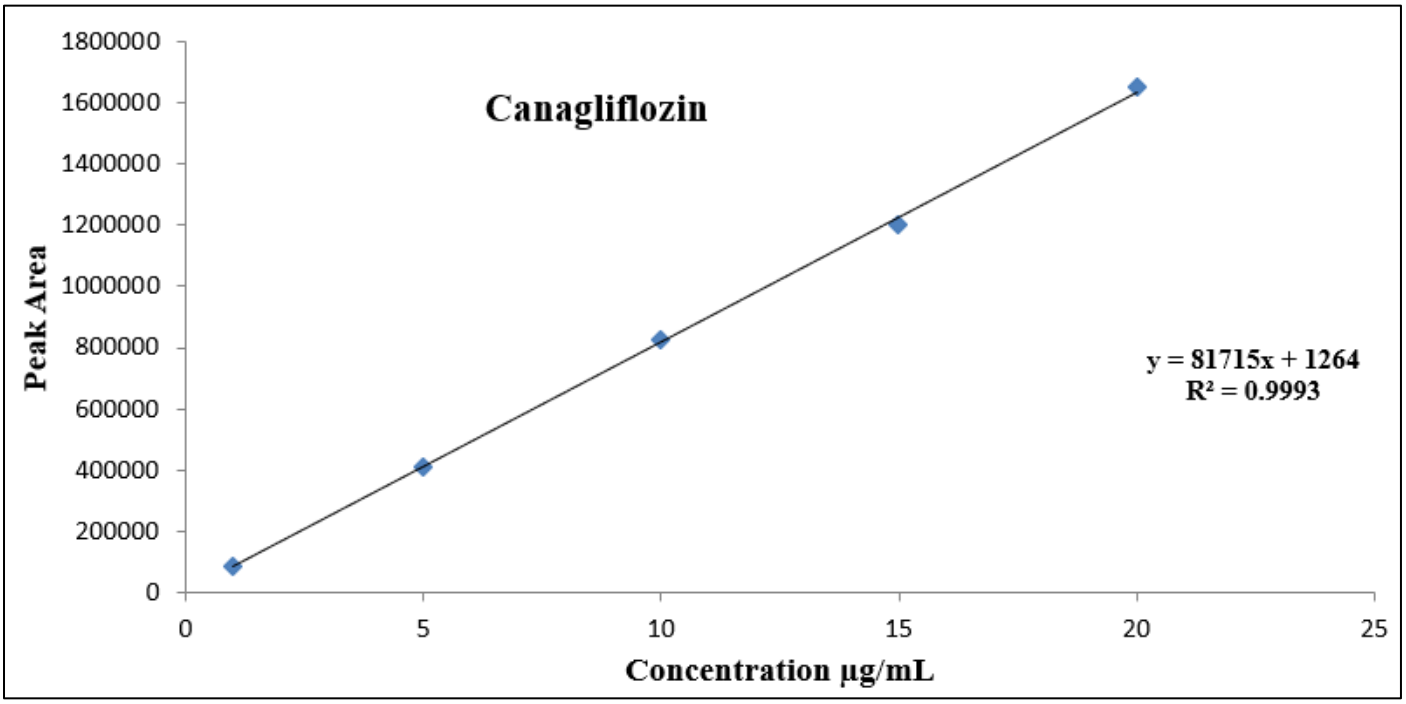

Fig 5 Standard Graph for Canagliflozin

Both drugs showed excellent linearity over the studied range with correlation coefficients greater equals to 0.999, indicating a direct proportional relationship between concentration and peak area.

➢ *Precision*

Precision was evaluated as repeatability by analyzing six replicate injections of the same sample solution.

Table 6 Precision Data for Metformin

Injection	Peak Area
1	758900
2	761200
3	756800
4	759500
5	757900
6	760300
Mean	759100
%RSD	**0.22%**

Similarly, for Canagliflozin, %RSD was found to be 0.35%. Since %RSD values were less than 2%, the method is considered precise and reproducible.

➢ *Accuracy in Recovery Study*

The recovery assessments at 80%, 100%, and 120% levels were used to determine accuracy.

Table 7 Accuracy Data for Metformin

Level	Amount added (mg)	Amount found (mg)	% Recovery
80%	40	39.6	99.0
100%	50	50.4	100.8
120%	60	59.5	99.2

Table 8 Accuracy Data for Canagliflozin

Level	Amount added (mg)	Amount found (mg)	% Recovery
80%	4	3.96	99.0
100%	5	5.03	100.6
120%	6	5.95	99.2

The recovery values for both drugs were in the range of 98–102%, indicating that the method is accurate and free from interference by excipients.

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➢ *LOD and LOQ*

LOD and LOQ were determined based on the standard deviation of the response and slope method. The low LOD and LOQ values for Metformin 0.12µg/m Land 0.36µg/mL, whereas canagliflozin 0.2 µg/mL, and 0.73µg/mL, indicate that the method is sensitive enough for the estimation of both drugs in tablet dosage forms.

➢ *Assay of Tablet Formulations*

The assay results were within acceptable limits (98–102%), confirming the suitability of the method for routine quality control analysis and which was depicted in the table 8.

Table 9 Assay of Tablet Formulations

Drug	Amount found (mg)	% Assay
Metformin(500mg)	498.5	99.7%
Canagliflozin (Prominad) (100mg)	100.6	100.6%

➢ *Robustness Results for Both Drugs*

Table 10 Robustness Results for Both Drugs

Parameter Varied	Condition	%RSD (Metformin)	%RSD (Canagliflozin)	Observation
Flow rate	0.28 mL/min	0.64	0.72	No significant change
Flow rate	0.32 mL/min	0.59	0.68	Acceptable
Mobile phase	58:42 (v/v)	0.71	0.80	Good resolution
Mobile phase	62:38 (v/v)	0.66	0.77	Stable peaks
Wavelength	223 nm	0.73	0.85	Acceptable response
Wavelength	227 nm	0.65	0.79	No interference

The %RSD of peak area values under all varied conditions was found to be less than 2.0%, indicating that the quantification of Metformin was not affected by these minor changes, whereas the %RSD of peak area values for Canagliflozin was also below 2.0%, confirming that the method remained precise and reliable and were showed them results in the table, 9.

V. CONCLUSION

The simultaneous measurement of metformin and canagliflozin in tablet dose form was accomplished by the successful development and validation of a straightforward, quick, accurate, and precise UPLC method. The technique is suitable for routine quality control analysis in the pharmaceutical industry and conforms to ICH guidelines.

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