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Original Article

RP-HPLC Method for the Estimation of Canagliflozin in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A unique RP-HPLC method become developed and proven for the estimation of Canagliflozin in pharmaceutical dosage forms. The RP-HPLC of Canagliflozin become achieved on Symmetry C_{18} column having 250mm×4.6 mm ID dimension, 5µm particle size) and Agilent LC1220 HPLC machine with UV detection (VWD detector) at 293nm become HPLC gadget. The mobile segment becomes decided on as Methanol: Phosphate buffer (P^H adjusted to 4 with orthophosphoric acid) (65:35 v/v) with flow rate at 1ml / min. Chromatogram indicates the retention time of 2.980min. The RP-HPLC method turned into proven as consistent with ICH recommendations and the linearity was found in the concentration range of 10-125µg/ml with Correlation coefficient was 0.999 and the regression equation was determined to be Y = 12680x+13722. The LOD and LOQ of Canagliflozin had been discovered to be 0.9 µg / ml and 2.7 µg / ml respectively. Recovery of Canagliflozin was found to be in the range of 99.33-99.92%. For this reason, the proposed technique turned into efficaciously implemented for the estimation of Canagliflozin in pharmaceutical dosage forms as per ICH guidelines.

Key words: Canagliflozin, RP-HPLC, VWD detector, ICH recommendations.

1. INTRODUCTION

Canagliflozin is an orally administered sodium-glucose co-transporter-2 (SGLT2) inhibitor used in the remedy of sufferers with type 2 diabetes by means of inhibiting the transporter protein SGLT2 inside the kidneys, canagliflozin reduces renal glucose reabsorption, thereby increasing urinary glucose excretion and reducing blood glucose levels[1]. Canagliflozin is chemically known as (1S)-1, 5-

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anhydro-1-C-(3-{[5-(4-fluorophenyl) thiophen-2-yl] methyl]}-4-methylphenyl)-D-glucitol become shown in Figure 1. Literature overview tells that very few analytical methods have been reported for the determination of Canagliflozin which includes High performance liquid chromatography[2-4], Liquid chromatography-Mass spectroscopy[5], and Pharmacokinetics studies[6]. The prevailing look at became aimed to develop a novel, easy, economic and demonstrated RP-HPLC method for the estimation of Canagliflozin consistent with ICH recommendations[7].

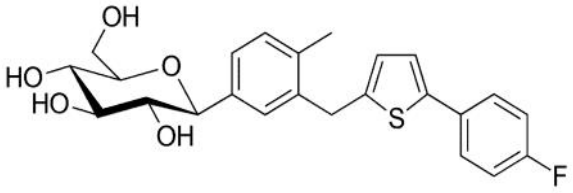

Fig 1: Chemical structure of Canagliflozin

2. MATERIALS AND METHODS

Chemicals and Reagents:

Canagliflozin bulk drug had been procured from USV Limited, Mumbai, India. Potassium dihydrogen phosphate (Merck Chemical Company, GR-Grade), Orthophosphoric acid (Merck Chemical Company, GR-Grade), Water (Merck Chemical Company, HPLC-Grade) and Methanol (Merck Chemical Company, HPLC-Grade) had been used inside the study. Sulisent® tablet contain Canagliflozin 100mg is acquired from Welcome Healthcare Pvt. Ltd, Mumbai, India.

Instrumentation:

The RP-HPLC technique become advanced in Agilent LC1220 HPLC machine, ready with VWD detector and EZ Chrome software, Symmetry C_{18} column (250mm×4.6 mm, 5µm particle size) was used as stationary phase. All weights were taken on digital balance (version: CA123, Make: Contech), pH Meter (Model: 3 Star, Make: Global) and Sonicator (Model: UCB 70, Make: Life care) had been used within the study.

Chromatographic conditions:

The RP-HPLC approach changed into completed in Symmetry C_{18} column (250mm × 4.6 mm i.d., 5 µm particle size) as stationary phase and mobile phase was a mixture of Methanol: Phosphate buffer (P^H adjusted to 4 with orthophosphoric acid) (65:35 v/v) was passed at a flow rate of 1.0 mL/min at 293 nm detector wavelength. 20µl is the injection volume and the run time become 5min and the retention time of Canagliflozin becomes observed to be 2.980min.

