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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF CANAGLIFLOZIN IN BULK AND TABLET DOSAGE FORM

P.Shravani Mary, V.Kiran Kumar

Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

Abstract:

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 INERTSIL C18 column (150mm×4.6mm,5µm) with a mobile phase combination of Methanol: Acetonitrile: Water (30:50:20 v/v/v) at a flow rate 1.0ml/min and the eluents were monitored at 250nm. Under these LC conditions Canagliflozin peak was eluted at 3.367 min. The developed method was validated as per ICH guidelines. The correlation coefficient values in linearity were found to be 0.999 and concentration range of 20-60µg/ml for canagliflozin and the mean percentage assay was found to be 99.89%. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: *Canagliflozin, RP-HPLC, Method development, Validation*

Corresponding author:

P.Shravani Mary,

M. Pharmacy,

Department of pharmaceutical Analysis,

Mother Teresa College of Pharmacy,

Ghatkesar, NFC Nagar, Hyderabad,

Telangana,India

E-mail: shravaniraju31@gmail.com

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INTRODUCTION:

This drug is used in conjunction with diet and exercise to increase glycemic control in adults diagnosed with type 2 diabetes mellitus Label. Another indication for canagliflozin is the prevention of major cardiovascular events (myocardial infarction, stroke, or death due to a cardiovascular cause) in patients with type 2 diabetes, as well as hospitalization for heart failure in patients with type 2 diabetes.[1-2] In addition to the above, canagliflozin can be used to lower the risk of end-stage kidney disease and major increases in serum creatinine and cardiovascular death for patients with a combination of type 2 diabetes mellitus, diabetic nephropathy, and albuminuria.[3] The sodium-glucose co-transporter2 (SGLT2), is found in the proximal tubules of the kidney, and reabsorbs filtered glucose from the renal tubular lumen. Canagliflozin inhibits the SGLT2 co-transporter. This inhibition leads to lower reabsorption of filtered glucose into the body and decreases the renal threshold for glucose (RTG), leading to increased glucose excretion in the urine. IUPAC name is 2-(3-{[5-(4-fluorophenyl) thiophen-2-yl] methyl}-4-methylphenyl)-6-(hydroxymethyl) oxane-3,4,5-triol. Molecular formula $C_{24}H_{25}FO_5S$. Molecular Weight is 444.51. Canagliflozin is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas. The solubility of canagliflozin in these solvents is approximately 30 mg/ml.

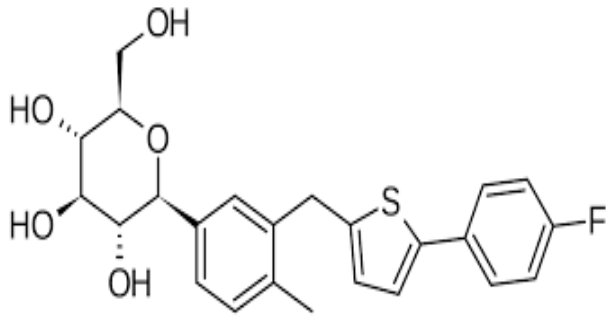

Figure 1: Structure of Canagliflozin

Literature review of Canagliflozin shown that there were several analytical methods like were several analytical methods like UV spectroscopy [3], LCMS [4], HPLC [5-11], HPTLC [12] and only few methods were reported for RP-HPLC for the estimation of this drug in bulk and in its formulation. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC method for the determination of Canagliflozin in API and formulation. The developed method validated as per ICH guidelines. [13-15]

MATERIALS AND METHODS:

Chemicals and Reagents: Canagliflozin were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 250 nm with column INERTSIL column, C18(150x4.6 ID) 5µm, dimensions at Ambient temperature. The optimized mobile phase consists of Methanol: Acetonitrile: water (30:50:20 v/v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of mobile phase

A mixture of 30 volumes of Methanol and 50 volumes of Acetonitrile and 20 volumes of water were prepared. The mobile phase was sonicated for 10min to remove gases.

Preparation of mixed standard solution of Canagliflozin:

Weigh accurately 10mg of Canagliflozin in 25ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of Canagliflozin is prepared by diluting 1ml of Canagliflozin to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution of Canagliflozin:

5 tablets (each tablet contains 100mg of Canagliflozin) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 40µg/ml were prepared by dissolving weight equivalent to 10mg of Canagliflozin dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 25ml with mobile phase. Further dilutions are prepared in 5 replicates of 40µg/ml of Canagliflozin was made by adding 1ml of stock solution to 10 ml of mobile phase.

