Research Article on Development and Validation of RP-HPLC Method for Estimation of Canagliflozin

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Abstract

To develop an accurate, precise, specific high performance liquid chromatography method for quantification of Canagliflozin in bulk and dosage forms. A C18 column (250mm X 4.6mm; 5µm phenomenex) was used with mobile phase containing Acetonitrile-0.1% sodium acetate buffer (pH-4.6), (20:80) in isocratic mode. The flow rate maintained was 1.0ml/min and the U.V detector was operated at 291nm. The retention time of Canagliflozin was 3.307min and showed a good linearity in concentration range of 2-14µg/ml with correlation coefficient of 0.999. The average percent recovery was found to be 99.98%. The developed method follows validation parameters such as system suitability, linearity, precision, accuracy, limit of detection and limit of quantification and robustness as per ICH guidelinesQ2(R1). The proposed method was found to provide faster retention time with sharp resolution with linearity at a lowest concentration as compared to previous methods and this method is validated as per International conference on harmonization guidelines and successfully applied for bulk and pharmaceutical dosage form.

1. INTRODUCTION

Canagliflozin is a novel, potent, and highly selective sodium glucose co-transporter (SGLT) 2 inhibitor. It has been proved that Canagliflozin can increase urine glucose excretion by reducing the renal glucose threshold and by decreasing the filtered glucose re-absorption. Canagliflozin was approved by FDA in March 2013. The chemical name (IUPAC) of Canagliflozin is (2S, 3R, 4R, 5S, 6R)-2-{3-[5-(4-flurophenyl)-thiophen-2-ylmethyl]-phenyl} 6 hydroxy methyl tetra hydro-pyran-3, 4, 5-triol. The structure was shown in figure 1. It is white to off white solid with melting point of 95-105°. Canagliflozin is soluble in phosphate buffer, methanol, dimethyl sulfoxide, acetonitrile, etc. but insoluble in aqueous media. It is a product of a division of Johnson and Johnson and marketed with the brand names of INVOKANA®,SULISENT® in strengths 100 and 300mg respectively.(1)

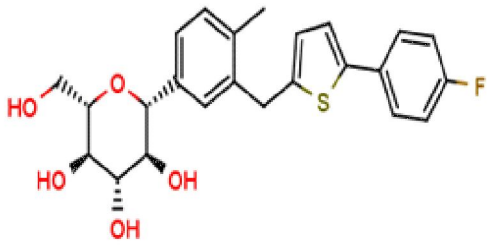

Fig.1: Canagliflozin

1.1 Mechanism of Action

Canagliflozin is an inhibitor of subtype 2 sodium glucose transport proteins (SGLT-2), which is responsible for atleast 90% of renal glucose reabsorption. Blocking the transporter causes up to 119grams of blood glucose per day to be eliminated through the urine, corresponding to 476 kilocalories. Additional water is eliminated by osmotic dieresis, resulting in a lowering of blood pressure. This mechanism is associated with a low risk of hypoglycemia compared to other types of anti-diabetic drugs such as sulfonylurea derivatives and insulin.[2]

2. MATERIALS AND METHODS

2.1 Instrumentation

Chromatography was performed with SCHIMADZU-LC 20AD with Rheodyne injection port, U.V detector to provide compact and convenient for LC with class LC-solutions software.

2.2 Reagents and Chemicals

The reference sample of Canagliflozin was collected from Laurus laboratory limited. HPLC grade Acetonitrile, HPLC grade water and other chemicals was obtained from SD-fine chemicals limited. Commercial tablets (SULISENT-100mg) were purchased from the local pharmacy.

2.3 Chromatographic Conditions

The mobile phase consisted of Acetonitrile and 0.1% sodium acetate buffer adjusted to pH 4.6 was taken in ratio of 20:80 at a flow rate of 1.0ml/ minute mobile phase was degassed by ultrasonic bath and was filtered through a 0.45µm membrane filter under vacuum. PHENOMENEX C18 column (4.6mm X 250mm, 5µ) was used as the stationary phase. The eluents were detected and quantified at 291nm.

2.4 Preparation of Standard Stock Solution

Canagliflozin standard stock solution (1000µg/ml): A 10 micro grams of standard Canagliflozin was weighed and dissolved in Acetonitrile and made up to mark to 10 micro liter volumetric flasks.

Canagliflozin working solution (100µg/ml): from stock solution, further 1ml was transferred in10ml volumetric flask and made up to mark with Acetonitrile.

