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# A novel validated RP-HPLC method for the estimation of canagliflozin in bulk and pharmaceutical dosage forms

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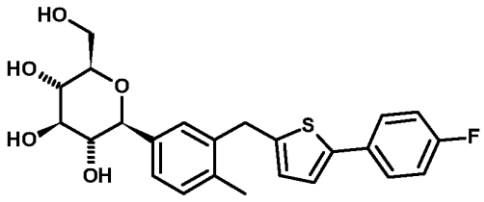
## Abstract

The objective of the present study was to develop a simple, specific and accurate reverse phase high performance liquid chromatographic method for the determination of Canagliflozin in bulk and pharmaceutical dosage forms. The method is optimized on an inertsil ODS-3(250×4.6mm, 5µ) column with a mobile phase combination of 0.02% Formic acid: Acetonitrile (40:60) at a flow rate 1.2ml/min and the eluents were monitored at 230nm. Under these LC conditions Canagliflozin peak was eluted at 4.4 min. The developed method was validated as per ICH guidelines. The calibration curve was linear over a concentration range of 10-50µg/ml ($R^2$ =0.999) and the mean percentage assay was found to be 98.2. The statistical data proved that proposed method is accurate, precise and reproducible. The method which is LC-MS compatible can be adopted in the routine analysis of Canagliflozin in bulk and pharmaceutical dosage forms.
**Keywords:** Canagliflozin, RP-HPLC Method development, Method validation.

## 1. Introduction

Canagliflozin is an antidiabetic drug used to improve glycemic control in patients with type 2 diabetes. Canagliflozin is an inhibitor of subtype 2 sodium-glucose transport protein (SGLT2) which is responsible for at least 90% of the glucose reabsorption in the kidney (SGLT1) being responsible for the remaining 10%). Canagliflozin is chemically known as (2S, 3R, 4R, 5S, 6R)-2-{3-[5-[4-Fluoro-phenyl)-thiophen-2-ylmethyl]-4-methyl-phenyl}-6-hydroxy methyl-tetrahydro-pyran-3, 4, 5-triol. Literature review revealed that there were several analytical methods like HPLC [1-6], LC-MS [7], UV spectroscopy [8], HPTLC [9] and only few RP-HPLC methods were reported for the estimation of Canagliflozin in bulk and tablet dosage forms and these methods used non-volatile buffers which reduces column efficiency. Hence the present work aimed at the development of a new sensitive, accurate and precise RP-HPLC method for the estimation of Canagliflozin in bulk and tablet dosage forms and by using mobile phase that is compatible with LC-MS and to validate the same as per ICH guidelines [10-12].




**Structure of canagliflozin**

## 2. Experimental Section

### 2.1 Materials and Methods

#### 2.1.1 Chemicals and reagents

Canagliflozin procured from LAURUS LABS Pvt Ltd., Hyderabad and marketed Canagliflozin tablets Invokana (B-NO: FLZSN00), was bought from a local pharmacy with a labelled amount of 100mg. Methanol, Acetonitrile, Water, Formic acid, other chemicals and reagent used were of HPLC grade.

**2.1.2 Equipment**

A Shimadzu prominence HPLC system provide with LC-10AT binary pump, SIL-20AHT auto sampler, and SPD-10A UV detector was used. Data acquisition was carried out using LC solutions software.

**2.2 Chromatographic conditions**

Inertsil ODS-3 column (250×4.6mm, 5µ) and a mobile phase consisting of 0.02% Formic acid and acetonitrile in the ratio of 40:60 (v/v) at a flow rate of 1.2ml/min were employed. For quantitative analytical purpose, the eluents were monitored by the UV detector at a wavelength of 230nm with an injection volume of 10µl and maintaining ambient temperature conditions.

**2.3 Preparation of stock and standards**

10mg of canagliflozin was accurately weighed and transferred to a clean and dry 10ml volumetric flask containing 5mlof methanol, sonicated for 1minute and the volume is made up to the mark with methanol to obtain a final concentration of 1mg/ml. Calibration standards in the concentration range of 10-50µg/ml were prepared by adequate dilution of the stock solution with 0.02% Formic acid as diluent.

**2.4 Method validation**

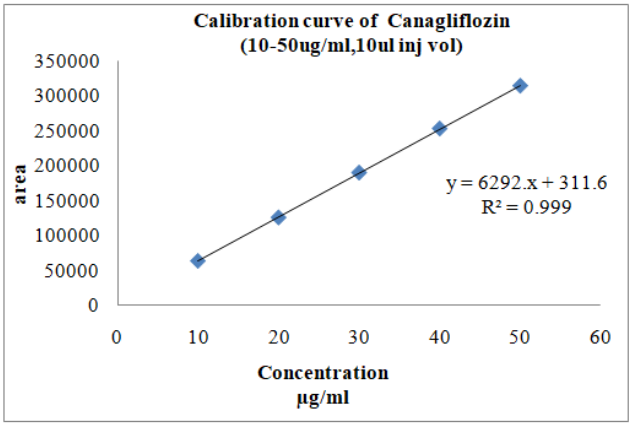
**2.4.1 Specificity**

The specificity of the method was established by comparing the chromatograms of drug substance and drug product with that of blank and placebo. Absence of peaks other than the drug peak such as those of impurities or excipients ascertains the specificity of the method.

