Development and Validation of Reverse Phase High Performance Liquid Chromatography Method for the Estimation of Canagliflozin in Bulk and its Pharmaceutical Formulation

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ABSTRACT
Objectives: Study was initiated to develop simple, specific, precise, selective and accurate reverse phase high performance liquid chromatographic method for the estimation of canagliflozin in bulk and pharmaceutical formulation. Sodium glucose co-transporter 2 inhibitors for glycemic control activity are categorized under type 2 classification under antidiabetic drugs. Methods: Development of method was initiated by determining solubility in acetonitrile and detection at 290nm for the drug canagliflozin. The chromatographic separation was achieved on Shimadzu LC-20AT (Shimadzu Corporation, Japan). The system for chromatographic analysis was equipped with binary low-pressure mixing pump (LC-20AT) and an UV-detector (Shimadzu SPD-20A), a Princeton C, column (250 x 4.6mm; 5µm), 20 µl sample loop volume. Results: Concurrent results were obtained in the developed and optimized method. Linearity was found to be 0.998 over the range of 0.089µg/ml– 50µg/ml. Sensitivity of the developed method was 98ng/ml. The mobile phase optimized for method was composed of acetonitrile: water (50:50, %v/v) at a flow rate of 1.0 ml/min. Canagliflozin was detected at 290 nm with retention time of 7.3±0.2 min in concurrent manner. Quantitative assay of the marketed formulation had been carried out and the percentage of drug was determined in the limits of specification. Percentage recovery was found between 98.9 to 99.8%. Accuracy and precision studies performed had achieved metrics under the specified limits. The validation was successfully performed according to ICH guideline Q2R1. Conclusion: The developed method can be employed for the estimation of canagliflozin in pharmaceutical formulation and also for a bioequivalence study to evaluate its applicability further. Key words: Canagliflozin, Method development, RP-HPLC, SGLT-2 Inhibitor, Validation.

INTRODUCTION
Canagliflozin is the first compound under novel class of drug that acts as sodium glucose co-transporter 2 (SGLT-2) inhibitors. It inhibits renal glucose reabsorption by increasing urinary glucose excretion which leads to lower adrenal threshold for glucose and reduced blood glucose levels in patients with type 2 diabetes mellitus. Canagliflozin is an orally active, potent and exhibits approximately 2×10-fold higher selectivity for SGLT-2 over SGLT-1 and are approved at doses of 100 and 300mg for the treatment of type 2 diabetes mellitus worldwide. SGLT-2 inhibitors are a promising new class of anti-diabetic drugs currently approved in Europe, the US and Japan. They allow weight-reducing and effective glycemic control combined with a low risk of hyperglycemia. Canagliflozin is white crystalline powder, almost odorless, slightly soluble in water, freely soluble in acetonitrile, methanol and is chemically named as (2S, 3R, 4R, 5S, 6R)-2-([5-(4-fluorophenyl) thiophen-2-yl] methyl)-4-methylphenyl)-6-(hydroxyl methyl) oxane-3, 4, 5-triol having molecular formula C_{24}H_{25}FO_{5}S. A detailed survey of literature revealed the determination of canagliflozin by UV spectroscopy, HPTLC, HPLC and LC-MS/MS in simultaneous estimation of drugs and in biological fluids (i.e. human and rat plasma). This study describes a new highly sensitive, rapid, simple, accurate, precise, reproducible, economical and stable chromatographic method for the determination of canagliflozin (Figure 1) in pharmaceutical formulation.

MATERIALS AND METHODS
Chemicals and Reagents
HPLC grade acetonitrile was acquired from Merck industries (Mumbai, India). HPLC grade water was generated employing a Milli Q water system (Bangalore, India). The reference standard of Canagliflozin was received as a gift sample from Aurabindo Pharma, Ltd., Hyderabad, India. The marketed formulation was purchased from a local pharmacy, The Nilgiris, Tamil Nadu.

Instrumentation
Experiments pertaining to HPLC were exercised on Shimadzu LC-20AT system (Shimadzu Corporation, Japan). The system for chromatographic analysis was equipped with a binary low-pressure mixing pump (LC-20AT) and an UV-detector (Shimadzu SPD-20A), a Princeton C, column (250 x 4.6mm; 5µm), 20µl sample loop volume. The software, Lab Solutions data station was employed for data collection and analysis. A shimadzu 1700(E) spectrometer was engaged for recording the UV spectrum.

