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Analytical Method Development and Validation for the Estimation of Canagliflozin in Bulk and Formulation by RP- HPLC

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ABSTRACT

A simple and sensitive reverse phase high performance liquid chromatographic method was developed and successively validated for the estimation of Canagliflozin. In the new method, Canagliflozin separation was carried out by the nonpolar inertsil ODS-3 (250 × 4.6 mm, 5 μ) column with a mobile phase composition of Ammonium acetate buffer (pH-4.5) and Acetonitrile in the ratio of 30:70% v/v. Canagliflozin was determined at 252 nm using UV detection and the compound was eluted at the retention time of 4.5 min. As per International Conference on Harmonization (ICH) guidelines, the method was validated and the parameters were precision, accuracy, linearity, limit of detection, limit of quantitation and robustness. The chromatographic method was accurate, linear, specific, precise and robust. The results of method concluded that the proposed RP-HPLC method is useful, convenient and reliable in regular analysis of Canagliflozin in bulk and its formulation.

Keywords: Canagliflozin, RP-HPLC, Antidiabetic agents, Validation, ICH guidelines.

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INTRODUCTION

Chromatography is an analytical tool for the determination of drugs in pharmaceutical formulations. At present HPLC is the most commonly used of

Canagliflozin is an antidiabetic and it used to control body glucose levels in the patients suffering from type-2 diabetes. In the kidney 90 percentage of glucose reabsorbed by sodium-glucose transport protein

subtype 2 (SGLT -2) and remaining 10 percentage is reabsorbed by SGLT-1. Canagliflozin inhibit SGLT-2 and prevent the reabsorption of glucose in the kidney. Canagliflozin chemically denoted as (2S, 3R, 4R, 5S, 6R) - 2 - {3- [5- [4-Fluoro-phenyl]-thiophen-2-ylmethyl]-4-methyl-phenyl}-6-hydroxy methyl-tetrahydro-pyran-3, 4, 5-triol. It was contraindicated in the patients suffering from type-1 diabetes, diabetic ketoacidosis, renal impairment, end-stage renal disease and patients

on dialysis. Literature review of Canagliflozin shown that there were several analytical methods like were several analytical methods like UV spectroscopy [1], LC-MS [2], HPLC [3-8], HPTLC [9] 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. [10-12]

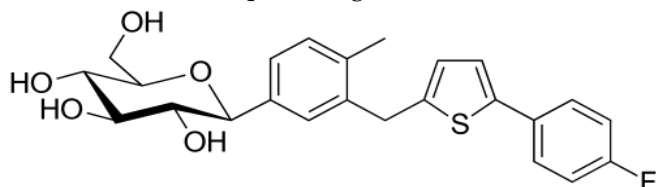


Fig. 1: Structure of Canagliflozin

Table 1: Chromatographic Conditions for Canagliflozin

Flow rate	1 mL/min
Wavelength	252 nm
Injection volume	20 μ L
Retention time	4.5
Column Temperature	Ambient
Run time	7 min
Column	inertsil ODS-3 (250 $\times$ 4.6 mm, 5 μ) column
Mobile phase	Ammonium acetate buffer (pH-4.5) and Acetonitrile in the ratio of 30:70% v/v

MATERIALS AND METHODS

Materials and reagents

A gift sample of Canagliflozin from Aurobindo Pharmaceuticals, Hyderabad used as Standard drug. INVOKANA (Canagliflozin 100 mg) film coated tablet dosage form bought from the local market. Other chemicals like HPLC grade Acetonitrile, Ammonium acetate and phosphoric acid were bought from SD Fine Chemicals, Mumbai, India

Chromatographic conditions and Instrumentation

The LC system with HPLC pump model (Jasco PU 2080 Plus) with auto sampler programmed at 20 μ L capacity per each injection was used. The HPLC system consisted of an UV-detector (Jasco UV 2075 Plus). Separation was carried out by the nonpolar inertsil ODS-3 (250 $\times$ 4.6 mm, 5 μ) column. Chromatographic conditions were cited in Table 1.

Preparation of Ammonium acetate Buffer pH 4.5

Accurately weighed 0.77 g of Ammonium acetate was dissolved in few mL of HPLC grade water in a 1000 mL volumetric flask and make up to the volume with HPLC grade water. Solution pH was adjusted to 4.5 by using orthophosphoric acid and the buffer was filtered through 0.45 micron membrane filter to remove any fine particles present in the solution.