Chromatographic Parameters:

Equipment : Agilent LC1220 HPLC system, equipped with VWD detector
Column : Symmetry C_{18} (250mm×4.6 mm, 5µm particle size)
Flow rate : 1ml/min
Wavelength : 293 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 5 Minutes

Preparation of mobile phase:

Solution A: 1.368g of Potassium di-hydrogen phosphate (KH_2PO_4) was taken into 1000ml volumetric flask and dissolved to 1000ml with HPLC grade water and sonicated in ultrasonic water bath and then it become filtered through 0.45µm filter out the usage of vacuum filtration and the pH become adjusted to 4 with orthophosphoric acid.

Solution B: Methanol HPLC-Grade

Mobile Phase: Volume of Solution (A) and solution (B) taken in ratio 35:65 (v/v) and mixed properly and filtered through 0.45µm membrane filter out and degas for 10 minutes.

Preparation of diluent:

Mobile phase was used as diluent.

Preparation of Standard Stock Solution:

100mg of Canagliflozin was taken into 100ml volumetric flask and then it was dissolved in 100ml distilled water; the final volume was making up to the mark with distilled water to get the standard solution concentration of 1000µg/ml. Those stock solutions are further diluted to specific concentrations.

Preparation of Sample Solution:

Canagliflozin is available with brand name SULISENT® (100mg, Welcome Healthcare, India). Twenty tablets of Canagliflozin were taken and made into a fine powder and the powder equivalent to 100mg of Canagliflozin turned into taken and transferred into a 100ml volumetric flask after which it changed into dissolved in mobile phase and sonicated for half hour and make up to the mark. Then it changed into filtered through 0.45 micron Whatmann filter paper (No. 41) and the final solution was make up to the mark with mobile phase to get the solution of 1000µg/ ml. From that 10ml of above solution was transferred to 100ml volumetric flask, volume was made with mobile phase to produce 100µg/ml [8, 9]. From the above solution 5ml was pipetted out separately into 10ml volumetric flask to get 50µg/ml. 20µl of standard and sample solution was injected into the HPLC system and the peak areas were measured for Canagliflozin was shown in Figure 2 and 3 respectively. The % Assay was calculated through evaluating the peak area of standard and sample chromatogram and the assay result was proven in Table 1.

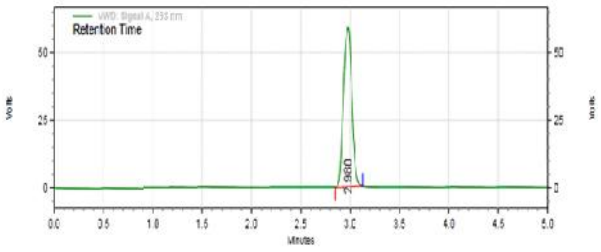

Fig 2: Standard chromatogram of Canagliflozin

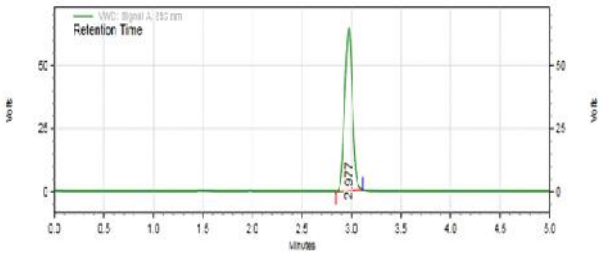

Fig 3: Sample chromatogram of Canagliflozin

Table 1: Assay of marketed formulation of Canagliflozin

Drug	Sulisent® Label Claim (mg)	Amount Found (mg)	% Label Claim ± % RSD (n=3)
Canagliflozin	100	100.09	100.09±0.21

System Suitability:

For system suitability research, one blank followed by using six replicates of a single calibration standard solution of 50µg/ml of Canagliflozin was injected into the HPLC device to check the system suitability for the proposed method, the parameters consisting of retention time, theoretical plates and peak asymmetry have been measured [10] and the effects have been presented in Table 2.

Table 2: System suitability test parameters for Canagliflozin

Parameter (n=6)	Canagliflozin
Retention Time (Mins)	2.980
Theoretical plates	4853
Tailing factor	1.1

Specificity:

The specificity of the RP-HPLC method was established through injecting the blank and placebo solution into the HPLC system. The representative chromatogram of blank and placebo become proven in Figure 4 and 5 respectively.