Selection of Wavelength
A standard solution containing 1mg/ml Canagliflozin in acetonitrile was prepared. From the stock solution a secondary solution was prepared by dissolving 1.0ml of the standard solution in the mobile phase. The standard solution was scanned at wavelengths ranging from 200-400nm.
Standard and sample preparations

10mg of Canagliflozin API was dissolved in acetonitrile HPLC grade in a 10ml volumetric flask (1mg/ml) for preparing the primary standard stock solution. From this standard stock solution, a series of dilution viz.; 100 µg/ml, 10 µg/ml and 1µg/ml was prepared in the mobile phase, which then served as 100% target concentration.

Method Development

The proposed method was designed by optimizing the chromatographic conditions by pertaining to various trial runs altering the mobile phase composition, ratio of the mobile phase, pH, column type and dimensions to attain symmetrical analyte peak at a sufficiently short run time. Acetonitrile was used as an organic modifier in the mobile phase. Initially, various ratios of acetonitrile and water employed as the mobile phase for separations, exhibited peak asymmetry. Finally, a symmetric analyte peak with an acceptable short run time was achieved employing acetonitrile and water in a ratio of 50:50 %v/v at a flow rate of 1ml/min, with a Princeton C_{18} (250 x 4.6mm, 5µm) being utilized as the stationary phase and the eluents were monitored at a wavelength of 290nm. Canagliflozin was eluted at 7.3±0.2 min. The mobile phase was prepared by filtering through a 0.45µ PTFE (Poly tetra fluoro ethylene) membrane filter before incorporating in the HPLC system. Lab solutions data station recorded and processed the chromatograms.

System suitability

System suitability parameters play a key role in development and validation of an analytical method ensuring the optimal performance of the system. Chromatographic parameters viz; number of theoretical plates (N), Retention time (R), Resolution (R) and Peak asymmetric factor (A) were scanned on injecting 6 replicates of the standard Canagliflozin at a concentration of 10 µg/ml.

Method validation

The RP-HPLC method developed was targeted for quantifying Canagliflozin and validation of the designed method was carried out in accordance to the ICH guidelines for the parameters namely linearity, precision, specificity, accuracy, robustness, detection limit and quantitative limit.16

Accuracy and Precision studies

Intraday and inter-day studies assess the precision of the designed method. Six independent injections of three different concentrations i.e. [Low Quality Control (LQC- 1µg/ml), Medium Quality Control (MQC- 4 µg/ml) and High-Quality Control (HQC- 16 µg/ml level)] were utilized in the study of the proposed method. Intraday precision as well as repeatability was examined by analyzing the samples on the same day and the interday precision was done by analyzing these same samples (as used in intraday study) on three different days. Calculations for mean and %RSD were carried out by using the values obtained from interday and intraday studies.

Recovery studies elicit the accuracy of the method. The accuracy of the method was assessed by the standard addition method, i.e. analysis of the sample spiked with the known concentration of the standard under optimized chromatographic condition. The recovery results acquired from the 3 different levels (LQC- 1µg/ml; MQC- 4 µg/ml; and HQC- 16 µg/ml) of concentrations made way for the calculations of percentage mean recovery, standard deviation and percentage relative standard deviation.

Specificity/ selectivity

Specificity of the method was illustrated by injecting the diluents, standard solution of canagliflozin and the sample solution extracted from the tablet formulation for any co-eluting peaks within the retention time of the drug.

Linearity and Range

Linear proportionality of the acquired response to that of the analyte concentration specifies the linearity of the said analytical method. Selected range of concentrations [98 ng/ml to 50 µg/ml] were injected in threefold under chromatographic conditions that has been optimized and the respective chromatograms were documented. The linearity was established based on the co-relation co-efficient obtained by plotting a graph with concentration (with an average of six determinations each) in µg/ml at the x-axis and peak area of drug at the y-axis.

Limit of Detection and Limit of Quantification

Sensitivity of a method determines how capable is the method for detecting the lowest possible concentration of analyte without any noise. This is assessed by the parameter of LOD and LOQ. Limit of Detection (LOD) is the smallest concentration of the analyte that can be detected by the developed method which evoke a computable response (signal to noise ratio 3) whereas Limit of quantification (LOQ) is the least concentration of the analyte which generates a response that can be precisely quantified (Signal to noise ratio 10).

