A validated stability indicating RP-HPLC method for the quantification of Canagliflozin

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Abstract

A simple, authentic and stability indicating high performance liquid chromatographic method for determination of Canagliflozin in bulk and pharmaceutical formulations was developed and validated as per ICH Q2 R1 Guidelines, A C18 Column (250 mm length×4.6 mm diameter × 5 μm particle size) with a mobile phase consisting of Acetonitrile: 1-octanesulphonic acid in a ratio of 70:30 v/v was employed for the chromatographic study. A flow rate of 1.0 mL/min with an injection volume of 20 μL was selected for this study and the proposed method was validated with different parameters such as Linearity, Precision, Accuracy, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ). Canagliflozin was eluted at 3.4 ± 0.5 min and detected at 245 nm. The method is linear over the concentration range of 10-100 μg/mL with correlation co-efficient r = 0.9997. The plate count and tailing factor was found 5398 and 1.05 respectively. The LOD and LOQ were found to be 0.0170 μg/mL and 0.1705 μg/mL respectively. The percentage recovery was achieved in the range of 98-102%, which was within the acceptance criteria. Developed method was employed to determine the amount of Canagliflozin present in dosage form (Sulisent). The stability of the method was demonstrated by forced degradation studies of drug in which it was degraded under conditions of hydrolysis (acidic and alkaline), oxidation, photolytic and thermal stress as per ICH guideline Q1A (R2).

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INTRODUCTION

Canagliflozin (C_{24}H_{25}F_{0.5}S) is a white to off white solid with melting range of 95-105°C is chemically named as (2S,3R,4R,5S,6R)-2-[3-[[4-fluorophenyl] thiophen-2-yl] methyl]-4-methylphenny]-tetrahydro-6-hydroxymethyl-2H-pyran-3,4,5-triol (Figure 1). It is soluble in many organic solvents (methanol, Dimethyl sulfoxide) but insoluble in aqueous media. Canagliflozin is an oral anti-diabetic agent which belongs to a newly developed class. It has an inhibitory action on sodium-glucose co-transporter 2 (SGLT2). It received approval by US FDA in March-2013 for treating the patients having type-II diabetes (Harsharan PS, 2015 & Neumiller JJ, 2010).
Canagliflozin reduces reabsorption of filtered glucose by stopping Sodium-glucose co-transporter 2 (SGLT2) and brings down the renal limit for glucose (RTG) and in this way increments urinary glucose discharge (Elkinson S, 2013; Bailey C.J, 2011).

![Structure of Canagliflozin](image)

**Figure 1: Structure of Canagliflozin**


In continuation of our previous work on Canagliflozin (Sreenivasulu S, 2017) method development and validation the stability studies in terms of specificity of method was described in this Paper. The aim was to study the ability of the proposed method to measure the analyte response in presence of its degradation products.

**EXPERIMENTAL**

**Instruments**

The two LC system, used for method development and validation were: (i) Shimadzu LC-10AT (Shimadzu Corporation, Japan) connected to Variable Wavelength Detector (UV-Vis). Lab Solutions software (Version) was used for data acquisition and system suitability calculations. (ii) The Agilent 1100 series (Agilent Technologies Inc., USA) connected to Variable Wavelength Detector (UV-Vis). The output was checked and reported utilizing chemstation programming.

Metrohm computerized pH meter, model 780 was utilized for the pH alterations. The reproducibility of the estimations is inside 0.01 pH. Mettler Toledo XP6 model electronic micro balance having maximum limit of 6.1 g, sensitivity of ± 0.01 mg was utilized for standard and test weighing. Sartorius BS/BT 2245 model electronic Analytical balance having maximum limit of 220 g, sensitivity of ± 0.1 mg and was utilized for the purpose of chemicals weighing.

**Chemicals & Reagents**

Canagliflozin (API) gift sample was obtained from Mitsubishi Tanabe Pharma, Japan. HPLC grade of Acetonitrile, HPLC grade O-phosphoric acid and HPLC grade water was obtained from Rankem Limited, India. HPLC grade 1-octane sulphonic acid sodium salt monohydrate was obtained from SD Fine Limited. Market samples of Sulisent 100 mg tablets of Canagliflozin was compassionately provided by USV Limited.