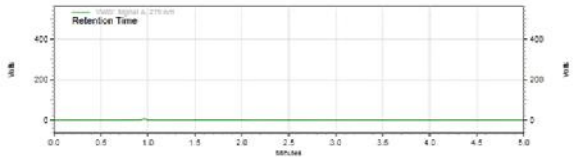

Fig 4: Chromatogram of Blank

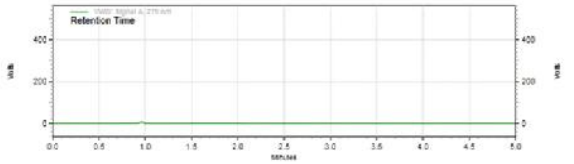

Fig 5: Chromatogram of Placebo

Linearity:

Aliquots of 0.1, 0.25, 0.5, 0.75, 1.0, 1.25 ml had been pipette out and transferred into10ml volumetric flasks from stock solution (1000µg/ml) and diluted up to the mark with diluent to get the concentration range of 10-125 µg/ml. 20µl of each concentration was injected into the HPLC system [11, 12] for 3 times. The peak area and retention time have been recorded and the mean values of peak areas have been plotted in opposition to concentrations. The linearity data is presented in Figure 6 and Table 3.

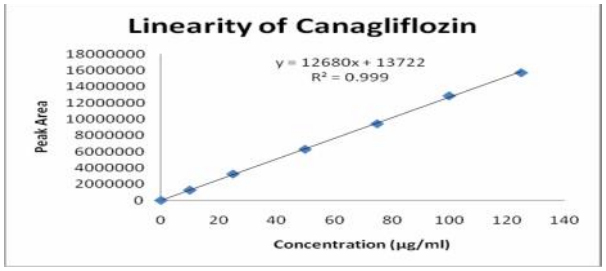

Fig 6: Linearity graph of Canagliflozin

Table 3: Linearity data for Canagliflozin

Linearity of Canagliflozin	
Concentration (µg/ml)	**Peak Area**
10	1260197
25	3250493
50	6300987
75	9451480
100	12901974
125	15752467

Accuracy studies:

The accuracy of the approach changed into performed through calculating recovery of Canagliflozin by way of the approach of standard addition. Recognized quantity of standard solution of Canagliflozin at 50%, 100% and 150% changed into delivered to a pre-quantified sample solution and injected into the HPLC device [13, 14]. The mean percentage recoveries of Canagliflozin at each level become calculated and the results were offered in Table 4.

Table 4: Recovery study data of Canagliflozin

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Statistical Analysis
S_1:50%	25	24.82	99.28	Mean-99.33
S_2:50%	25	24.89	99.56	S.D-0.21
S_3:50%	25	24.79	99.16	%RSD-0.21
S_4:100%	50	49.68	99.36	Mean-99.57
S_5:100%	50	49.96	99.92	S.D-0.3
S_6:100%	50	49.72	99.44	%RSD=0.3
S_7:150%	75	75.07	100.09	Mean-99.92
S_8:150%	75	74.82	99.76	S.D-0.17
S_9 :150%	75	74.94	99.92	%RSD-0.17

Precision studies for Canagliflozin:

Method precision (Repeatability):

Canagliflozin tablet powder equivalent to 100mg had been taken into 100ml volumetric flask after which turned into added and sonicated to dissolve it absolutely and volume was made up to mark with diluents and filtered through 0.45 µm nylon membrane filter. From the above solution 0.5ml become pipette out and transferred into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 50µg/ml of Canagliflozin. A six replicates of the above solution turned into injected into HPLC device and the %RSD for the area of six replicate injections was calculated as cited in Table 5.

Table 5: Method precision data for Canagliflozin

Canagliflozin		
S.No.	Concentration (µg/ml)	% Assay
1	50	101.09
2	50	101.26
3	50	101.75
4	50	100.93
5	50	101.40
6	50	101.52
Average		**101.49**
SD		**0.57**
%RSD		**0.56**

System precision:

50µg/ml of Canagliflozin standard solution become injected six times into the HPLC and the %RSD for the area of six replicate injections turned into calculated as noted in Table 6.

Table 6: System precision data for Canagliflozin

Canagliflozin		
S.No.	Concentration (µg/ml)	Peak Area
1	50	6290987
2	50	6291779
3	50	6298775
4	50	6294118
5	50	6293384
6	50	6289004
Average		**6293007**
SD		**3354.36**
%RSD		**0.05**

Intermediate precision/ruggedness:

The intermediate precision of the method become evaluated by performing precision on different laboratory by different analyst and different days. 50µg/ml of Canagliflozin became injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as cited in Table 7.

