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Research Article

DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHOD FOR ESTIMATION OF CANAGLIFLOZIN IN API AND TABLET DOSAGE FORM

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ABSTRACT

A simple, specific, precise and accurate chromatographic method for estimation of canagliflozin in API and tablet dosage form was developed by C_{18} column having 250 mm length, 4.6 mm internal diameter, 5µ particle size. Peak was observed in mobile phase consist of Methanol: Acetonitrile: 0.1 % Ammonium Acetate in the proportion of 40:40:20 v/v/v. The flow rate was 1ml/min. The estimation was carried out at 290 nm. The retention time was found to be 4.1 minute for Canagliflozin. Linearity was found in range of 100-300 µg/ml. The method was validated as per ICH guideline Q2R1. All validation parameters were found to be within accepted range specified in ICH guideline Q2R1.

INTRODUCTION

Canagliflozin is oral selective Sodium-Glucose co-transporter2 (SGLT2) inhibitor used for the management of type 2 Diabetes Mellitus.[1] The chemical name (IUPAC) of Canagliflozin is (2S, 3R, 4R, 5S, 6R)-2-{3-[5-(4-fluoro-phenyl)-thiophen-2-ylmethyl]-4-methyl-phenyl}-6hydroxy methyltetrahydro-pyran-3,4,5-triol with molecular formula $C_{24}H_{25}FO_5S$. It is white to off white solid with melting point of 95-105ºC.[2-4] It is soluble in many organic solvents (Methanol, Acetonitrile, Dimethyl sulfoxide) but insoluble in aqueous media. It curbs the transporter protein SGLT2 present in the proximal tubules of the kidney which curtails renal glucose absorption, thereby increasing urinary glucose excretion and lowering blood glucose levels. [4-5] it is a product of Mitsubishi Tanabe Pharma and Janssen Pharmaceuticals, a division of Johnson and Johnson and marketed with the brand name of INVOKANA in strengths of 100 and 300 mg respectively. [6]

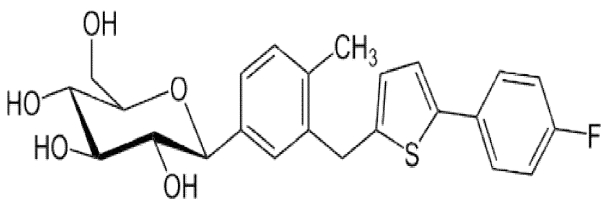

Fig 1 Structure of Canagliflozin

MATERIALS AND METHOD

Instruments

Shimadzu Model SPD-20A instrument, series RP-HPLC system with UV detector. Phenomex Column C_{18}, 250 X 4.6 mm, 5µm (particle size). Sonicator of Digital Ultrasonic cleaner (LMUC series LABMAN) and analytical balance of Libror AEU-210 (shimadzu)

Chemicals

Canagliflozin standard was supplied by Johnson & Johnson, Pharmaceutical Company. Acetonitrile, Methanol, Distilled Water and Ammonium Acetate of HPLC grade was purchased from Merck (India) Ltd., Mumbai. Canagliflozin tablets (Containing 100 mg) was procured from local market.

HPLC conditions

A chromatographic separation of drug was achieved using Phenomex, 250 X 4.6 mm, 5µm (particle size) C_{18} column with Mobile phase of Acetonitrile: Methanol: 0.1 % Ammonium acetate (40:40:20 V/V). Drug was monitored at detection wavelength of 290nm, the flow rate was 1 ml/min, and injection volume was 20 µl. Retention time of Canagliflozin was about 4.1 minute respectively.

Preparation of Mobile Phase

0.1 % Ammonium acetate was prepared by dissolving 0.1 gm of Ammonium acetate in 100 ml of water. Methanol, Acetonitrile and Ammonium acetate were sonicated for 5 min for degassing and filtered through 0.45 µ Millipore filter.

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Preparation of Standard Solution

20 mg of Canagliflozin was taken and transferred to 100 ml volumetric flask separately and volume was made up with diluent (Stock solution-200 µg/ml)

Preparation of Sample Solution

For the analysis of tablet formulation (CANAGLIFLOZIN Tablet, 100 mg), Twenty tablets were weighed, powdered. Tablet powder equivalent to 100 mg of Canagliflozin was transferred to 100 ml volumetric flask and the volume was made up to the mark and from above stock solution further dilutions were made to make 200 µg/ml. The solution was filtered through 0.45 µ Millipore filter.

Method Validation [7]

Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the matrix. Chromatogram of standard, test and blank is done.

Linearity and Range (n=5)

Linearity response to determine by analyzing different concentrations for calibration curve in range of 100-300 mcg/ml for Canagliflozin. Peak areas were measured at each level. Peak areas were plotted against concentration and equation of straight line and correlation co-efficient was determined.

Accuracy (n=3)

The accuracy of the method was determined at 0%, 50%, 100%, & 150% by calculating recoveries of Canagliflozin by the standard addition method. Known amount of standard solutions of Canagliflozin were added to pre-quantified sample solution of Canagliflozin. Each solution was injected in triplicate and the percentage recovery was calculated by measuring the peak areas and fitting these value into the regression equation of the respective calibration curves.