2.5 Preparation of 0.1% Sodium Acetate Buffer

Dissolve 27grams of sodium acetate in 250micro litres of HPLC grade water and add 4.8 micro litre of glacial acetic acid into sodium acetate until pH-4.6 and dilute with water to 500micro litre.

2.6 Preparation of Sample Solution

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 10micro litre volumetric flask, 5micro liter of diluent added and sonicated for 30 minutes, further the volume made up with diluent and filtered. From the filtered solution 0.1micro liter was pipette out into a 10micro liter volumetric flask and made up to mark with diluent.

2.7 Preparation of Calibration Curve

From working solution, appropriate dilutions were made to get the final concentration of 2, 4,6,8,10µg/ml and absorbance was taken at λ max 291nm. Averages of such 5 sets of values were taken for standard calibration curve, and the calibration curve was plotted.

2.8 Method Validation

Parameters such as linearity, accuracy, precision, robustness, ruggedness, LOQ, LOD were performed according to the ICH guidelines Q2 (R1).

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2.9 Method development and optimization of chromatographic conditions

To achieve a good peak different parameters and different proportions of solvents like methanol, acetonitrile and water were tested with binary and tertiary eluents. Finally 0.1% sodium acetate buffer adjusted to pH 4.6: A CN mix in ratio of 80:20% V/V mobile phases at a flow rate of 1ml/min and detection at 291nm achieved good satisfactory peak. The chromatogram of optimized standard mixture is shown in fig.2 the system suitability parameters such as retention time, resolution and theoretical plates for optimized standard mixture are given in table.1

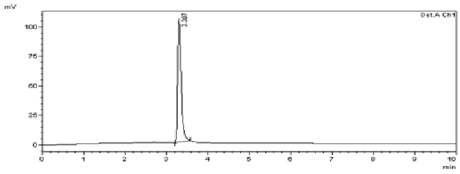

Fig.2: Chromatogram of Canagliflozin

3. METHOD VALIDATION

The method was validated for system suitability, accuracy, precision, linearity, limit of detection, limit of quantification and robustness as per ICH guidelines Q2R1.

3.1 System Suitability

It is done for checking the system performance parameters of developed HPLC method by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor. Resolution(R), were determined (Table-1) the results were found to be within limits.

Table-1 : System Suitability Results

Parameter	Canagliflozin
Retention time	3.307mins
Theoretical plates	6499.641
Area	626369
Tailing factor	1.599

3.2 Specificity

Specificity is the ability to determine between the analyte of interest and other components that are present in the sample. Specificity of the method was as curtained from the overlay of the standard, sample, blank, placebo chromatograms. Blank chromatogram does not show any disturbance peak at the retention time of Canagliflozin and hence the method is specific which is shown in fig.3

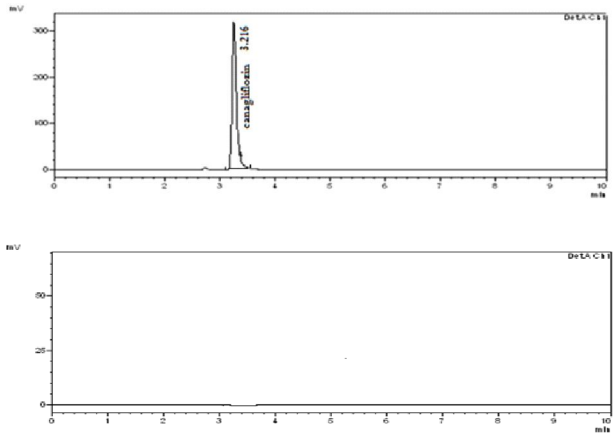

Fig.3: Blank Chromatogram

3.3 Linearity

Linearity was established over a concentration of 2-15µg/ml by plotting a graph of concentration versus respective peak areas. From working standard solution 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.5ml of solutions were pipette out separately and transferred to 10ml volumetric flask. The final volume was made up to mark with diluent. This gives 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml, 15µg/ml concentration solutions respectively. The retention time were noted at 291nm. The regression coefficient for the analysis was 12848X+63686 with coefficient correlation r^2= 0.999.the summary of parameters are shown in table.2

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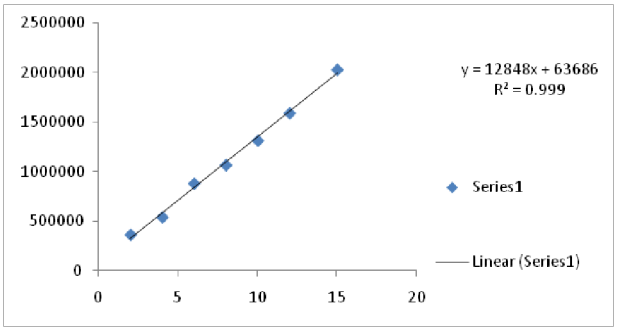