LOD and LOQ can be calculated by the formula:

\[
LOD = \frac{3.3 \cdot \sigma}{S}
\]

\[
LOQ = \frac{10 \cdot \sigma}{S}
\]

Where \(\sigma\) = Standard deviation of the response; \(S\) = Slope of the deviation curve.

Robustness

The parameter of ruggedness and robustness of the developed method was scaled by bringing about slight changes in the stated experimental conditions like minute deviations in analyte concentrations, source of reagent, various brands of columns and marginal variability in ratio of the mobile phase, pH of aqueous buffer, flow of eluents etc.

Assay of the marketed formulation

10 film-coated tablets of canagliflozin (100mg) were weighed, finely powdered in a mortar and pestle and a quantity equivalent to the average weight of the formulation was transferred to 100ml volumetric flasks, dissolved in about 60 ml of mobile phase and sonicated for about 10 min. These solutions were then filtered through whatman filter paper and the volume was adjusted to 100ml with mobile phase (considered as solution A). 1.0 ml of solution A was transferred to a 100ml volumetric flask and the volume was adjusted with mobile phase to obtain a working standard having a concentration of 10µg/ml. The solution was analysed in triplicate using optimized chromatographic conditions. The chromatograms were

Figure 1: Structure of Canagliflozin.
recorded and the amount of the drug present was assessed, standard deviation and the %RSD (Relative Standard Deviation), were calculated and reported.

RESULTS

Canagliflozin is a sodium glucose co-transporter (SGLT-2) inhibitor. (SGLT-2) inhibition is believed to reduce blood glucose levels by increasing the amount of glucose excreted in the urine. This drug was approved by U.S. Food and Drugs Administration (USFDA) on 2013 through brand Invokana from Janssen Pharmaceuticals.17

In Method development and validation of the drug Canagliflozin, High Performance Liquid Chromatography (HPLC) technique was opted for the method using shimadzu LC-20AT, HPLC system (Shimadzu Corporation, Japan). The system for chromatographic technique was equipped with a binary low-pressure mixing pump (LC-20AT), an UV-detector (Shimadzu SPD-20A), a Princeton C18 column (250 x 4.6mm; 5µm) and 20µl sample loop volume. The software of Lab Solutions data station was employed for data collection and analysis. Validation of the method was performed including all parameters specified under ICH guideline Q2R1.

Concurrent results were achieved in the developed and optimized method. Drug canagliflozin was preliminarily assessed for parameters like solubility in acetonitrile and detection wavelength of 290nm was determined from UV spectrum. After few trials, the mobile phase was optimized with a composition of acetonitrile: water (50:50, %v/v) at a flow rate of 1.0 ml/min. Canagliflozin was detected at 290 nm with retention time of 7.3±0.2 min in concurrent manner. A check carried out for specificity was observed that there was no interference in retention time of drug by mobile phase or diluent used in method. Mobile phase had been used as diluent in overall method which had nullified the interference in chromatogram obtained. The method was found to be linear with an R² value of 0.998 over the range of 0.098µg/ml– 50µg/ml. Sensitivity of the developed method was 98ng/ml. Quantitative assay of market formulation had been carried out and the percentage of drug was determined as 98.90 ± 0.04% w/w which was within the limits of the marketed formulation had been carried out and the percentage of drug present was assessed, standard deviation and the %RSD (Relative Standard Deviation), were calculated and reported.

DISCUSSION

Canagliflozin in oral dosage form of tablet is specifically indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. Canagliflozin in tablet dosage form (Invokana) was approved to reduce the risk of major adverse cardiovascular effects in adults with type 2 diabetes mellitus and established cardiovascular disease. Canagliflozin in brand of Invokana was approved in September 2019 to reduce the risk of end stage kidney disease (EKSD), worsening of kidney function, cardiovascular (CV) death and hospitalization of heart failure in adults with type 2 diabetes and diabetic kidney disease (nephropathy) with a certain amount of protein in the urine. This sodium-glucose co-transporter 2 (SGLT-2), in proximal renal tubules is responsible for the majority of the reabsorption of filtered glucose from the tubular lumen. It reduces the reabsorption of filtered glucose and lowers the renal threshold for glucose (RTG) and thereby increases urinary glucose excretion (UGE). It increases the delivery of sodium to the distal tubule by blocking SGLT-2-dependent glucose and sodium reabsorption. This drug is believed to increase tubuloglomerular feedback and reduce intraglomerular pressure.17 A valuable endeavor has been made for this RP-HPLC technique to accomplish estimation of canagliflozin. Advancement has been done after preliminaries executed through factor sections and versatile stages for accomplishing a practical and acclimatized conditions. The structure of the technique relies on the idea of the example (ionic or ionisable, hydrophobic or hydrophilic) and its level of solvency. Canagliflozin exhibited maximum absorbance at 290nm (Figure 2). Thus it was stipulated as the λmax for surveilling the chromatographic eluents. Since Canagliflozin is seen as promptly dissolvable in polar solvents, thus RP-HPLC strategy was considered to be suitable for its detachment as switched stage chromatography. It is a method utilizing alkyl groups covalently clung to the stationary phase particles so as to make a hydrophobic stationary phase, which has a more grounded partiality for hydrophobic or less polar mixes. Reverse phase chromatography utilizes a polar mobile phase. Subsequently,