Precision

The repeatability of the proposed method was determined by measuring the corresponding responses 6 times. The intra-day and inter-day precisions of the proposed method was determined by measuring the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of Canagliflozin.

LOD and LOQ

The LOD is estimated from the set of 3 calibration curves used to determine method linearity.

LOD was calculated as, LOD =3.3 × (SD/Slope)
SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.
The LOQ was estimated from the set of 5 calibration curves used to determine method linearity.

LOQ was calculated as, LOQ =10 × (SD/Slope)
SD = Standard deviation of the Y- intercepts of the 5 calibration curves.
Slope Slope = Mean slope of the 5 calibration curves.

Robustness

The Robustness study was performed by altering the method parameters like changing flow rate. The change in the response of Canagliflozin was noted.

Flow rate: was changed from 1ml/min to 0.9ml/min and 1.1ml/min.

Assay

For the analysis of tablet formulation (CANAGLIFLOZIN Tablet, 100 mg), Twenty tablets were weighed, powdered. Tablet powder equivalent to 100 mg of Canagliflozin was transferred to 100 ml volumetric flask and the volume was made up to the mark and from above stock solution further dilutions were made to make 200 µg/ml. The solution was filtered through 0.45 µ Millipore filter.

RESULTS AND DISCUSSION

The analytical method was found to be specific as there was no interference of any excipients or impurities which can be shown from figure 2, 3 and 4. Overlay of linearity was shown in figure 5 in the range of 100-300 mcg/ml and regression coefficient was found to be 0.9998 which is shown in figure 6 and calibration data are shown in table 1 and regression data is shown in table 2. The %RSD for repeatability was found to be 1.57 as mentioned in table 3. The %RSD for intraday precision was found to be 0.92-1.20 of Canagliflozin mentioned in table 4. The %RSD for interday precision was found to be 1.06-1.30 mentioned in table 5. Mean percentage recovery of Canagliflozin was found to be in range of 98.04-100.27 mentioned in table 6. The % RSD for robustness was found to be 1.29-1.70 for Canagliflozin as mentioned in table 7. The % assay was found to be 99.88-101.84% as mentioned in table 8.