**Chromatographic Conditions**

Chromatographic column employed for separation of analyte was Phenomenex Gemini-NX C18 (250 mm length × 4.6 mm diameter, 5μ particle size).

The mobile phase was composed of sodium 1-octane sulphonate buffer 0.05 M and Acetonitrile (30:70, v/v). This solution was degassed by filtering using a 0.45 micron Millipore filter paper under vacuum filtration and pumped at room temperature, at a flow rate of 1.0 mL/min by using UV detector at a wavelength maximum 245 nm.

**Chromatographic Parameters**

Equipment: Agilent 1100 series HPLC system with VWD detector.

Wavelength: 245 nm.

Injection volume: 20 μL.

Flow rate: 1.0 mL/minute.

Run time: 10 Minutes.

Column: Phenomenex Gemini-NX C18 (250 mm × 4.6 mm, 5μ).

Column oven Temperature: Ambient.

**Solutions and sample preparation**

**Preparation of 1-Octane Sulphonate buffer:** A 0.05M sulphonate buffer was prepared by dissolving 5.88 gm of sodium 1-octane sulphonate in 500 mL of HPLC grade water and pH was adjusted in accordance with 3.5 using 1.0 N O-phosphoric acid. The buffer was filtered through 0.45μ Millipore membrane filter paper to evacuate fine particles and gasses.
Preparation of Mobile Phase
Mobile phase was prepared by mixing 30% of 1-octane sulphonate buffer and 70% of HPLC grade Acetonitrile (v/v) and filtered through 0.45µ Millipore film filter paper and degassed by sonication.

Preparation of diluent: Acetonitrile was utilized as diluent.

Preparation of Standard Stock Solution (100 µg/mL):
Standard stock solution was prepared by dissolving 100 mg of Canagliflozin in 10 mL of Acetonitrile taken in a clean and dry 100-mL volumetric flask and diluted up to the volume with acetonitrile (the concentration of resulting solution is 1000 µg/mL) and sonicated for 8 mins, filtered using 0.45 µ Millipore film filter paper.

1.0 mL of above Standard stock solution of 1000 µg/mL Canagliflozin was further diluted to 10 folds with acetonitrile to get 100 µg/mL Canagliflozin. All other further dilutions were carried out using the working solution (100 µg/mL Canagliflozin) for the method development and validation parameters.

Preparation of Sample Solutions
In a mortar, 10 tablets were taken and squashed finely. Tablet powder identical to 50 mg Canagliflozin were taken in 50 mL clean and dry volumetric flask, few mL of diluent was added and sonicated to dissolve totally and volume was made up to mark with Acetonitrile. Resultant solution was sonicated for 8 min then the solution was filtered utilizing 0.45µ Millipore channel paper. 600 µL of above stock solution was pipetted out into a 10 mL volumetric flask and diluted up to the mark with acetonitrile to get the 60 µg/mL concentration.

Procedure
20 µL of each blank, standard and sample solutions were injected into the chromatographic system. The peak areas were calculated for Canagliflozin and the % assay was considered by comparing the peak area of standard and sample chromatogram by using the formula given below.

\[
\text{Assay} \% = \frac{AT}{AS} \times \frac{WS}{WT} \times \frac{DT}{DS} \times \left(\frac{P}{100}\right) \times \frac{Avg.WT}{Label \ Claim} \times 100
\]

In the above formula, AT = Average peak area obtained from Test or Sample preparation, AS= Average peak area obtained from standard preparation, WS = Weight (mg) of the standard taken, WT=Weight (mg) of the Test or sample taken, P = %purity of working standard, DS= Dilution factor for standard preparation, DT=Dilution factor for sample preparation.

Assay methods were reviewed by Bakshi M. et al and Blessy M et al (Bakshi M, 2002; Blessy M, 2014). In continuation of our research work, Stress examines were done under ICH suggested conditions (ICH Q2 R1, 2005) to survey the stability indicating property of the developed HPLC method. Forced degradation of Canagliflozin was completed by exposing the bulk sample to acidic hydrolysis, alkaline hydrolysis, oxidative, photolytic, thermal conditions.