Preparation of Mobile Phase

Mobile phase was prepared by mixing the Ammonium acetate buffer (pH 4.5) and Acetonitrile in the ration of 30:70% v/v and the mixture degasified by vacuum filtration using 0.45 μ filter and sonication. Mobile phase used as diluent for the preparation of standard and sample stock and working solutions.

Preparation of stock and working standard solution

100 mg of Canagliflozin standard was weighed and transferred to 100 mL volumetric flask containing few mL of Diluent. Solution was sonicated for 5 min for dissolving the drug and finally make up to the volume with diluent. From the above stock solution 20 ppm of final standard solution was prepared by serial dilutions.

Preparation of sample solution

20 tablets of Canagliflozin (INVOKANA) were weighed and powdered. From the tablet powder 100 mg equivalent amount of Canagliflozin was transferred into a 100 mL volumetric flask. 50 mL of diluent was added and sonicated for 20 min to dissolve the entire drug and finally made up to the final volume with diluent. Solution was filtered and from the filtrate 20 ppm of final sample solution was prepared by serial dilution by using diluent.

Assay

20 μ L of Canagliflozin standard and sample solutions were injected through autosampler into chromatographic system and peak areas were measured. The percentage of assay of tablets calculated and results were given in the Table 2.

Method Development

To optimize the HPLC method parameters, mobile phase ratios of different solvents were tried. Good separation and peak symmetry for Canagliflozin were developed with combination of Ammonium acetate buffer (pH 4.5) and Acetonitrile in the ratio of 30:70% v/v. System flow rate was confirmed as 1 mL/min. The peak was eluted at the retention time of 4.5 min at 252 nm wavelength.

Analytical Method Validation

The developed method was validated as per ICH guidelines Q-2, R-1. The typical parameters were Accuracy, precision, repeatability, intermediate precision, specificity, detection limit (LOD), quantitation limit (LOQ), linearity and robustness.

System suitability

This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting six standard solutions of Canagliflozin and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity

This parameter performed by injecting blank, placebo, standard and sample solutions and any interference with excipient and mobile phase were observed.

Linearity and range

These parameters were studied by injecting 5, 10, 15, 20, 25 and 30 μ g/mL solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area in the concentration range of 5 – 30 μ g/mL.

Accuracy

This parameter studied by preparing 50%, 100% and 150% concentration solutions of Canagliflozin in

triplicate by spiking the standard drug to the placebo and calculated the percentage recovery of Canagliflozin.

Precision

Repeatability

In this parameter closeness of six assay values were measured by calculating % RSD. For this, six sample solutions were prepared from single batch as given in sample preparation above by taking the tablet formulation.

System precision

This parameter carried out to determine whether the HPLC instrument working perfectly or not. System precision studied by injecting standard Canagliflozin 20 µg/mL solution six times and % RSD was calculated from the peak areas.

Intermediate precision

Intermediate precision also called as Ruggedness of the method. It was studied by performing the system precision in different days, by different analyst and by different equipment and determined by calculating % RSD for assay values.

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

LOD and LOQ were determined as per ICH guidelines by using the formulas $3.3 \times SD/S$ and $10 \times SD/S$ respectively. In the formulas SD is the response standard deviation and S is the calibration curve slope. A signal-to noise ratio for LOD is between 3 or 2:1 and LOQ is 10:1.

Robustness

Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for six injections peak area values of each change in condition.

Table 2: Assay of Canagliflozin marketed tablet

Canagliflozin	Peak area	Invokana label claim	% label claim
Standard	735334		
Sample	731657	100 mg	99.5

RESULTS AND DISCUSSION

Specificity

Specificity was evaluated by observing the chromatograms of blank, placebo, sample and standard Canagliflozin. The chromatograms of blank, standard, sample and placebo showed no peaks were interfering with a drug retention time of 4.5 min. Chromatograms of blank, placebo and 20 ppm standard and sample canagliflozin were given in Figure 2, 3, 4 and 5.

Precision

Repeatability

Repeatability was determined by calculating % RSD of six assay values and the values are given the Table 3. The % RSD value was found to be 0.3.

System precision

Standard solution of canagliflozin (20 ppm) was injected six times and % RSD from peak areas was

calculated as 0.23. System precision values were given in Table 4.