Figure 2: UV Spectrum of Canagliflozin.

Table 1: Assay of Marketed Formulations.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample*</th>
<th>Label claim†</th>
<th>Amount present± in mg±SD; %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Formulation -I</td>
<td>100 mg</td>
<td>98.9± 0.04</td>
</tr>
</tbody>
</table>

Table 2: System Suitability Parameters.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>Canagliflozin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Retention time (min)*</td>
<td>7.3</td>
</tr>
<tr>
<td>2</td>
<td>Theoretical plates (N)*</td>
<td>4250</td>
</tr>
<tr>
<td>3</td>
<td>Tailing Factor(t)*</td>
<td>0.993</td>
</tr>
<tr>
<td>4</td>
<td>Asymmetry factor (A)*</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>Regression coefficient (R²)*</td>
<td>0.998</td>
</tr>
<tr>
<td>6</td>
<td>Regression equation*</td>
<td>y = 72144x + 33801</td>
</tr>
<tr>
<td>7</td>
<td>Linearity and Range*</td>
<td>0.098 µg/ml - 50µg/ml</td>
</tr>
<tr>
<td>8</td>
<td>Limit of Detection (LOD)*</td>
<td>0.048 µg/ml</td>
</tr>
<tr>
<td>9</td>
<td>Limit of Quantification (LOQ)*</td>
<td>0.098 µg/ml</td>
</tr>
</tbody>
</table>

* mean of 03 determinations.
†a. 20 numbers of tablet dosage form.
b. Content of drug present in each tablet dosage form.
c. Average amount of drug present in each tablet dosage form.
 SD: Standard Deviation.
 %RSD: Percentage of Relative Standard Deviation.

a, b, c, d, h, i: decisive parameters for the suitability of the developed method.
e, f, g: Parameters assessing statistical linear regression.
hydrophobic atoms in the polar phase will adsorb to the hydrophobic stationary phase and hydrophilic particles in the versatile phase will go through the column and are eluted first. The strategy has accomplished ideal framework appropriateness in parameters and a basic portable period of isocratic mode was performed where other revealed strategies are with different approaches like temperature and pH conditions. The LOD and LOQ were found to be 0.048µg/ml and 0.098µg/ml respectively indicating the sensitivity of the method. These results have been tabulated in Table 2. Method got built up with dependable framework appropriateness in parameters. Approval for the strategy was executed according to ICH guideline Q2R1. Accuracy was accomplished utilizing tablet formulation (Table 3) and Precision was performed in intraday and interday for the LQC, MQC and HQC in triplicate and each have achieved <2.0%RSD values in breaking points (Table 4). Regarding specificity no co-eluting peak was observed, a sharp and symmetric peak shape demonstrated the specificity of the method with a retention time of 7.3 ± 0.2 min and is shown in Figure 3. The chromatograms of the standard and sample solutions were depicted in Figure 4, Figure 5. The system suitability parameters were found to be within limits and are summarized in Table 2. The linearity graph had showed excellent co-relation between the concentrations and peak area when observed within the range of (0.098-50µg/ml) for Canagliflozin (Table 5). The co-relation co-efficient for canagliflozin was ≥ 0.998, the slope and the intercept were depicted at 72144 and 33801 respectively. The graph depicting linearity of the method is represented in Figure 6. Results of

Table 3: Accuracy studies of Canagliflozin.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Actual Concentration* (µg/ml)</th>
<th>Recovered Concentrationb (µg/ml) ± SD; %RSD</th>
<th>Percentage Recoveredc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.98±0.005; 0.67</td>
<td>98.9%</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>3.96±0.015; 0.38</td>
<td>99%</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>15.97±0.013; 0.09</td>
<td>99.8%</td>
</tr>
</tbody>
</table>

*a. Spiked standard concentration to formulation.
b. Recovered standard concentration from formulation.
c. Percentage value of b from a.

