RESEARCH ARTICLE

Preformulation and UV-Spectrophotometric Analytical Method Validation Studies for Assessment of Canagliflozin

Saurabh Shukla[1], Neelam Sharma[2*], Sukhbir Singh[2*], Sandeep Arora[3], Anita Rani[1,4]

[1]*Chitkara College of Pharmacy, Chitkara University, Punjab, India*
[2]*Department of Pharmaceutics, MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana, India*
[3]*Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India*
[4]*Janta College of Pharmacy, Butana, Sonipat, Haryana, India*

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ABSTRACT

The objectives of the present investigation were the physicochemical examination of canagliflozin to authenticate the drug's characteristics and validation of straightforward and rapid ultraviolet-spectroscopy technique for evaluation of canagliflozin in phosphate buffer, pH 6.8. X-ray diffraction study revealed numerous peaks i.e. 11.41, 16.35, 16.5, 16.65, 18.53, 18.58, 19.55, 20.11, 20.46, 20.71, 21.62, 23.96, 24.17, 24.77, 26.5, 27.08, 27.68, 28.22, 28.73 and 32.6 at °2θ which demonstrated extremely crystalline character of drug. The regression equation for the standard plot was Y = 0.01957X + 0.036, and correlation coefficient was 0.9936. The method was found linear as indicated by correlation coefficient values of 0.9933, 0.9938, and 0.9943 for three consecutive standard plots. The average recovery of Canagliflozin was found to be 100.033% which was inside the prerequisite specification of the range 98 to 102%. The percentage relative standard deviation for repeatability, inter-day precision, intermediate precision, and robustness was less than 2%. The limit of detection (LoD) for Canagliflozin was 2.38 µg/mL, and the limit of quantitation was 7.24 µg/mL, which established the sensitivity of the UV-spectroscopy analytical method. In conclusion, the validation parameters like linearity, accuracy, repeatability, inter-day precision, intermediate precision, robustness, and sensitivity were found acceptable for the UV-spectroscopy analytical technique for analysis of Canagliflozin.

Keywords: Canagliflozin, Intermediate precision, Inter-day precision, Limit of detection, Limit of quantitation, Robustness, Ultraviolet-spectroscopy

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INTRODUCTION

Canagliflozin (CGF) (Figure 1) is orally available inhibitor of sodium-glucose cotransporter-2 (SGLT-2) prescribed for type-2 diabetes mellitus therapy.[1-3] Various techniques have been established for the estimation of CGF alone or in combination with metformin in pharmaceutical and bulk products. The liquid chromatography,[4] reverse phase-high performance liquid chromatography (RP-HPLC),[5,6] and HPLC[7,8] were reported for the estimation of CGF. Several analytical procedures were also established for the estimation of CGF[9] or simultaneous bio-analytical estimation of Metformin and CGF in human plasma like RP-HPLC[10] and liquid chromatography/tandem mass spectrometry (LC-MS/MS).[11] In another research, the comparative investigation between ultraviolet-visible spectroscopy and HPLC for the estimation of CGF in organic solvent was established.[12] In the present investigation, physicochemical characterization and x-ray diffraction analysis of CGF was executed to confirm drug's solubility profile and crystalline nature. Furthermore, the ultraviolet-spectroscopy technique was established for the estimation of CGF in phosphate buffer, pH 6.8. The objective of the study includes validation of a straightforward and rapid UV-spectroscopic method in phosphate buffer, pH 6.8 according to International Conference of Harmonization guidelines Q2 (R1).[13]

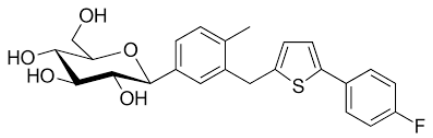

Figure 1: Chemical structure of canagliflozin

**Author for Correspondence: singh.sukhbir12@gmail.com*

MATERIALS AND METHODS

Materials

Canagliflozin was purchased from Tripurrte Pharmaceuticals Private Limited, Hyderabad, Telangana. Sodium hydroxide and potassium dihydrogen phosphate were purchased from Loba Chemie Pvt. Ltd, Mumbai (India). All the chemicals utilized were of analytical grade.