Table 7: Ruggedness data for Canagliflozin

Ruggedness Data for Canagliflozin								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Conc. (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
50	99.47	100.84	99.28	100.76	99.74	99.46	100.07	99.94
50	99.58	100.69	99.73	100.75	99.23	100.09	101.56	100.00
50	100.03	100.35	99.54	99.00	100.27	101.12	99.28	101.50
50	99.49	101.07	101.21	100.37	100.34	100.44	101.47	99.02
50	99.06	99.05	99.90	100.54	100.60	99.81	99.08	99.42
50	99.95	100.76	101.14	100.93	101.16	101.55	99.57	99.24
Average	**99.59**	**100.46**	**100.13**	**100.39**	**100.22**	**100.42**	**100.17**	**99.85**
SD	**0.354**	**0.729**	**0.833**	**0.708**	**0.671**	**0.784**	**1.092**	**0.894**
%RSD	**0.355**	**0.725**	**0.831**	**0.706**	**0.670**	**0.784**	**1.091**	**0.895**
Intermediate precision within-laboratories variations (n=24)								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
Average	100.14				**Average**	100.16		
SD	0.656				**SD**	0.860		
%RSD	0.65				**%RSD**	0.85		
Reproducibility between laboratories (n=48) (% Assay)								
Average	100.15							
SD	0.758							
%RSD	0.75							

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3.3×SD/S and 10×SD/S respectively as consistent with ICH guidelines, in which SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD and LOQ of Canagliflozin was calculated and shown in Table 8.

Table 8: Summary of validation parameter for Canagliflozin

Parameters	**RP-HPLC method**	
	Canagliflozin	
Linearity range (µg/ml)	10-125	
Slope	12680	
Intercept	13722	
Correlation coefficient	0.999	
LOD (µg/ml)	0.9	
LOQ (µg/ml)	2.7	
Method Precision (% RSD, n=6)	0.56	
System precision (% RSD, n=6)	0.05	
Ruggedness (% RSD, n=24)	Lab-1	Lab-2
	0.65	0.85
Reproducibility (% RSD, n=48)	0.75	
% Accuracy	99.33-99.92	
Robustness (% RSD, n=6)	Less Flow rate	More Flow rate
	0.3	0.4
	Less Organic phase	More Organic phase
	0.23	0.45

Robustness:

Robustness became executed with the aid of deliberate change within the flow rate and mobile phase proportion became made to assess the impact on the method. The effects are summarized in Table 9.

3. RESULTS AND DISCUSSION

RP-HPLC parameters have been optimized with the aid of investigating numerous mobile phase compositions. An amazing separation and peak symmetry for Canagliflozin have been done with a mobile phase of Phosphate buffer (pH adjusted to 4 with orthophosphoric acid) and Methanol (35:65, v/v) was delivered at a flow rate of 1mL/min with VWD detection at 293nm. The retention time of Canagliflozin was determined to be 2.980min. Linearity becomes established in the range of 10-125µg/ml with correlation coefficient 0.999 and means accuracies were 99.33% to 99.92%, which suggests accuracy of the proposed method. The % RSD values of accuracy had been found to be < 2 %. The % RSD value of method precision was 0.56% and % RSD value of system precision was 0.05%. The % RSD value of reproducibility is 0.75% display that the proposed method is precise. The LOD and LOQ value were discovered to be 0.9µg/ml and 2.7µg/ml respectively. The % RSD values of robustness studies have been determined to

be < 2% reveal that the method is robust enough. These statistics shows that the proposed approach is unique, specific and sensitive for the determination of Canagliflozin

Table 9: Summary of Robustness (Change in Flow Rate and mobile phase) for Canagliflozin

Parameters	Mean peak Area(n=3)	S.D	%R.S.D	RT	Theoretical plates
Flow rate 0.9ml/min	6878060	21024.5	0.305	3.663	7619
Actual flow rate 1ml/min	6293007	3354.36	0.053	2.977	4853
Flow rate 1.1ml/min	6461927	26328.2	0.407	2.707	4217
10% less organic (58:42)	6920519	16567.65	0.239	2.633	7609
Actual mobile phase (65:35)	6293007	3354.36	0.053	2.977	4853
10% more organic (72:28)	6236215	28314.8	0.454	3.280	4281

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4. CONCLUSION

As in keeping with the ICH guidelines, RP-HPLC technique for the estimation of Canagliflozin of their bulk and pharmaceutical dosage form was established and validated. Linearity was done for Canagliflozin in the range of 10-125µg/ml with correlation coefficient 0.999. The percentage recovery of drug was achieved in the range of 98-102% which become within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the advanced method. The developed method is simple and unique. For this reason it may be used for the routine evaluation of Canagliflozin of their bulk and pharmaceutical dosage form.

5. ACKNOWLEDGEMENT

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