New Analytical Method Development and Validation of Canagliflozin (Anti-diabetic) drug by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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Abstract: A new Reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Canagliflozin in bulk and pharmaceutical dosage form. Chromatographic separation of Canagliflozin was achieved by using Agilent Eclipse plus C_8 (150mm ×4.6, 5µm) column. Detection was carried out at 290nm with a flow rate of 1ml/min with an injection of 20 µl was selected using mobile phase Acetonitrile and Trifluoroacetic acid buffer (45:55).The standard curve was linear over a working range of 5-30 µg/ml and gave an average correlation factor 0.997 for Canagliflozin. The system was operated at ambient temperature and the retention time was observed at 4.23 min for Canagliflozin. The method was validated with different parameters such as Linearity, Precision, Accuracy, Robustness, Limit of detection (LOD), Limit of quantification (LOQ).The Limit of detection and Limit of quantification was found to be 0.69µg/ml and 2.30µg/ml respectively of Canagliflozin. The relative standard deviations of intra and inter day assay less than 2 and the method was conveniently used for routine analysis of Canagliflozin in bulk and tablet dosage forms.

Keywords *- Canagliflozin, RP-HPLC, LOD, LOQ, Precision, Accuracy, Linearity*

I. INTRODUCTION

Canagliflozin Anhydrous is the anhydrous form of canagliflozin, a C-glucoside with a thiophene ring that is an orally available inhibitor of sodium-glucose transporter 2 (SGLT2) with antihyperglycemic activity. Canagliflozin is also able to reduce body weight and has a low risk for hypoglycemia[1].

Canagliflozin is chemically known as (2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl)thiophen-2-yl] methyl]-4-methylphenyl]tetrahydro-6-hydroxymethyl-2H-pyran-3,4,5-triol,[2] molecular formula is $C_{24}H_{25}FO_5S$ and molecular weight is 444.52.[3]

It inhibits renal glucose reabsorption by increasing urinary glucose excretion which leads to lowered renal threshold for glucose and reduced blood glucose levels in patients with type 2 diabetes mellitus[4]. The mean absolute oral bioavailability of canagliflozin is approximately 65%. It is extensively bound to proteins in plasma (99%), mainly to albumin [5, 6].

Canagliflozin is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. Use in type 1 diabetes mellitus patients or in treatment of diabetic ketoacidosis is not recommended. Invokana is indicated for the treatment of adults with insufficiently controlled type 2 diabetes mellitus as an adjunct to diet and exercise.

- as monotherapy when metformin is considered inappropriate due to intolerance or contraindications
- in addition to other medicinal products for the treatment of diabetes.[7]

Mechanism Of Action:[8]

Canagliflozin is in class of drugs called sodium-glucose transport protein 2(or SGLT2) inhibitors, which drugs work by increasing in the amount of glucose that gets passed out in the urine. When blood passes through the kidneys, the kidneys filter glucose out of the blood and SGLT proteins then help reabsorb glucose back into the blood.SGLT2 proteins are responsible for 90% of the glucose that is reabsorbed, so by blocking the action these proteins, less glucose is reabsorbed and so more glucose is excreted via the urine.

