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VALIDATION OF A STABILITY-INDICATING REVERSE PHASE HPLC METHOD FOR DETERMINATION OF CANAGLIFLOZIN API

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ABSTRACT

A new simple, rapid and sensitive RP-HPLC method has been developed for the determination of Canagliflozin Active Pharmaceutical Ingridient (API). The method employs X-bridze C18 column (150mm×4.6 mm, 5mm particle size) column for the chromatographic separation and orthophosphoric acid (0.1% w/v) : acetonitrile (60:40, v/v) was used as a mobile phase. Separation was completed within 10 min with a flow rate of 1 ml/min and detection was at 230 nm. The retention time of canagliflozin was found to be 8.70 min. The proposed method was found to have the linearity in the concentration range of range of 25-75 µg/ml. Linear regression coefficient was not less than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Canagliflozin. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH Guidelines and USP requirements. The method was found to have suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS: Canagliflozin, RP-HPLC Estimation, Analytical Method Validation, API.

INTRODUCTION

Canagliflozin is an anti diabetic drug used to improve glycemic control in patients with type II diabetes.[1-5] Chemically it is (2S,3R,4R,5S,6R)-2-(3-{[5-(4-fluorophenyl)thiophyl]methyl}-4-methylphenyl-6-(hydroxymethyl)oxane-3,4,5-triol (fig. 1). This drug showed a promising effect in stabilizing the glycemic fluctuations in patients with type 2 diabetes[6,7] throughout the day and suppresses the diabetic complications. It inhibits

SGLT2, reduces reabsorption of filtered glucose and lowers the renal threshold for glucose (RTG), and thereby increases urinary glucose excretion.[8-11]

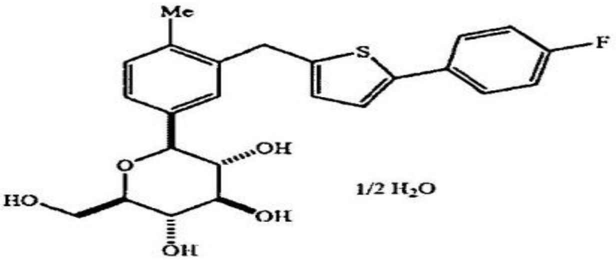

Fig. 1: Structure of Canagliflozin.

In clinical trials, Canagliflozin produced a dose-dependent reduction in HBA1C of 0.6 to 0.9 greater than that obtained with placebo when administered as mono therapy, in combination with metformin, or in combination with metformin and sulfonylurea.[12]

Various analytical methods including UV Spectroscopy[13] HPLC– MS/MS methods[14], RP-RP-HPLC,[15-20] are available for the estimation of canagliflozin in pharmaceutical dosage forms. We tried to develop a simple method for the estimation of canagliflozin API by RP-HPLC method. The proposed method was optimized and validated as per the International Conference of Harmonization (ICH) guidelines.[21]

MATERIAL AND METHODS

Analytically pure sample of Canagliflozin API was obtained from Aktteva Biopharma Ltd., Ahmedabad, Gujarat. India. Methanol, Acetonitrile (HPLC Grade) was obtained from MERCK Germany. Analytical reagent grade orthophosphoric acid was purchased from Rankem, Mumbai,India.

a) Chromatographic conditions

Canagliflozin was analysed in X-bridze C18 column (150mm×4.6 mm, 5mm particle size) column for the chromatographic separation. The mobile phase was composed of 0.1% w/v orthophosphoric acid and Acetonitrile (60:40, v/v). Filtered through 0.22 mm membrane filter under vacuum filtration and degassed. The mobile phase is pumped at ambient temperature, at a flow rate of 1 ml/min with UV detection wavelength at 230 nm. Injection volume was 10µl. The run time was 10 min. The mobile phase was used as diluent.

a) Preparation of stock solution

Accurately weighed quantity of Canagliflozin (50 mg) was transferred to a 100 ml volumetric flask, dissolved and diluted up to the mark with diluent and was ultra-sonicated for 5 min (5 0 µg/ml).

b) Preparation of standard working solution

Standard working solution was prepared by taking 5 ml of stock solution into 50 ml volumetric flask and the final volume was made up with diluent (50 µg/ml). The solution was filtered and then diluted immediately before use to appropriate concentration levels by using mobile phase.

RESULTS AND DISCUSSION

i. Method development and optimization of chromatographic conditions

To achieve good peak different buffer pH-conditions and different proportions of solvents like methanol, acetonitrile and water were tested with binary and tertiary eluents. However, 0.1% OPA in Water: ACN mixed in the ratio of 60:40 % v/v mobile phase at a flow rate of 1 ml/min and detection at 230 nm achieved good satisfactory results. The chromatogram of optimized standard mixture is shown in fig. 2. The system suitability parametres such as retention time, resolution and theoretical plates for optimized standard mixture are given in table 1.