**2.4.2 Linearity**

Calibration curves were plotted to establish linearity of the method by employing five standard solutions in the concentration range of 10-50µg/ml each in triplicate using an injection volume of 10µl. Statistical analysis of the resultant data was performed and parameters such as slope, intercept, and regression equation were calculated.

**Figure 2: Calibration curve of Canagliflozin**




**2.4.3 Precision**

**System precision**

It was measured in terms of repeatability of application of a homogenous solution by injecting six replicates of standard solution (30µg/ml) of drug substance.

**Method precision**

It was measured in terms of repeatability of application by giving six replicates of sample solution of drug product, 30µg/ml. In each case, standard deviation and percentage relative standard deviation were evaluated to ascertain the repeatability of the method.

**2.4.4 Accuracy**

Accuracy of the developed method was confirmed by performing standard addition and recovery studies. In this method, the sample solution was spiked with known concentrations of standard solutions within the linearity range at three different levels, 80, 100 and 120% of test concentration each in triplicate and analysed. The percentage recovery and percentage relative standard deviation were tabulated at each level.

**2.4.5 Limit of detection (LOD), limit of quantification (LOQ)**

The LOD and LOQ were determined from the calibration curve by standard deviation of the response and slope method using the formula LOD= 3.3 σ/s and LOQ= 10 σ/s, where, σ=standard deviation of the intercepts and S= mean of slopes obtained in the calibration plots.

**2.4.6 Robustness**

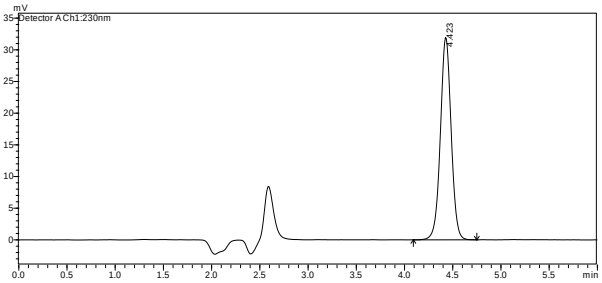
Robustness of the proposed method was determined by making small deliberate changes in flow rate (±0.2ml/min) and mobile phase (±2%) of the optimized method. The deviation in parameters such as tailing factor, theoretical plates at each condition was tabulated.

**2.4.7 System suitability**

System suitability of the method was established by injecting increasing volumes (10-50µL) of the same homogenous standard solution, 30µg/ml. The percentage relative standard deviation of retention time, tailing factor and theoretical plate number with change in volume was evaluated.

**2.4.8 Stability of solution**

Stability of stock solution was determined at a temperature of 2-8$^{o}$C by analysing the stock solution at regular time intervals over a period of 24hours.



**2.5 Assay**

Canagliflozin tablets (Invokana-100mg) were taken to estimate the suitability of proposed method to estimate Canagliflozin in tablet formulation. For this 20 tablets were taken, coating was removed, tablets were weighed individually to determine the average tablet weight. Tablets are then powdered using mortor and pestle, the powder equivalent to 10mg of Canagliflozin was accurately weighed and transferred into a 10ml volumetric flask containing 5ml methanol. The final volume was made up to the mark with methanol. The solution was sonicated for 1min to ensure solubility of drug. The solution was centrifuged for 2min and supernatant was collected, adequate dilution was done using 0.02% formic acid obtain a sample solution with a final concentration of 30µg/ml. The drug content in the formulation was quantified by comparing the peak area obtained with that of the standard solution.

## 3. Results and discussion

Literature survey revealed that there were several analytical methods like Spectrophotometry, HPLC, LC-MS, LC, GCMS and Only few RP-HPLC methods were reported for the estimation of Canagliflozin in bulk and tablet dosage forms and these methods used non-volatile buffers which reduces column efficiency. Hence the present work aimed at the development of a new sensitive, accurate and precise RP-HPLC method for the estimation of Canagliflozin in bulk and tablet dosage forms and by using mobile phase that is compatible with LC-MS.

**3.1 Method development**

Different trials were performed with various columns, mobile phase combination and diluents for optimum elution of Canagliflozin. Intial trial was carried with INERTSIL ODS-3 column (250×4.6mm, 5µ) using a mobile phase of 0.02% formic acid and acetonitrile in the ratio of 50:50(v/v) at a flow rate of 1ml/min. With these LC conditions, Canagliflozin peak was eluted preceding the solvent front with a tailing factor (2.45). The mobile phase combination was further changed to 40:60(v/v) of 0.02% formic acid and acetonitrile at a flow rate of 1ml/min. With these LC conditions, the Canagliflozin was eluted as sharp peak at 4.43min which showed ideal peak properties. The standard chromatogram of Canagliflozin was shown in figure.1.