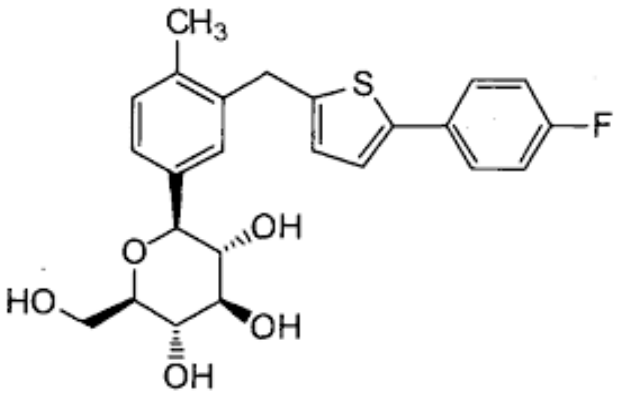

Figure: 1:-Structure of Canagliflozin

II. MATERIALS AND METHODS

Instrumentation

The Agilent 1120 Compact LC HPLC system consisting of gradient pump (LC-10AT vp pump) (4MPa or 400barr), Standard cell, UV variable wavelength detector, rheodyne injector, and agilent syringe was used. Sonicator (EQUITRON230VAC, 50Hz), Analytical weighing balance (Shimadzu AUX 220) was used for weighing, , vacuum pump (SUPER FIT), filtration kit (TARSONS) and Nylon membrane filter (Merck Millipore) for solvents and sample filtration were used throughout the experiment. The separations were achieved on Agilent Eclipse plus C8 column (5µm 4.6x150mm), column length is 25 cm with UV detection at 290nm. Double beam UV-Visible spectrophotometer (SHIMADZU-UV 1700) was used for wavelength detection. The EZ Chrome Elite software-dual channel was used for evaluation, acquisition and storage of chromatographic data.

Chemicals and reagents

Invokana is a tablet dosage form each contains 100mg of Canagliflozin. . HPLC grade Acetonitrile (Merck), Analytical grade Trifluoroacetic acid (TFA) buffer was used as the solvents throughout the experiment. HPLC grade water obtained by using Direct-Q water purification system (Millipore, Milford, USA) was used in HPLC study. Pharmaceutical formulation Canagliflozin tablet (label claim contain 100mg) was used in HPLC analysis.

Buffer preparation

0.1% of Trifluoroacetic acid buffer: - It was prepared by dissolving 0.5 ml of Trifluoroacetic acid and dissolved in 500ml of HPLC grade water then pH maintained to 4.5 with glacial acetic acid.

Chromatographic condition

After several trials with the different combination and ratio of solvents, the mobile phase trifluoro acetic acid buffer (buffer): Acetonitrile (55:45v/v) at PH-4.5. Retention time (R_t) 4.23 min for Canagliflozin. Wavelength was selected by scanning the standard drug over a wide range of wavelength 200 nm to 400 nm. The component shows reasonably good response and maximum peak at 290nm.

Standard solutions for HPLC estimation of Canagliflozin

A tablet is powdered which contain 100 mg of active ingredient is transferred into 10 ml of volumetric flask and is dissolved in mixture of acetonitrile and the buffer(45:55) volume were made up to the mark with same solvent. This gave the concentration of 1000 µg ml^{-1} of canagliflozin (Stock-1). From stock solution 1, 6 dilution was prepared between 5-30 µg ml^{-1} which is working concentration.

III. METHOD VALIDATION AND DEVELOPMENT

The developed method was validated according to ICH guidelines with respect to accuracy, precision, linearity, specificity, robustness, limit of detection (LOD), limit of quantification (LOQ), ruggedness and system suitability.

Specificity and selectivity

Specificity is a procedure to detect quantitatively the analyte in presence of the components that may be expected to be present in the sample matrix. While selectivity is a procedure to detect the analyte qualitatively in presence of components that may be expected to be presented in the sample matrix. The excipients in tablet formulation were spiked in pre weighted quantity of drugs and then absorbance was measured and calculations were done to determine the quantity of the drugs.