Acid and alkali hydrolysis
Aliquot of 1.0 mL of Canagliflozin solution (1000 µg/mL) was transferred into a small round bottom flask. To it 9.0 mL of 0.1 N hydrochloric acid or 0.1 N sodium hydroxide was added and mixed well. The prepared solutions were maintained at 35°C for 48 hrs. The samples were cooled to room temperature (25°C), neutralized by adding an equal amount of base or acid. Diluted with Acetonitrile and filtered through a 0.45 µm membrane filter.

Oxidation
Aliquot of 1.0 mL of Canagliflozin solution (1000 µg/mL) was transferred to a small round bottom flask. To the contents in flask, 9.0 mL of 30% hydrogen peroxide solution were added and the reaction mixture was maintained at 35°C with intermittent shaking for 48 hrs. The samples were cooled to room temperature (25°C). Diluted the sample with acetonitrile and filtered through a 0.45 µm membrane filter.

Irradiation with UV light
Canagliflozin sample was exposed to Ultra Violet light (wavelength = 365 nm) for 48 hrs. The sample was dissolved in 10 mL acetonitrile. Resultant sample solution was suitably diluted with acetonitrile and filtered through a 0.45 µm membrane filter.

Thermal degradation
Canagliflozin sample was exposed to a temperature of 70°C for 48 hrs in a refluxing apparatus. The stressed sample was suitably diluted with acetonitrile and filtered through a 0.45 µm membrane filter.
Figure 2: Typical chromatogram of Blank injection at 245 nm

Figure 3: Typical chromatogram of Canagliflozin standard Solution at 245 nm

Figure 4: Typical chromatogram of Canagliflozin sample Solution at 245 nm
**Figure 5**: Calibration curve of Canagliflozin

\[ y = 6753.039x - 1661.22, \text{ Correlation } (r^2) = \]

**Figure 6**: A typical chromatogram representing acid hydrolysis degradation behaviour of Canagliflozin

**Figure 7**: A typical chromatogram representing alkaline hydrolysis degradation behaviour of Canagliflozin
RESULTS AND DISCUSSION

Method Development

Various ratios of Acetonitrile and water as mobile phase were tried to develop a simple reverse phase liquid chromatography method. pH of mobile phase becomes important factor to improve the peak shape and tailing factor. Improved peak shape and separation was achieved at pH = 3.5 with sodium 1-octane sulphonate buffer. Thereafter, Acetonitrile and sodium 1-octanesulphonic acid were taken in a ratio of 70:30 v/v and flow rate of 1.0 mL/min was employed. Phenomenex Gemini-NX C18 Column 250 mm length × 4.6 mm diameter, 5 µ particle size was selected as the stationary phase to improve separation and the tailing of the peak was reduced considerably. Canagliflozin showed maximum absorption at 245 nm of wavelength, therefore 245 nm was selected as the detection wavelength. The retention time was found to be 3.4 ± 0.5 min with plate count and tailing factor as 5398 and 1.05 respectively. The resulting chromatograms of blank, Canagliflozin standard and sample were shown in Figure 2, Figure 3 and Figure 4 respectively.

Analytical Method Validation

The developed Analytical method was validated with different parameters like System Suitability, precision (intra and inter-day), linearity, accuracy, robustness, Limit of detection (LOD) and Limit of Quantification (LOQ) (Shabir GA, 2003; USP 2004; Miller JM, 2000) as per ICH Q2A and Q2B guidelines ("Validation of Analytical Procedures: Text and Methodology Q2 (R1)", Geneva, 2005).

System Suitability

Carried out to evaluate the system suitability parameters for replicate injections. The system suitability results (such as, Retention time, Capacity factor, Plate count & Tailing factor results obtained as 3.4 ±0.5, 2.38, 5398 & 1.05 respectively) with in the acceptance criteria .

Precision

Precision is measured by injecting six replicates of a single calibration standard solution (60 µg/mL concentration). The intra-day and inter-day precision studies (intermediate precision) were carried out and the result are reported in terms of relative standard deviation of Peak Area were presented in Table-1.