The developed method was validated as per ICH guidelines.

**3.2 Method validation**

**3.2.1 Specificity**

Specificity of the method was as certained from the overlay of the standard, sample, blank, placebo chromatograms. No interference by the matrix, excipients or any other impurities was observed at the elution time of Canagliflozin implying the specificity of the method for detection of Canagliflozin.

**3.2.2 Linearity**

Linearity was established over a concentration range of 10-50µg/mL by plotting a graph of concentration versus respective peak areas. Regression analysis of the data thus obtained showed a good regression coefficient of 0.999 with a regression equation y=6292.x-311.6. The standard deviation and percentage relative standard deviation values were calculated at each concentration which were found to be within limits i.e., less than 2. The data obtained was shown in table 1.

**3.2.3 Precision**

System and method precision studies were carried out by giving six replicate injections of 30µg/mL standard solution of drug and sample solution of drug product respectively. The data obtained was shown in table 1. The percentage relative standard deviation obtained was found to be less than 2% for peak areas as per specifications

**3.2.4 Accuracy**

Accuracy of the method was determined by employing standard addition method. The amount of recovery and percentage relative standard deviation at each level i.e., 80, 100 and 120% of test concentration was calculated and it was found to be less than 2 percent. The accuracy data was represented in table 1.

**Table 1: Linearity, Precision and Accuracy data**

| **Validation data of Canagliflozin** | | |
|---|---|---|
| **Linearity** | **Range**<br>**Regression equation**<br>**Regression coefficient ($R^2$)** | 10-50µg/ml<br>Y= 6292.4±311.6<br>0.999 |
| **Accuracy (n=3)** | **Level of Addition (percent)** | **Mean Recovery (RSD)** |
| | 80<br>100<br>120 | 98.44(0.087)<br>99.21(0.040)<br>100.31(0.017) |
| **Precision (n=6)** | | |
| **System Precision** | **Average Peak area of the standard sample (RSD)** | 189694(0.7622) |
| **Method Precision** | **Average peak area of the Assay sample (RSD)** | 188928.33(0.709) |
| **Percent assay (n=3)** | **Mean ± SD** | 99.57 (0.705) |

**3.2.5 LOD and LOD**

Limit of detection and limit of quantification values were calculated employing the average slope and standard deviation ratio method. The LOD and LOQ values obtained were 0.00136µg/mL and 0.00414µg/mL respectively.

**3.2.6 Robustness**

Robustness studies were carried out by making small deliberate changes in the flow rate, (±0.2mL/min) and

mobile phase composition (±2%) and %RSD was calculated for parameters such as number of theoretical plates, tailing factor with respect to the changes were tabulated. The results obtained were satisfactory and shown in table 2.

**Table 2: Robustness data**

| Chromatographic parameters | Retention time(min) | Theoretical plates (N) | Tailing factor |
|---|---|---|---|
| **Mobile phase composition ratio (0.02% Formic acid: Acetonitrile)** | | | |
| 42:58 | 4.749min | 7494.864 | 1.013 |
| 40:60 | 4.423min | 6938.178 | 1.041 |
| 38:62 | 4.12min | 6932.838 | 1.063 |
| **Flow rate (mL/min)** | | | |
| 1 | 5.246min | 8290.151 | 1.041 |
| 1.2 | 4.423min | 6938.178 | 1.041 |
| 1.4 | 3.818min | 6141.29 | 1.033 |

**3.2.7 System suitability**

System suitability testing is an integral part of the analytical procedure. System suitability studies were carried out by injecting five times a 1µg/mL standard concentration of Canagliflozin at different injection volumes ranging from 10-50µL. The %RSD for system suitability test parameters like tailing factor and theoretical plate number were less than 2 indicating the present conditions were suitable for the analysis of Canagliflozin.

**3.3 Assay**

The percentage assay of the drug in the formulation was obtained by comparing the peak areas of the sample with that of the standard and they were found to be 98.25 respectively.

**3.4 Stability of the analytical solution**

The stability of the stock and standard solution were determined by analysis the samples under refrigeration (8±1$^{o}$ C) at different time intervals up to 24hours. The percent variation in assay values at different time interval were found to be less than 2 when compared with the initial zero time interval solution , thus indicating that the solution were stable for a period of 24hours when stored at 8$^{o}$ C.

## 4. Conclusion

Canagliflozin is an antidiabetic drug used to improve glycemic control in patients with type 2 diabetes. A sensitive RP-HPLC-PDA method was developed for the determination of Canagliflozin in bulk and pharmaceutical dosage form with LC-MS compatible mobile phase composition in a short run time. The developed method was validated as per ICH guidelines ($Q_2R_1$)

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