SD: Standard Deviation.
%RSD: Percentage of Relative Standard Deviation.

Table 4: Precision studies of Canagliflozin.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Concentration* (µg/ml)</th>
<th>Intraday Mean±SD; %RSD</th>
<th>Interday Mean±SD; %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 (LQC)</td>
<td>0.9917±0.0090; 0.915</td>
<td>0.9875±0.0077; 0.787</td>
</tr>
<tr>
<td>2</td>
<td>4 (MQC)</td>
<td>3.9209±0.0031; 0.079</td>
<td>3.9701±0.0723; 1.822</td>
</tr>
<tr>
<td>3</td>
<td>16 (HQC)</td>
<td>15.9556±0.0335; 0.210</td>
<td>15.9000±0.0713; 0.448</td>
</tr>
</tbody>
</table>

*a mean of 03 determinations.

(LQC – Low Quality Control; MQC – Middle Quality Control; HQC – High Quality Control samples)
a. Selected concentrations for precision analysis.
b. Mean values on the same day analysis.
c. Mean values on the following day of analysis.

SD: Standard Deviation.
%RSD: Percentage of Relative Standard Deviation.

Figure 3: Typical chromatogram of the Blank solution.

Figure 4: Typical chromatogram of the standard solution (Canagliflozin– 10 µg/ml).

Figure 5: Typical chromatogram of the sample solution extracted from tablet dosage form (100 mg tablet).

Linearity Plot of Canagliflozin

\[ y = 72144x + 33801 \]

\[ R^2 = 0.9986 \]

Figure 6: Linearity Plot of Canagliflozin.
Table 5: Linearity data of Canagliflozin.

<table>
<thead>
<tr>
<th>Concentration of drug* (µg/ml)</th>
<th>Peak Areaab</th>
</tr>
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<tbody>
<tr>
<td>50</td>
<td>3589648</td>
</tr>
<tr>
<td>25</td>
<td>1934300</td>
</tr>
<tr>
<td>12.5</td>
<td>938536</td>
</tr>
<tr>
<td>6.25</td>
<td>484096</td>
</tr>
<tr>
<td>3.125</td>
<td>304849</td>
</tr>
<tr>
<td>1.563</td>
<td>152086</td>
</tr>
<tr>
<td>0.78</td>
<td>58477</td>
</tr>
<tr>
<td>0.39</td>
<td>44042</td>
</tr>
<tr>
<td>0.195</td>
<td>24519</td>
</tr>
<tr>
<td>0.098</td>
<td>14721</td>
</tr>
</tbody>
</table>

Regression Co-efficient: \[ y = 72144x + 33801 \]

Table 6: Robustness Results.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters*</th>
<th>Retention time (min)ab</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Flow rate</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>(ml/min)</td>
<td>7.36± 0.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>7.29± 0.2</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>7.33± 0.2</td>
</tr>
<tr>
<td>2.</td>
<td>Mobile Phase (Water: ACN %v/v)</td>
<td>7.37± 0.2</td>
</tr>
<tr>
<td></td>
<td>50:50</td>
<td>7.28± 0.2</td>
</tr>
<tr>
<td></td>
<td>48:52</td>
<td></td>
</tr>
</tbody>
</table>

Sl. No. | Parameters* | Peak area (micro volts)c |
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wavelength (in nm)</td>
<td>285</td>
</tr>
<tr>
<td></td>
<td></td>
<td>290</td>
</tr>
<tr>
<td></td>
<td></td>
<td>295</td>
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Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest.

Abbreviations

SGLT-2: Sodium Glucose co-transporter 2; HPLC: High Performance Liquid Chromatography; PTFE: Poly Tetra Fluoro Ethylene; % RSD: Percentage Relative Standard Deviation; LOD: Limit of Detection; LOQ: Limit of Quantification; USFDA: U.S. Food and Drugs Administration; ESKD: End Stage Kidney Disease; CV: Cardiovascular; RTG: Renal Threshold for Glucose; UGE: Urinary Glucose Excretion; MQC: Middle Quality Control; HQC: High Quality Control.

References


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