Linearity and Range

A series of linearity solutions for Canagliflozin were prepared in the concentration range of 10-100 µg/ml. 20 µL of each standard was injected in triplicate and the results (chromatograms) were recorded for all the linearity standards under the optimized chromatographic conditions. The graph plotted (Figure 5) between peak area vs concentration was obeying linear regression equation \( Y = 6753.039x -1664.22 \) and co-efficient for drug was found to be 0.9997, thus falls within the acceptance limits. Analysis of Variance (ANOVA) results derived from linearity such as Regression sum of squares and Residual sum of squares are 277421522616 and 137317336 respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of the developed method were determined from the standard deviation (SD) of the response and slope (m). The limit of detection and limit of quantitation results of Canagliflozin were found to be 0.0170 µg/mL and 0.1705 µg/mL respectively.

Accuracy:

A definite concentration of standard drug (80%, 100% & 120 % level) were added to pre-analysed sample solution and the percentage recovery was studied. 80% and 120% levels were prepared by considering 60 µg/mL concentration as 100%. The % Mean recovery for Canagliflozin are 98.73 and these results are within acceptable limit of 98-102. The % RSD for Canagliflozinwas 0.49 is within limit of ≤2. Hence, the proposed method is accurate.

Robustness

The robustness of proposed method was established by varying the flow rate, pH, Column temperature and mobile phase composition within allowable limits from established chromatographic conditions. The obtained results shows that percentage assay result of the samples are within acceptable limit of 98 - 102, also there were no obvious change in mean retention time (Rt), mean % assay and %RSD obtained for assay results are within acceptable limit of ≤2. The plate count and tailing factor were found to be in acceptable limits i.e., > 2500 and < 2.0 respectively.

Hence, the method is consistent with variations in the analytical conditions. The results were presented in Table 2.

Stability of Mobile phase, standard and sample solution

Established the stability of standard, sample solution and mobile phase which was used in estimation of Canagliflozin over a period of 3 days. The sample and standard solutions injected at initial time, 24 hrs and 48 (stability sample) by keeping at controlled room temperature 25°C. %RSD results obtained are within limit of ≤ 2
Figure 8: A typical chromatogram representing oxidative degradation behaviour of Canagliflozin

Figure 9: A typical chromatogram representing irradiation with UV light degradation behaviour of Canagliflozin

Figure 10: A typical chromatogram representing Thermal degradation behaviour of Canagliflozin
and hence the Mobile phase, standard stock and sample are stable for 48 hrs at Controlled room temperature.

**Tablet Analysis**

The Content of Canagliflozin in the Sulisent 100 mg tablets was determined by the proposed method. Percentage RSD results 0.39% is within limit of ± 2 and the amount of Analyte found in the Tablet sample is 98.95 mg against the label claim 100 mg. (Sreenivasulu S, 2017).

**Specificity**

The HPLC chromatograms of Canagliflozin were recorded by injecting the 20 μL of each resultant solution of acid & Base hydrolysis, oxidative degradation, irradiation with UV and thermal degradation samples into the HPLC system, which were shown in Figure 6 to 10 respectively. The % degradation was calculated to assess the stability of Canagliflozin drug product under various stress conditions. % degradation was considered by relating the peak area of untreated or unexposed sample area and treated or exposed sample peak area by using the formula given below.

\[
\% \text{ degradation} = \left( \frac{A_u - A_e}{A_u} \right) \times 100
\]

Where; \( A_u \) = Peak area of untreated / unexposed stock solution, \( A_e \) = Peak area of treated / exposed stock solution.
The retention time and the % of degradation of the Canagliflozin were tabulated in Table-3. The proposed method has a high level of specificity because of its capacity to isolate the analyte from its degradation products.

**CONCLUSION**

The proposed new method for the determination of Canagliflozin by High Performance Liquid Chromatography using Variable Wavelength Detector (UV) & Phenomenex Gemini-NX C18 Column was developed and validated based on the ICH guidelines. Various analytical method validation parameters results were summarised in Table-4.

The %RSD got was inside the specification criteria i.e., not more than 2. The % accuracy as far as exactness was accomplished in the scope of 98 - 102%, which was within the acceptance criteria. The mobile phase, standard and sample stock solutions were observed to be steady up to 48 hrs at controlled room temperature. The technique was observed to be accurate, precise, robust and specific as the drug peak did not interfere with the extra peaks aroused during the forced degradation studies. In the meantime the chromatographic elution step is attempted in a brief span (10 min). Henceforth, the newly developed and validated method can be utilized for the regular and stability analysis.

**REFERENCES**


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