Physicochemical Characterization of Canagliflozin

Melting point of CGF was estimated through the capillary method.[14] The drug was heated at 105°C for three hours, and percentage loss on drying was calculated using Eq. 1.[15] Partition coefficient was determined using n-hexane as the organic phase, and water as the aqueous phase by the shake flask method using Eq. 2.[16] Solubility study was executed by the equilibrium solubility method. The excess quantity of the drug was dissolved in water, methanol, ethanol, dimethyl sulfoxide, and phosphate buffers in screw-capped vials and agitated for 24 hours in an orbital shaker (Remi, India) at 37°C to attain equilibrium. The samples were passed across 0.45 µm filter and analyzed.[17,18]

$$\%\ \text{Loss on drying} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100 \qquad \text{Eq. (1)}$$

$$\text{Partition coefficient} = \frac{\text{amount of drug in organic phase}}{\text{amount of drug in aqueous phase}} \qquad \text{Eq. (2)}$$

X-ray Diffraction (XRD) Study

XRD of CGF was acquired using a diffractometer (Xpert-Pro) in a constant scan manner at 2θ scale from 5° to 50° using 1.54 A° and 1.39 Ao radiations emitted from CuKα and CuKβ, respectively, produced through tube operated at 45 kV and 40 mA.

Preparation of Standard Plot

A total of 10 mL of CGF was dissolved in 100 mL phosphate buffer (pH 6.8) in a 100 mL volumetric flask to prepare a 100 µg/mL buffer strength. Different aliquots of CGF in 0.5–2 mL range were relocated into a succession of 10 mL volumetric flasks, and the level was adjusted till the mark with buffer to acquire concentrations of 5–40 µg/mL, respectively. The standard plot was constructed as concentration versus absorbance.[19]

Analytical Method Validation

Validation is the technique of establishing benchmark verification which imparts prominent assurance that developed analytical techniques can generate a required outcome. The UV spectroscopy method was validated in requisites of linearity, accuracy, repeatability, inter-day precision, intermediate precision, sensitivity, and robustness.[20]

Linearity

The absorbance of CGF solutions 5–40 µg/mL was estimated in triplicate. The three standard plots were acquired using different absorbance values and estimated for regression equation and correlation coefficients to determine linearity.[19,21]

Accuracy

The amount of drug was analyzed in 5–40 µg/mL solutions of CGF and calculated for the percentage of drug recovered using a regression equation to assess the accuracy of an analytical method. To establish the accuracy of the analytical technique, the calculated percentages of drug recovered should be within the constraint specification of 100 ± 2%.[20]

Repeatability and Inter-day Precision

The absorbance of 5–40 µg/mL CGF solutions was estimated at three different time periods within a day (intra-day) and on three different days (inter-day). The %RSD was calculated for absorbance values to investigate intra-day and inter-day precision. The validation was established on the predetermined standard of %RSD should be less than 2%.[22]

Intermediate Precision

The intermediate precision was validated to ascertain that the analytical method remains unaltered under conditions of different analysts or equipment. The absorbance values of 5–40 µg/mL CGF were determined through different scientists on identical equipment and also by single analysts using different versions of Systronics equipment (AU-2701 and 2202), Ahmedabad, India. The %RSD was calculated to determine intermediate precision. The set standard for the establishment of validation of intermediate precision was that RSD should be < 2%.[23]

Robustness

LoD, and LoQ

UV-spectroscopy analyzed the 5–40 µg/mL samples of CGF at three wavelengths, *i.e.*, 290 ± 15 nm, and temperatures, *i.e.*, 25 ± 10°C. The %RSD was determined for estimated absorbance values. The following formula calculated the LoD and LoQ of CGF:

$$L\ (oD = 3.3\sigma)/S \qquad \text{Eq. (3)}$$

$$(LoQ = 10\sigma)/S \qquad \text{Eq. (4)}$$

Where 'S' and 'σ' are the slope and standard deviation of the *y*-intercept of the regression equation, respectively.[22,24]

RESULTS AND DISCUSSION

Physicochemical Characterization of Canagliflozin

The values of melting point, loss on drying, and partition coefficient of CGF were found 70 ± 2°C, 0.41%, and 3.1, respectively, which were found in compliance with theoretical specifications. The CGF was found to be slightly soluble in ethanol, dimethyl sulfoxide, methanol, and phosphate buffer (pH values 5.8/6.8/7.4). However, CGF was found practically insoluble in water (Figure 2).[15,25,26]

X-ray Diffraction Study

The numerous peaks *i.e.* 11.41, 16.35, 16.5, 16.65, 18.53, 18.58, 19.55, 20.11, 20.46, 20.71, 21.62, 23.96, 24.17, 24.77, 26.5, 27.08, 27.68, 28.22, 28.73 and 32.6 at °2θ were observed in XRD pattern of CGF which illustrated highly crystalline characteristics of drug (Figure 3 and Table 1).[27,28]

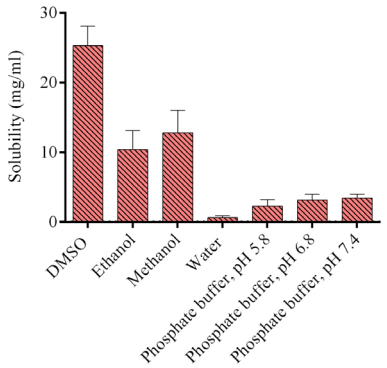

Figure 2: Solubility profile of canagliflozin in different organic and aqueous solvents