Preparation of standard stock solution of Canagliflozin

10mg of Canagliflozin was weighed and transferred in to 25ml volumetric flask and dissolved in methanol and then make 1 up to the mark with

methanol and prepare 40 µg /ml of solution by diluting 1ml to 10ml with methanol.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 30 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into INERTSIL column, C18(150x4.6 ID) 5µm column, the mobile phase of composition Methanol: Acetonitrile: water (30:50:20 v/v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Canagliflozin in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:
Linearity: The linearity study was performed for the concentration of 0 ppm to 60 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 75%, 100%,125%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Canagliflozin and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

Figure 2: Standard chromatogram

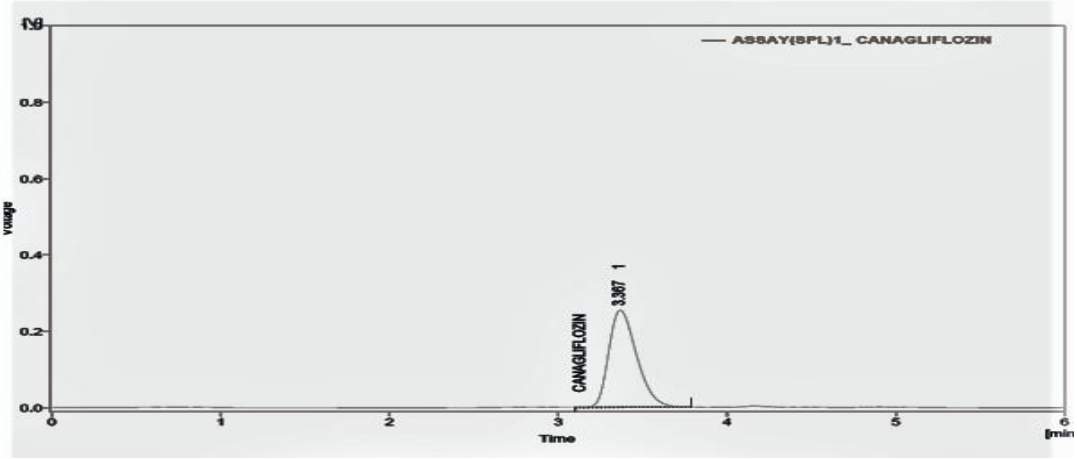

Figure 3: Sample chromatogram

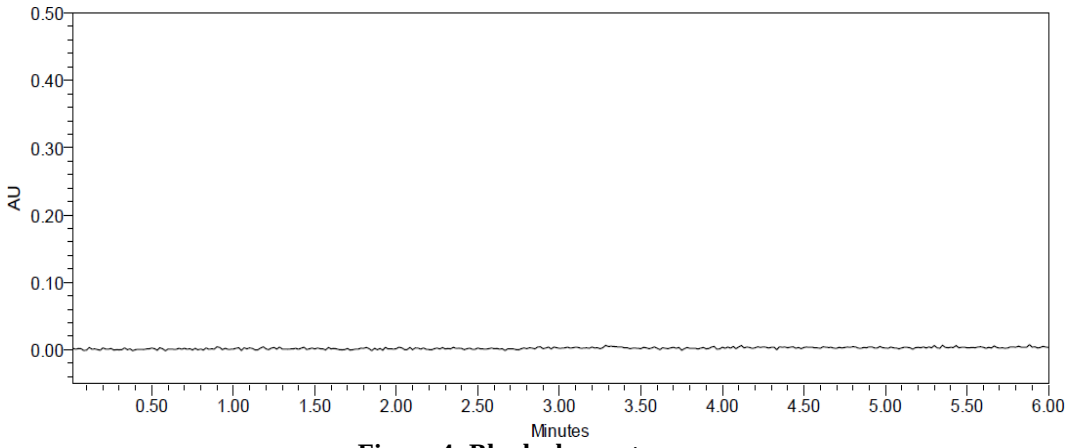

Figure 4: Blank chromatogram

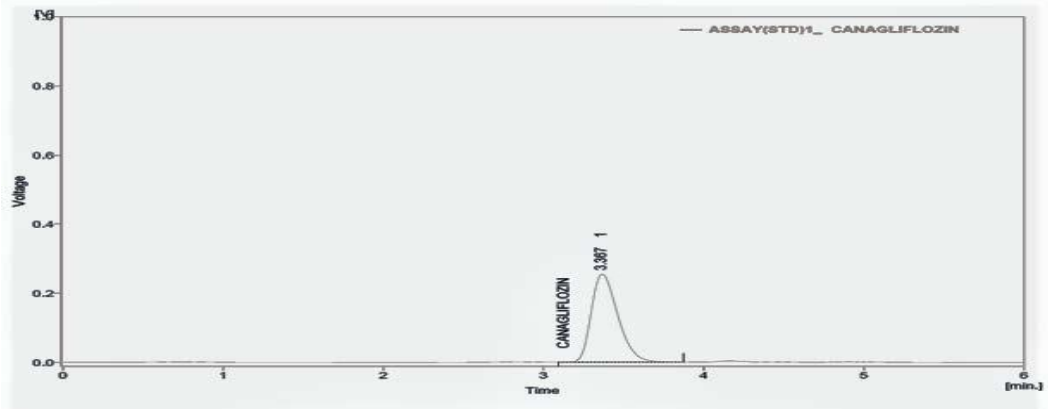

Table 1: System suitability parameters

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	3.337	2976.974	2476	1.500
2	3.367	2935.582	2554	1.477
3	3.343	2949.337	2550	1.512
4	3.353	2938.795	2576	1.477
5	3.327	2944.742	2567	1.512
Mean	3.3474	2949.086	-	-
SD	0.0224	14.469	-	-
%RSD	0.207	0.646	-	-

Table 2: Assay results for Canagliflozin

CANAGLIFLOZIN		
	Standard Area	**Sample Area**
Injection-1	2929.483	2915.223