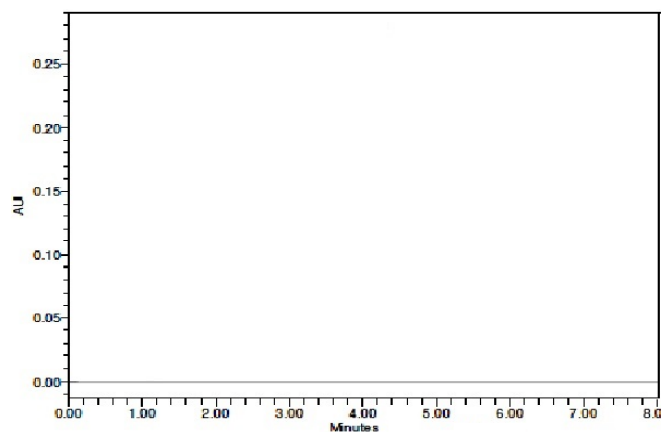


Fig. 2: Blank chromatogram of Canagliflozin

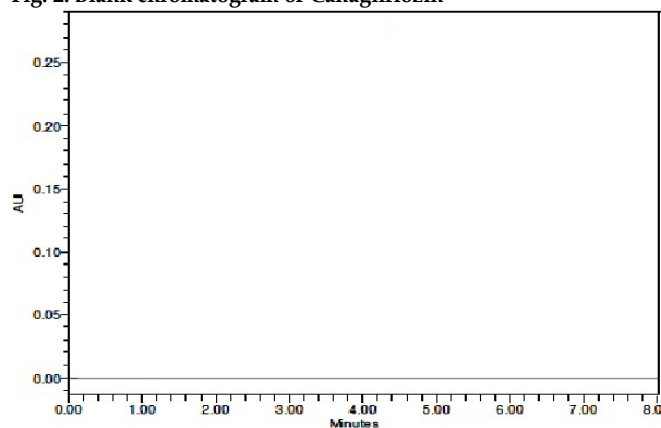


Fig. 3: Placebo Chromatogram of Canagliflozin

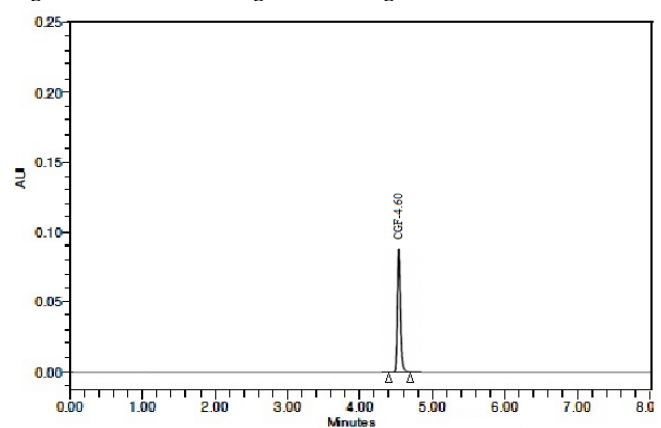


Fig. 4: Standard Chromatogram of Canagliflozin

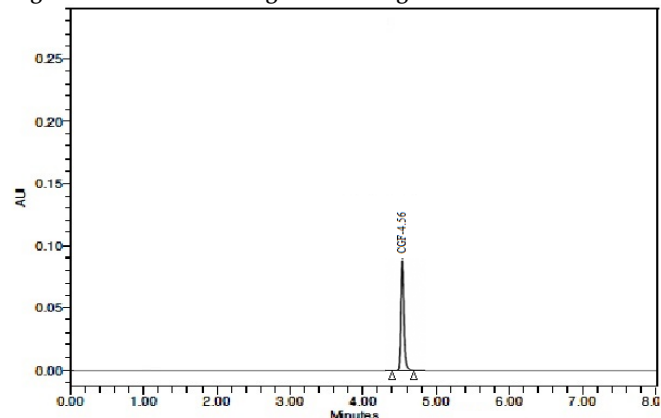


Fig. 5: Sample Chromatogram of Canagliflozin

Table 3: Method precision data for Canagliflozin

Concentration (µg/ml)	Peak area	% Assay
20	731356	99.35
20	731798	99.66
20	731498	99.47
20	735329	100.1
20	735334	100.17
20	731698	99.6
	AVG	99.725
	SD	0.306
	% RSD	0.3

Table 4: System precision data for Canagliflozin

Concentration (µg/ml)	Peak area
20	731376
20	731788
20	733498
20	735429
20	735355
20	731598
AVG	733174
SD	1711.93
% RSD	0.23