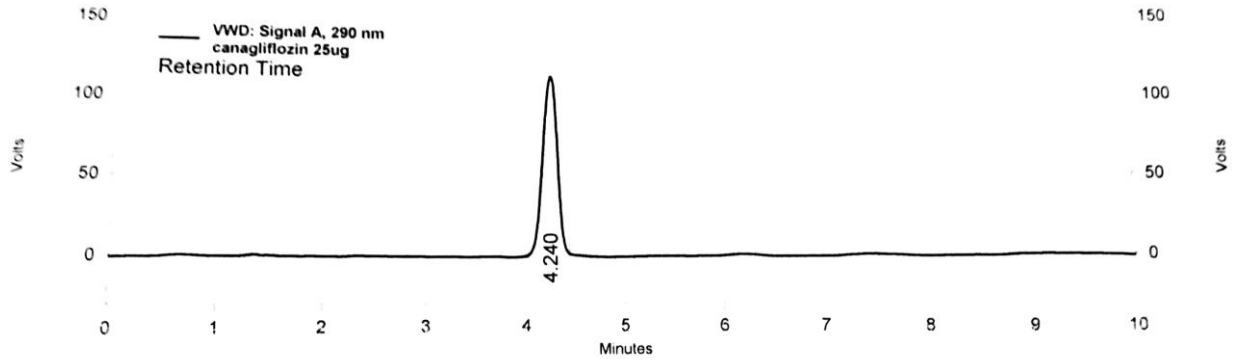

Figure 2- Standard chromatogram for Canagliflozin

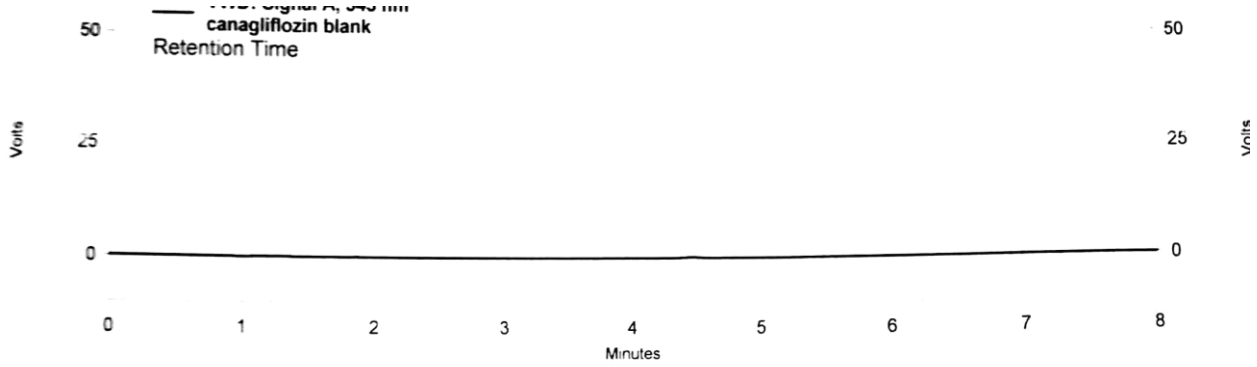

Figure 3: Blank chromatogram

Linearity

The procedure within a given range to obtain test result which are directly proportional concentration (amount) of analyte in the sample. The working standard were prepared, aliquots of different concentration such as 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25 µg/ml and 30 µg/ml with acetonitrile and buffer mixture. Six dilutions of each concentration were prepared separately. From above dilution, 20µl concentration were injected to the HPLC system and their chromatogram was observed. Peak areas were recorded for all the chromatograms and a standard calibration curve of peak area against concentration was plotted.

Table 1: Linearity data for Canagliflozin

Concentration(µg/ml)	Area
5	2818525
10	5115988
15	8127556
20	10652528
25	13482380
30	15362879

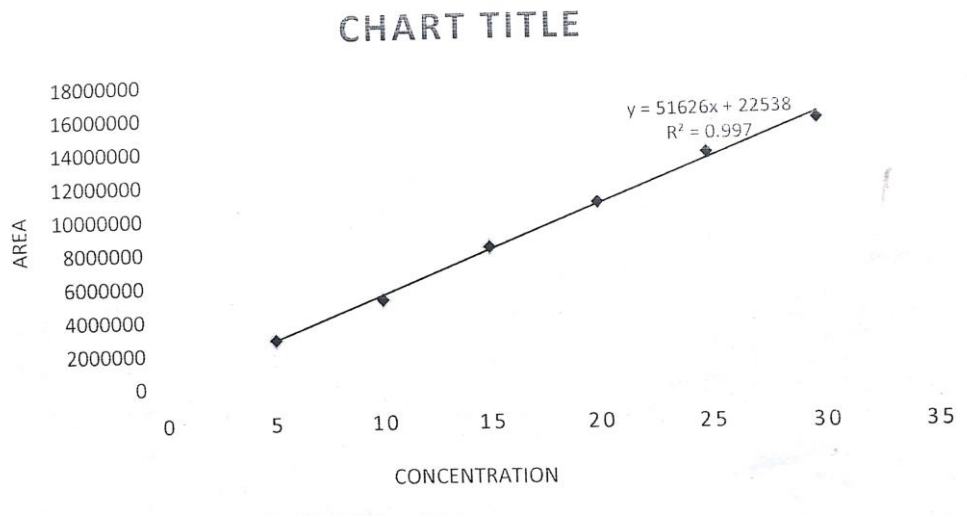

Figure 4:- linearity graph of Canagliflozin

Precision

The precision of an analytical procedure express the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample under the prescribed conditions.