Fig.4: Linearity curve of Canagliflozin

Table-2 : Linearity Results

Concentration	Area
2	358822
4	536107
6	876740
8	1064212
10	1311865
12	1590426
15	2030992

3.4 Precision

Precision was evaluated at three levels: repeatability, intermediate precision and reproducibility. Each level of precision is investigated by six replicates injections of concentration 10µg/ml of Canagliflozin. The retention time of this solution were noted at 291nm & the result of precision was expressed as % R.S.D given in table-3.

Table-3: Precision Results

Concentration	Absorbance
10µg/ml	1311768
10µg/ml	1311592
10µg/ml	1311689
10µg/ml	1311725
10µg/ml	1311562
10µg/ml	1311725
%R.S.D	**0.16%**

3.5 Accuracy

To determine the accuracy of the proposed method, recovery studies were conducted. It was conducted by following standard addition method (spiking standard to sample) in this method known amount of standard analyte was spiked to sample matrix at different levels. The concentration of analyte in original sample is then determined mathematically by calculating % recovery. In this standard is spiked at different levels to sample (50%, 100%, 150%).the obtained solutions were scanned at 291nm the results were tabulated in table-4.

Table - 4: Accuracy Results

Parameter	Amnt taken	%recovery	Mean recovery	%rsd
80%	14	101.82% 100.81% 100.85%	**100.82%**	**1.56**
100%	16	100.02% 100% 100.04%	**100.02%**	**0.02**
120%	18	98.51% 98.53% 98.64%	**98.51%**	**1.04**

3.6 Robustness

The robustness was unaffected by deliberate change in flow rate, pH, mobile phase composition and column temperature were performed. The retention time were noted and % R.S.D was calculated were tabulated in table-5.

Table-5: Robustness Results

Retention time	Absorbance	%RSD
0.9ml/min	1321897 1321762 1321824	**0.07%**
1.1m/min	13213624 13213786 13213536	**0.04%**

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3.7 Ruggedness

The ruggedness of the proposed analytical method was performed in different conditions like different columns, analyst, instrument, laboratory analysis of sample. 10µg/ml standard solution was injected at different days by different analysts. The results are within acceptance criteria which were tabulated in table-6.

Table-6: Ruggedness Results

day-1	Absorbance	**day-2**	absorbance
10µg/ml	13216598	10µg/ml	13215621
10µg/ml	13216836	10µg/ml	13216962
10µg/ml	13216456	10µg/ml	13215689
%R.S.D	**0.06**	**%R.S.D**	**0.02**

4. ASSAY OF CANAGLIFLOZIN

For estimating the Canagliflozin in marketed formulation, initially 10 tablets were accurately weighed and powdered. The average weight was calculated and powder equivalent to 10mg was weighed and transferred into 10ml volumetric flask and dissolved with diluent. It was sonicated for 10mins and diluted upto mark with Acetonitrile as diluent.

FORMULA:

$$\% \text{ Assay} = \frac{\text{Absorbance}_{sample}}{\text{Absorbance}_{standard}} \times \frac{\text{concentration}_{standard}}{\text{concentration}_{sample}} \times 100$$

$$= \frac{1311752}{1312768} \times \frac{10}{1000} \times \frac{1000}{20.7} \times \frac{99.8}{100} \times \frac{207}{100} \times 100 = \mathbf{99.69\%}$$

5. RESULTS & DISCUSSION

The analytical method was found to be specific which can be shown in figures below. The regression coefficient was 0.995. The %R.S.D for precision, robustness and ruggedness were found to be within acceptance criteria.

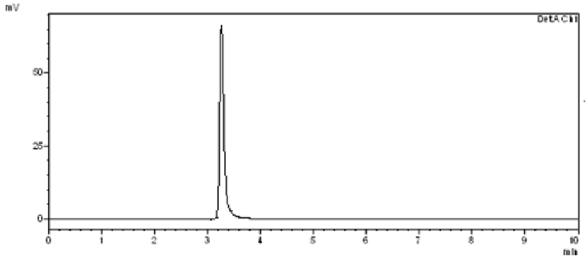

Fig.5: Linearity of Canagliflozin (2µg/ml)