Table 5: Intermediate precision data for Canagliflozin

	% Assay		% Assay		% Assay	
Concentration (µg/ml)	Analyt-1	Analyt-2	Day-1	Day-2	Equipment-1	Equipment-2
20	99.5	98.99	98.9	99.25	98.9	99.64
20	99.67	99.25	99.85	99.64	100.01	99.55
20	98.9	99.64	99.7	99.55	99.45	100.6
20	100.01	99.55	99.63	98.56	100.01	99.55
20	99.45	100.6	100.0	100.3	99.64	98.56
20	100.2	99.56	99.21	99.8	99.55	100.3
AVG	99.62	99.59	99.55	99.51	99.59	99.7
SD	0.4188	0.50	0.38	0.531	0.38	0.64
% RSD	0.41	0.5	0.38	0.53	0.3	0.64

Table 6: LOD and LOQ of Canagliflozin

Parameter	Result
LOD	0.01 ppm
LOQ	0.04 ppm

Table 7: Robustness of Canagliflozin

Parameter	Variation	% RSD
Robustness	i. Change in flow rate (± 0.1 mL/min)	0.92
	ii. Change in mobile phase (± 1 mL)	0.45

Table 8: Linearity data for Canagliflozin

Concentration (µg/mL)	Peak area
0	0
5	183892
10	366789
15	546642
20	743593
25	939576
30	1096525

Intermediate precision

Intermediate precision determined by performing the System precision in different days, by different analyst and by different equipment. Calculated % RSD values were less than 2 and values were tabulated in the Table 5.

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

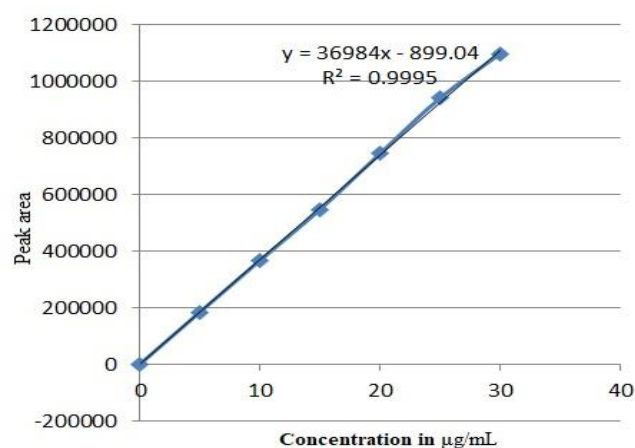
LOD and LOQ calculated as per ICH and the value were given the Table 6.

Robustness

Robustness was done by changing the actual mobile phase ratio and flow rate. Results were mentioned in Table 7 and the calculated % RSD values were less than 2.

Linearity and range

Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of 5, 10, 15, 20, 25 and 30 µg/mL solutions (prepared from standard stock solution). From the curve y-intercept is 899.04 and slope is 36984. Calibration curve shown in figure 6 and linearity values tabulated in Table 8.

**Fig. 6: Linearity of Canagliflozin****Table 9: Recovery data of Canagliflozin**

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	% recovery	% RSD
50% solution	10	9.98	99.8	0.64
50% solution	10	9.87	98.7	
50% solution	10	10.02	100.2	
100% solution	20	20.02	100.1	0.5
100% solution	20	19.94	99.7	
100% solution	20	19.78	98.9	
150% solution	30	29.76	99.2	0.45
150% solution	30	29.91	99.7	
150% solution	30	30.09	100.3	

Table 10: System suitability parameters for Canagliflozin

Parameter	Canagliflozin
Retention Time (mins)	4.5
% RSD	0.26
Theoretical plates	12854
Peak symmetry	1.16

Accuracy

Triplicate solutions of 50%, 100% and 150% concentration of Canagliflozin was prepared by spiking the standard to placebo and performed assay method. The results were depicted in the Table 9 and the % RSD was less than 2.

System suitability

Six standard solutions of Canagliflozin were injected into chromatographic system and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated. Results were given in the Table 10.

A new simple, sensitive and precise RP-HPLC method has been developed for the analysis of Canagliflozin in tablet formulation. The estimated %RSD values in different parameters like precision, accuracy, system suitability and robustness were found to be less than 2.0%, which indicates that the method is precise, accurate and robust. LOD and LOQ values for Canagliflozin were 0.01 and 0.04 µg/mL. Linearity and range of Canagliflozin were found between the concentration range of 5 to 30 µg/mL. The % recovery values were found between 98 -101% for Canagliflozin. The peak eluted for canagliflozin at 4.5 RT by using the mobile phase composition of Ammonium acetate buffer (pH-4.5) and Acetonitrile in the ratio of 30:70% v/v. This method used for an alternative method for the analysis of Canagliflozin in its tablet dosage forms.

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