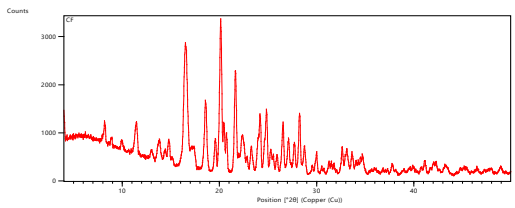

Figure 3: X-ray diffraction graph of canagliflozin at 2θ° scale

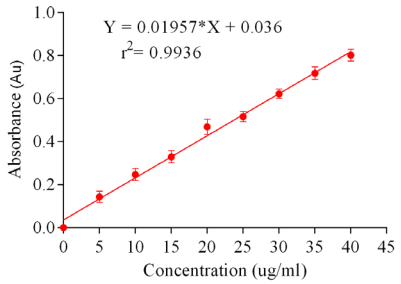

Figure 4: Calibration plot of canagliflozin in pH 6.8-phosphate buffer through UV- spectroscopy technique illustrating regression equation and regression coefficient

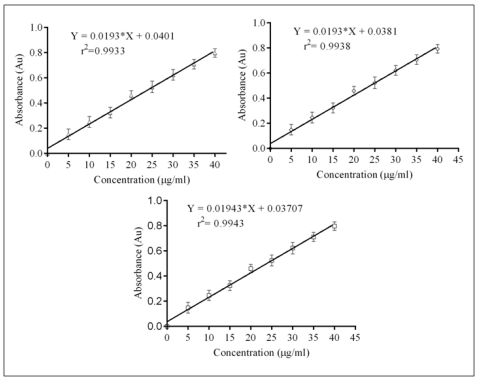

Figure 5: Illustrations for validation of linearity parameter of UV-spectroscopy process for assessment of canagliflozin in phosphate buffer, pH 6.8

Table 1: X-ray diffraction peaks and their relative intensity in canagliflozin

Position (2θ°)	*'d-spacing' in Å*	*Relative intensity (%)*	*Area (cts * 2θ°)*
11.4182	7.74342	23.42	244.37
16.3561	5.41511	30.49	266.24
16.5013	5.36780	45.75	264.46
16.6545	5.31877	39.83	438.89
18.5339	4.78343	19.90	176.24
18.5794	4.77184	26.36	127.19
19.5540	4.53612	19.69	104.29
20.1147	4.41094	100.00	687.02
20.4608	4.33710	31.19	103.65
20.7162	4.28421	24.86	99.29
21.6250	4.10616	66.48	384.67
23.9612	3.71085	22.00	178.68
24.1787	3.67795	38.98	189.41
24.7742	3.59088	37.72	287.23
26.5015	3.36062	33.85	236.32
27.0879	3.28919	24.82	199.69
27.6864	3.21943	22.44	156.45
28.2219	3.15955	44.20	263.31
28.7308	3.10473	18.45	127.38
32.6036	2.74424	21.42	148.71

Standard Plot of Canagliflozin

The absorbance of Canagliflozin concentration, *i.e.,* 5, 10, 15, 20, 25, 30, 35, and 40 µg/mL, was estimated at UV-spectrophotometer using predetermined lambda maximum of 290 nm (Systronics AU-2701, Ahmedabad, India). The graph was plotted between absorbance and concentrations of CGF to generate a standard plot (Figure 4). The regression equation and correlation coefficient for standard plot was Y = 0.01957*X + 0.036 and 0.9936, respectively. The *p*-value for plot was found < 0.0001 (*$p < 0.05$) which established statistical significance of anticipated analytical technique.[29,30]

Linearity

The correlation coefficients for standard plots of Canagliflozin in phosphate buffer, pH 6.8 were 0.9933, 0.9938 and 0.9943 as shown in Figure 5. These values were in compliance with the required specification (R^2 values not less than 0.95) and, therefore, established the linearity of the developed UV spectroscopy technique.[31,32]

Accuracy

The mean percentage CGF recovered from range of 5-40 µg/mL concentrations were found 100.6, 101.1, 100.87, 96.45, 100.44, 100.67, 100.51 and 99.63%, respectively (Figure 6). The estimations were carried out in triplicate (n=3). Since, the average percentage recovery of CGF was estimated to 100.033%, which was within the prerequisite specification of a range of 98–102%. Therefore, accuracy was demonstrated.[33-35]