The intra and inter day variation in the peak ratio of the drug solution was calculated in terms of co-efficient of variation (CV) and obtained by multiplying the ratio of the standard deviation to the mean with 100(CV=SD/MEAN × 100) shown in the graph

Table 2: Intraday precision of canagliflozin (morning)

Morning				
Injection (15µg/ml)	Areas	Average	SD	Rsd%
1	8297480	8143070	76582.64	0.94
2	8105281			
3	8134448			
4	8114841			
5	8103988			
6	8102383			

Table 3: Intraday precision of canagliflozin (afternoon)

Afternoon				
Injection (15µg/ml)	Areas	Average	Sd	Rsd%
1	8179372	8195140	78738.17	0.96
2	8290365			
3	8186601			
4	8105273			
5	8286326			
6	8122903			

Table 4: Interday precision of canagliflozin (day 1)

Day 1				
Injection (15µg/ml)	Areas	Average	Sd	Rsd%
1	8135338	8181191	63209.59	0.77
2	8196492			
3	8169000			
4	8177880			
5	8113608			
6	8294829			

Table 5: Interday precision of canagliflozin (day 2)

Day 2				
Injection (15µg/ml)	Areas	Average	Sd	Rsd%
1	8048328	8156403	84056.2	1.030
2	8180111			
3	8249294			
4	8242267			
5	8072953			
6	8145463			

Accuracy

The accuracy of analytical procedure expresses the closeness of test result between the value which is accepted either as true value or reference value. The procedure for the preparation of the solutions for Accuracy determination at 80%, 100% and 120% level were prepared in the acetonitrile.

For 80% Accuracy for canagliflozin:

80mg of the pure drug was added to 100mg of formulation

For 100% Accuracy for canagliflozin:

100mg of the pure drug is added to 100mg of formulation

For 120% Accuracy for canagliflozin:

120mg of the pure drug is added to 100mg of formulation

Table 6: - Accuracy data for estimation of Canagliflozin

S.N	Level of percentage recovery	Amount present (mg/tablet)	Amount of standard drug added	Area response	Mean	SD	RSD (%)	Total amount recovery	Recovery (%)
1.	80%	100	80	5165525	5181265	143252.82	0.27	10.09	100.09
				5184640					
				5193629					
2.	100%			8135692					

		100	100	8131829 8191799	8153107	33564.17	0.41	15.01	100.06
3.	120%	100	120	10895469 10922204 10880255	10899309	21236.54	0.20	20.45	102.25

Robustness

As defined by the ICH, the robustness of an analytical procedures describes to its capability to remain unaffected by small and deliberate variation in the chromatographic conditions and provides an indication of its repeatability. It was found to be unaffected by small variation ±0.1ml/min in flow rate of mobile phase, and wavelength ±5nm result are shown.

Table 7:-Robustness data for estimation of canagliflozin

S.N.	Parameter	Optimized	Used	Retention time (mins)
1.	Flow rate	1ml/min	0.9ml/min	4.590
			1.1ml/min	3.893
2.	Detection wavelength	290nm	295nm	4.213
			285nm	4.257

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

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples. A signal to noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Table 8: LOD and LOQ for estimation of Canagliflozin

Name of drug	LOD µg/ml	LOQ µg/ml
Canagliflozin	0.69	2.30

IV. CONCLUSION

From the above results, method was found to be accurate, precise, linear, specific, system suitable, robust proved to be sensitive, convenient and cost effective for the estimation of Canagliflozin in oral solid dosage

form. The proposed method has a run time of 10mintues, which makes the method simple, cost effective and suitable for the routine analysis of Canagliflozin in oral solid tablet dosage form.

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