Injection-2	2925.543	2928.592
Injection-3	2946.561	2945.457
Injection-4	2925.890	2923.218
Injection-5	2900.370	2915.166
Average Area	2933.862	2925.531
Assay(%purity)	**99.52%**	

Table 3: Linearity results of Canagliflozin

S.No.	Conc.(µg/ml)	Area
1	0	0
2	20	1438.77
3	30	2197.92
4	40	2882.59
5	50	3550.79
6	60	4255.54

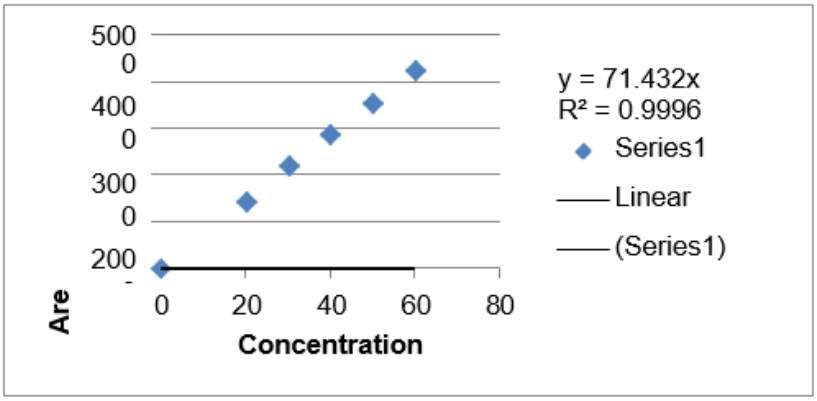

Figure 5: Linearity graph for Canagliflozin

Table 4: Showing accuracy results for Canagliflozin

Recovery level	Amount taken (mcg/ml)	Area	% Recovery	Average % Recovery
75%	30	2069.772	99.58	99.89
	30	2054.232		
	30	2058.565		
100%	40	2882.593	99.10	
	40	2875.659		
	40	2856.789		
125%	50	3535.008	101.01	
	50	3421.987		
	50	3551.001		

Table 5: Precision results for Canagliflozin

Canagliflozin		
S.No.	Rt	Area
1	3.367	2912.410
2	3.367	2932.566
3	3.533	2946.873
4	3.333	2920.975
5	3.777	2911.577
Avg	3.3474	2924.978
SD	0.0220	14.819
%RSD	**0.66**	**0.51**

Table 6. Ruggedness results of Canagliflozin

CANAGLIFLOZIN	%Assay
Analyst 01	99.67
Analyst 02	98.34
%RSD	0.94%

Robustness results

Table 7: Flow variation results for Canagliflozin

Parameter	Canagliflozin	
	Retention time(min)	**Tailing factor**
Flow		
0.8ml/min	4.187	1.717
1.2ml/min	2.830	1.656
Wavelength		
248nm	3.353	1.605
252nm	3.353	1.605

Table 8: LOD, LOQ of Canagliflozin

Drug	LOD	LOQ
Canagliflozin	7.35	22.29

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Canagliflozin in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Canagliflozin in pure and its pharmaceutical dosage forms.

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