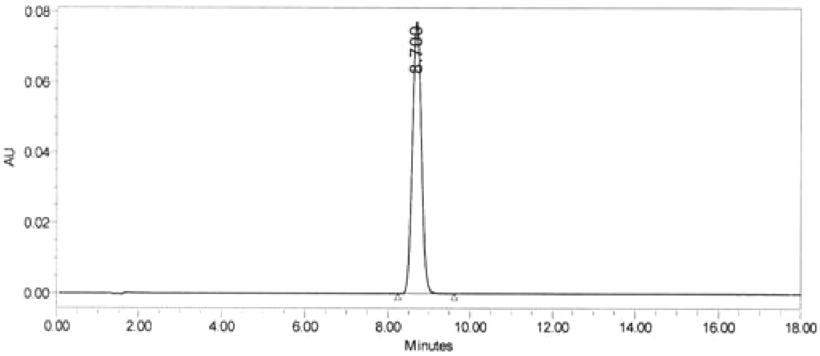

Fig. 2: Chromatogram of canagliflozin.

ii. Method validation

The method was validated for system suitability, accuracy, precision, linearity, detection limit, quantitation limit and robustness.

a) System Suitability

System Performance parameters of developed HPLC method was determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution(R), retention time (RT) and %RSD for replicate injections were determined (Table 1). The results were found to be within the limits.

Table. 1: System Suitability Results.

Parameter	Canagliflozin
Retention Time (min)	8.7
Theoretical plates	7476.23
Tailing Factor	1.06
Resolution	0.15
RSD of peak area	0.12
RSD of retention time	0.98

b) Specificity: Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. Studies are designed to evaluate the degree of interference, if any, which can be attributed to other analyte, impurities, degradation products, reagent "blanks" and excipients. Blank chromatogram does not show any disturbance peak at the retention time of canagliflozin (Fig.3) and hence the method is specific.

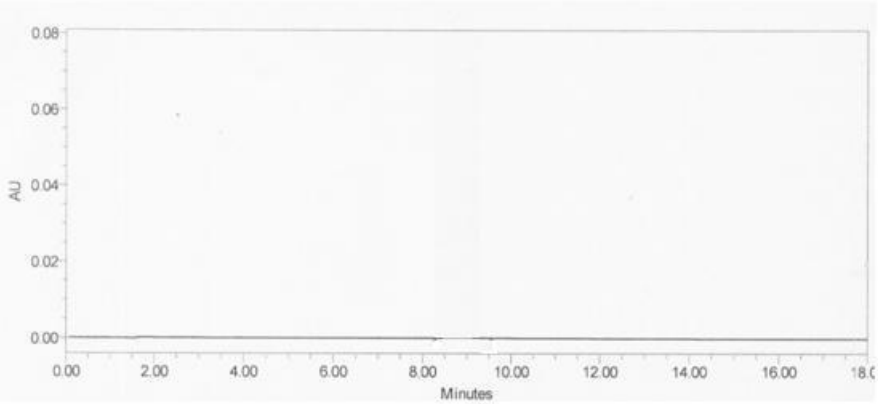

Fig. 3: Blank chromatogram.

c) Accuracy: To determine the Accuracy of the proposed method, recovery studies were conducted; known amount of pure drug concentrations was spiked in placebo at three different levels, ie, 50%, 100% and 150% and was calculated. Accuracy was calculated as the percentage of recovery. The results were tabulated in Table 2.

Table. 2: Accuracy data.

Parameter	Amount taken	Amount recovered	% Recovery	Mean Recovery	%RSD
canagliflozin					
50%	25	24.90	99.60	99.72	0.92
100%	50	49.88	99.76		
150%	75	74.85	99.80		

d) Precision: Precision was evaluated at three levels: repeatability, intermediate precision and reproducibility. Each level of precision was investigated by six replicate injections of concentrations of 50 µg/ml of canagliflozin. The result of precision was expressed as % RSD, given in Table 3.

Table. 3: Method precision Results.

Parameter	Results
	Canagliflozin
Repeatability	
Mean %RSD of Retention Time	0.15
Mean % RSD of Peak Area	0.21
Mean % Assay	100.22
Reproducibility	
Mean %RSD of Retention Time	0.14
Mean % RSD of Peak Area	0.22
Mean % Assay	100.8
Intermediate Precision	
Mean %RSD of Retention Time	0.16
Mean % RSD of Peak Area	0.25
Mean % Assay	99.96

e) Linearity

The linearity was evaluated by measuring different concentrations (80% to 130%) of the standard solutions. The solutions were examined by the assay procedure. The calibration curve was plotted using response factor (peak area ratio of the standard peak area and internal standard peak area) Vs concentration of the standard solution. From the calibration curve, the regression equation was computed. The summary of the parameters are shown in Table 4.