Conc.	Rt	area	Theoretical plate	Tailing factor
2µg/ml	3.256	358822	7015.43	1.487

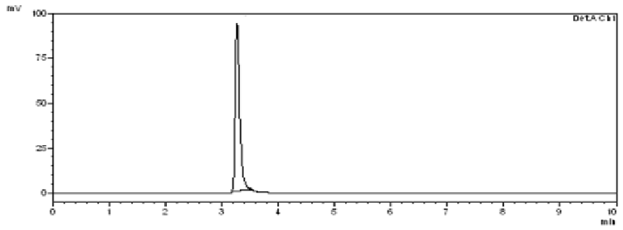

Fig.6 : Linearity of Canagliflozin (4 µg/ml)

Conc.	Rt	area	Theoretical plate	Tailing factor
4µg/ml	3.273	536107	6792.873	1.605

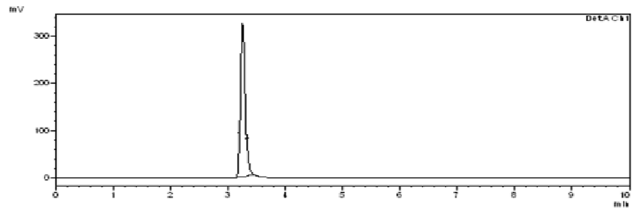

Fig.7 : Linearity of Canagliflozin (6 µg/ml).

Conc.	Rt	area	Theoretical plate	Tailing factor
6µg/ml	3.256	876740	1159.948	1.290

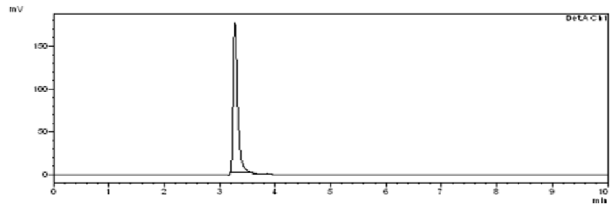

Fig.8 : Linearity of Canagliflozin (8µg/ml)

Conc.	Rt	area	Theoretical plate	Tailing factor
8µg/ml	3.273	1064212	6666.162	1.737

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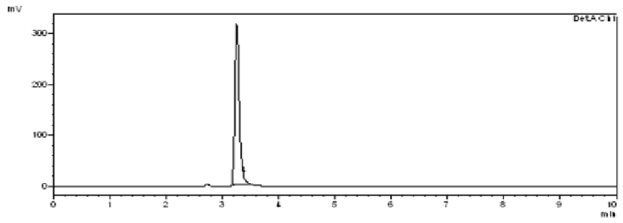

Fig.9: Linearity of Canagliflozin (10µg/ml)

Conc.	Rt	area	Theoretical plate	Tailing factor
10µg/ml	3.256	1311865	7470.144	1.260

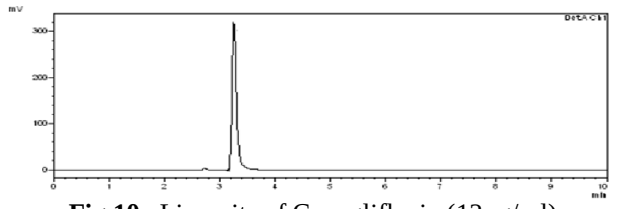

Fig.10 : Linearity of Canagliflozin (12µg/ml)

Conc.	Rt	area	Theoretical plate	Tailing factor
12µg/ml	3.286	1590426	167102.601	1.375

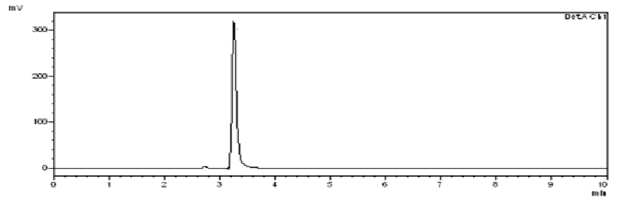

Fig.11 : Linearity of Canagliflozin (14µg/ml)

Conc.	Rt	area	Theoretical plate	Tailing factor
15µg/ml	3.292	2030992	8953.862	1.705

6. CONCLUSION

It could be concluded that the developed method for estimation of Canagliflozin in pharmaceutical dosage form and in bulk is simple sensitive, accurate, precise, reproducible and economical. The proposed method can be used for routine quality control analysis of Canagliflozin in bulk and pharmaceutical formulation.

7. ACKNOWLEDGEMENT

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8. ABBREVATIONS USED

UV-ultra-violet, **LOD**- limit of detection, **LOQ**- limit of quantification, **SGLT2**-sodium glucose co-transporter2, **µg**-microgram, **ICH**-international conference on harmonization, **RSD**-relative standard deviation.

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