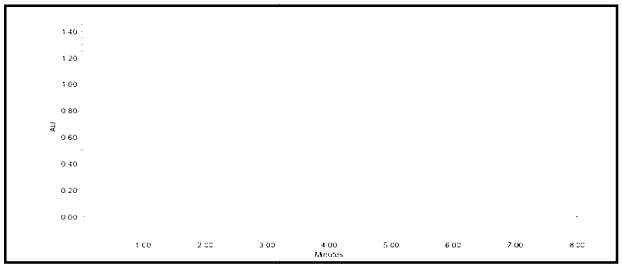

Figure 2 Chromatogram of blank

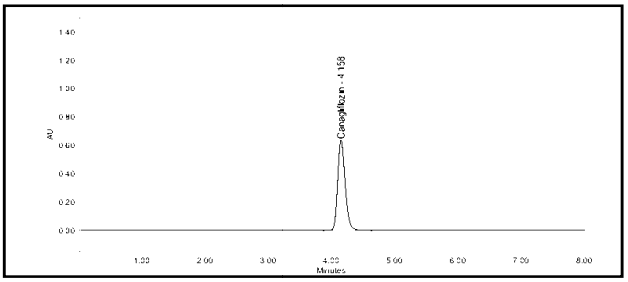

Figure 3 Chromatogram of standard solution of Canagliflozin

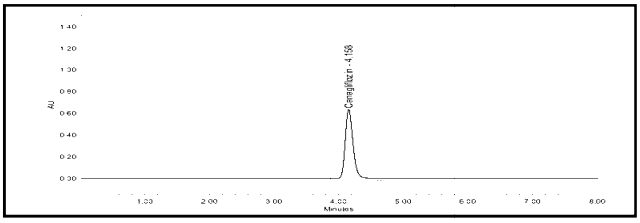

Figure 4 Chromatogram of Test Solution of Canagliflozin

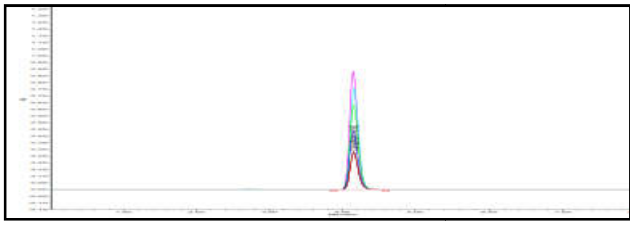

Figure 5 HPLC Overlay Spectra of Canagliflozin

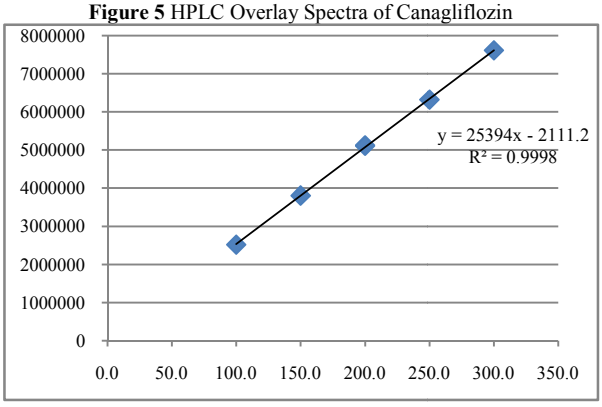

Figure 6 Calibration Curve of Canagliflozin for HPLC

Table 1 Calibration data for Canagliflozin at 290 nm

Conc. (mcg/ml)	Mean Response ± SD	% RSD
100	2522427±45029.81	1.78
150	3807541±27228.52	0.71
200	5117439±90438.91	1.76
250	6323402±39582.88	0.62
300	7613117±73081.05	0.95

Table 2 Data of regression analysis of Canagliflozin

Drug	Straight line equation of Calibration curve	Correlation Coefficient
Canagliflozin	Y = 25394x-2111.2	0.9998

Observation

A method is linear in a range of 100-300mcg/ml of Canagliflozin of standard solutions.

A correlation coefficient for Canagliflozin is 0.9998. The areas obtained were directly proportional to the concentration of analyte in the sample. The method can, therefore be termed as linear in the specified range.

Precision

Repeatability

The repeatability studies were carried out by measuring response for a single concentration for 6 times a day.

Intraday precision

Intraday precision was performed by analyzing three different concentration within range, three times in a day (3*3 determination).

Table 3 Repeatability data for Canagliflozin

Conc of Canagliflozin (mcg/ml)	Absorbance (n=6)
200	5070432
	5142459
	5189428
	5217435
	5257439
	5298042
Mean	**5195872**
SD	**81638.02**
% RSD	**1.57**

Table 4 Data of Intraday Precision for Canagliflozin

Canagliflozin		
Conc. (mcg/ml)	**Mean response ±SD**	**% RSD**
150	3771874±45501.81	1.20
200	5070773±50499.74	0.99
250	6181649±57016.32	0.92

Interday precision

Interday precision was performed by analyzing three different concentrations within linearity range, on different days.

Table 5 Data for Interday Precision for Canagliflozin

Canagliflozin		
Conc.	**Mean response ±SD**	**% RSD**
150	3859541±50381.71	1.30
200	5150773±66105.55	1.28
250	6281649±66994.11	1.06

Observation

Repeatability- The % RSD was found to be 1.57% for Canagliflozin. % RSD value was found to be less than 2.0 indicate that the method is precise.

Intraday precision- The %RSD was found to be 0.92-1.20% for Canagliflozin. % RSD value was found to be less than 2.0 indicate that the method is precise.

Interday precision- The % RSD was found to be 1.06-1.30% for Canagliflozin. % RSD value was found to be less than 2.0 indicate that the method is precise.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three levels (50%, 100%, and 150%) of standard addition

Table 6 Determination of Accuracy of Canagliflozin

Amount of Canagliflozin present (mcg/ml)	% Amount of std Canagliflozin added	Total amount of Canagliflozin present(mcg/ml)	Amount recovered mean (mcg/ml)	SD n=3	% Recovery
200	50	150	147.06	52456.65	98.04
	100	200	200.54	41629.77	100.27
	150	250	246.86	29961.24	98.74

Robustness: Change in Flow Rate

Table 7 Data of Robustness for Canagliflozin

Drug	Flow Rate	Mean ± SD	% RSD
Canagliflozin	0.9ml/min	4839852±62798.8	1.29
	1.1ml/min	4300097±73220.58	1.70

Assay

Table 8 Data of Assay for Canagliflozin

Amount of Canagliflozin present(mcg/ml)	Mean ±SD	% RSD	% Assay
200	5070605±78943.64	1.55	99.88
	5158007±84743.74	1.64	101.6
	5170533 ±77025.96	1.48	101.84

Observation: The Assay for Canagliflozin was shown in table 8 .The percentage Assay was found to be 99.88-101.84%.

Summary of Validation parameter for RP-HPLC

Table 9 Summary of validation parameters for RP-HPLC

Parameter	Canagliflozin
Linearity range (n=5)	100-300mcg/ml
Accuracy (%)	98.04-100.27
LOD (mcg/ml)	3.538
LOQ (mcg/ml)	10.722
Repeatability (n=6) %RSD	1.57
Intraday (n=3) %RSD	0.92-1.20
Interday (n=3) %RSD	1.06-1.30
Robustness(% RSD)	1.29 - 1.70
Assay (%)	99.88-101.84

CONCLUSION

The method was found to be simple, specific, accurate, economic and reproducible. Method can be successfully applied for routine QC analysis. It reveals that RP-HPLC method was validated as per ICH guideline Q2 (R1) as all validation parameters were found within the range.

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