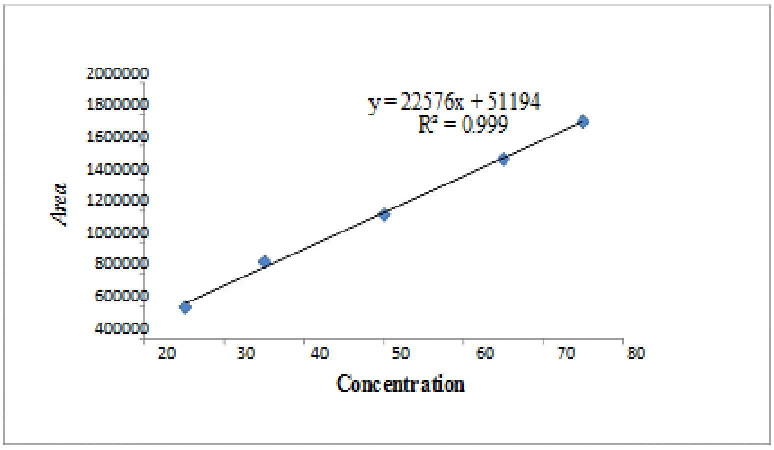

Fig. 4: Linearity curve of Canagliflozin.

Table. 4: Linearity parameters.

Parameter	**Canagliflozin**
Linearity Range(µg/ml)	25 -75
Correltion coeffecient	0.999
Slope	22576
Y-intercept	51194

f) Detection limit (DL) and quantitation limit (QL)

Estimation of DL and QL considered the acceptable signal-to-noise ratios 3:1 and 10:1 respectively. The limit of detection and quantitation determined was 3.58 and 10.85 µg/ml for canagliflozin.

g) Robustness and Ruggedness

The robustness of an analytical method was unaffected by deliberate change in the Flow rate, pH, mobile phase composition and column temperature were performed at 100% concentration.

The ruggedness of the proposed analytical method was performed in different condition like different columns, analyst, instrument, laboratories analysis of the same sample. The results are within acceptance criteria.

Analysis of marketed sample

The proposed method was applied for the analysis of canagliflozin API, the results were found to be 102%, results were summarized in Table 5.

Table. 5: Assay results.

Drug	Labelled amount	Amount found	% assay
Canagliflozin	50.53	49.47	102.14

iii. Forced degradation studies

To evaluate the stability indicating property of the developed HPLC method stress studies were carried out as per the ICH guidelines. Forced degradation of canagliflozin was carried out by exposing the bulk sample to acidic, alkaline, oxidative, photolytic, dry heat and neutral conditions.

a) Stock solution

Accurately weighed and transferred 50 mg of canagliflozin samples into a 100ml clean and dried volumetric flask added about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette out 5 ml of above stock sample solution into a 50ml volumetric flask and diluted up to the mark with diluent. Results of Forced degradation are shown in Table 6.

b) Acidic degradation

To 10 ml of stock solution, 10ml of 1N HCl was added and kept at 80ºC for about 12 hours in water bath, cooled and the volume was made up to 100ml with mobile phase. The solution was filtered through 0.22 micron membrane filter.

c) Alkali degradation

To 10 ml of stock solution, 10ml of 0.5N NaOH was added and kept at 80ºC for about 12 hours in water bath, cooled and the volume was made up to 100ml with mobile phase. The solution was filtered through 0.22 micron membrane filter.

d) Oxidative degradation

To 10 ml of stock solution, 5ml of 3% H_2O_2 was added and kept at 80ºC for about 12 hours in water bath, cooled and the volume was made up to 100ml with mobile phase. The solution was filtered through 0.22 micron membrane filter.

e) Thermal degradation

The 10 ml of stock solution was kept at 70ºC for about 10 days, cooled and the volume was made up to 100ml with mobile phase. The solution was filtered through 0.22 micron membrane filter.

Table. 6: Degradation studies of canagliflozin.

Condition	canagliflozin	%Rec
acid	1502998	95.08
base	1396675	89.63
peroxide	1566183	98.65
heat	1534041	95.83
UV	1494948	95.91

CONCLUSION

Based on the results of the above studies, it is concluded that the method for determination of assay of canagliflozin API is precise, linear over the concentration range, stability indicating, and rugged. The method is robust with respect to variation in flow rate, column temperature and buffer composition. The method is specific for the quantization of assay of canagliflozin API. So the developed method can be easily applied for routine analysis of canagliflozin API.

The method was found to be simple accurate economical and rapid and it can be applied for routine analysis in laboratories and suitable for the quality control of bulk and pharmaceutical formulations.

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