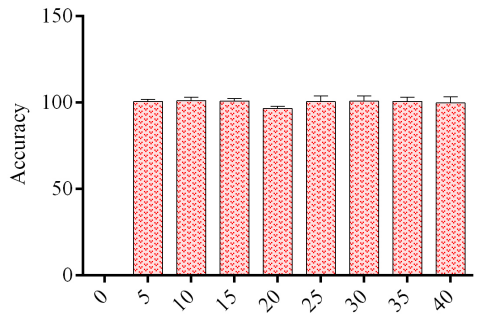

Figure 6: Accuracy validation of UV-spectroscopy method for assessment of canagliflozin

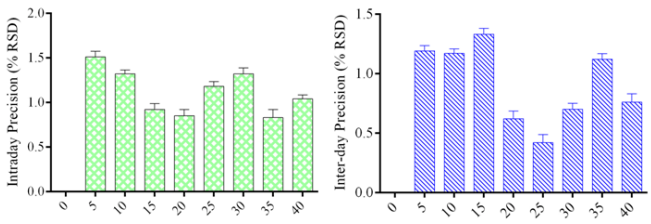

Figure 7: Manifestation of intra-day and inter-day precision of UV spectroscopy technique

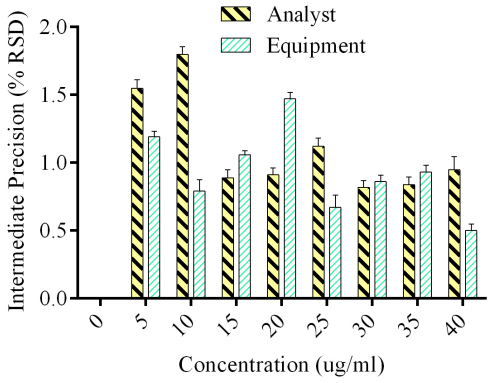

Figure 8: Demonstration of intermediate-precision of UV-spectroscopy procedure

Repeatability and Inter-day Precision

The %relative standard deviation (RSD) for absorbance values of 5, 10, 15, 20, 25, 30, 35, and 40 µg/mL CGF at intra-day was found 1.51, 1.32, 0.92, 0.85, 1.18, 1.32, 0.83 and 1.04% while on inter-day was found 1.19, 1.17, 1.33, 0.62, 0.42, 0.7, 1.12, and 0.76% which were found to be within the specified limits (< 2%) (Figure 7).[34,36]

Intermediate Precision

The intermediate precision validated intra-laboratories deviations in conditions of different analysts and equipment. The calculated %RSD for CGF solutions analyzed by separate equipments and *via* distinct analysts was stipulated within the requisite margins (< 2%), which validated (Figure 8).[37,38]

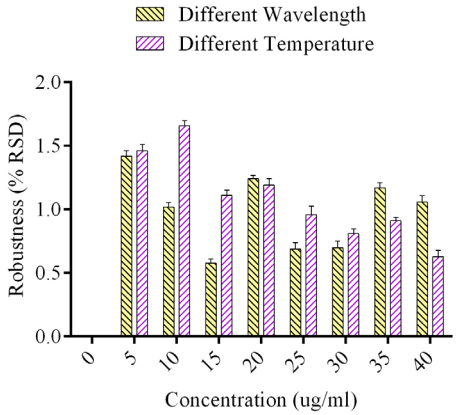

Figure 9: Diagrammatic revelation of the robustness of UV-spectroscopy system

Robustness

The % RSD of absorbance at diverse wavelengths as well as temperatures was less than 2% which revealed that the projected analytical process stayed unaltered through diminutive and intentional deviations in analytical technique parameters (Figure 9).[31,35]

Limit of Detection and Limit of Quantitation

Correspondingly, LoD and LoQ of CGF were found 2.38 and 7.24 µg/mL, which demonstrated enormous sensitivity of the spectroscopy analytical method.[39-41]

CONCLUSIONS

Canagliflozin is SGLT-2 inhibitor with potential use in the treatment of type 2 diabetes mellitus. X-ray diffraction study revealed numerous peaks at °2θ, which verified enormously crystalline nature of the drug. The values of correlation coefficients were 0.9933, 0.9938, and 0.9943 for three successive standard plots, which revealed the linearity of the method. The average recovery of Canagliflozin was 100.033% (within the range of 98–102%), which validated accuracy. The %RSD for inter-day precision, repeatability, intermediate precision, and robustness was <2%. LoD was 2.38 µg/mL, and LoQ was 7.24 µg/mL, which ascertained the sensitivity of current analytical method. Conclusively, this has been manifested that the developed UV-spectroscopy analytical technique was having linearity, accuracy, repeatability, inter-day precision, intermediate precision, robustness, and sensitivity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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