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ESTIMATION AND VALIDATION OF CANAGLIFLOZIN DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple and selective LC method is described for the determination of Canagliflozin dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Methanol: ACN: H2O (30:50:20v/v/v), with detection of 250 nm. Linearity was observed in the range 20-60 µg /ml for Canagliflozin (r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Canagliflozin, dosage form, LC method

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INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books. Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification,

Purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis. Qualitative analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half life etc. Quantitative analysis techniques are mainly used to determine the amount or concentration of analyte in a sample and expressed as a numerical value in appropriate units. These techniques are based on suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained the characteristic movement of a substance through a defined medium

under controlled conditions, electrical measurement or measurement of spectroscopic properties of the compound (1, 2).

Canagliflozin belongs to a new class of anti-diabetic drugs that works by inhibiting the sodium-glucose transport protein (SGLT2). This transport protein is found in the kidney and is responsible for reabsorbing glucose that has been filtered. Sodium-glucose co-transporter 2 (SGLT2), expressed in the proximal renal tubules, is responsible for the majority of the reabsorption of filtered glucose from the tubular lumen. Canagliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, canagliflozin reduces reabsorption of filtered glucose and lowers the renal threshold for glucose (RTG), and thereby increases urinary glucose excretion. Canagliflozin binds to SGLT2 more potently (250-times) than SGLT1 in vitro. The 50% inhibitory concentrations (IC50) are 2.2-4.4 nmol/L and 684 - 910 nmol/L for SGLT2 and SGLT1 respectively. Dose dependent decreases in renal threshold for glucose and increases in urinary glucose excretion were observed when single and multiple oral doses were administered to type 2 diabetes patients. Decreases in plasma glucose in a dose-dependent fashion were also noted as early as the first day of administration. When given to healthy and type 2 diabetic patients before a meal, a delay in intestinal glucose absorption and a reduction in postprandial glucose was observed. Canagliflozin does not prolong the QTc interval. 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 (1-4).

Aim is to develop new RP HPLC method for the estimation of Canagliflozin pharmaceutical dosage form.

MATERIALS AND METHOD (5-7)

Preparation of standard stock solution of Canagliflozin

10mg of Canagliflozin was weighed and transferred in to 25ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 40 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard solution

Weigh accurately 10mg of Canagliflozin in 25ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of Canagliflozin is prepared by diluting 1ml of Canagliflozin to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of Canagliflozin in 25ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of Canagliflozin is prepared by diluting 1ml of Canagliflozin to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

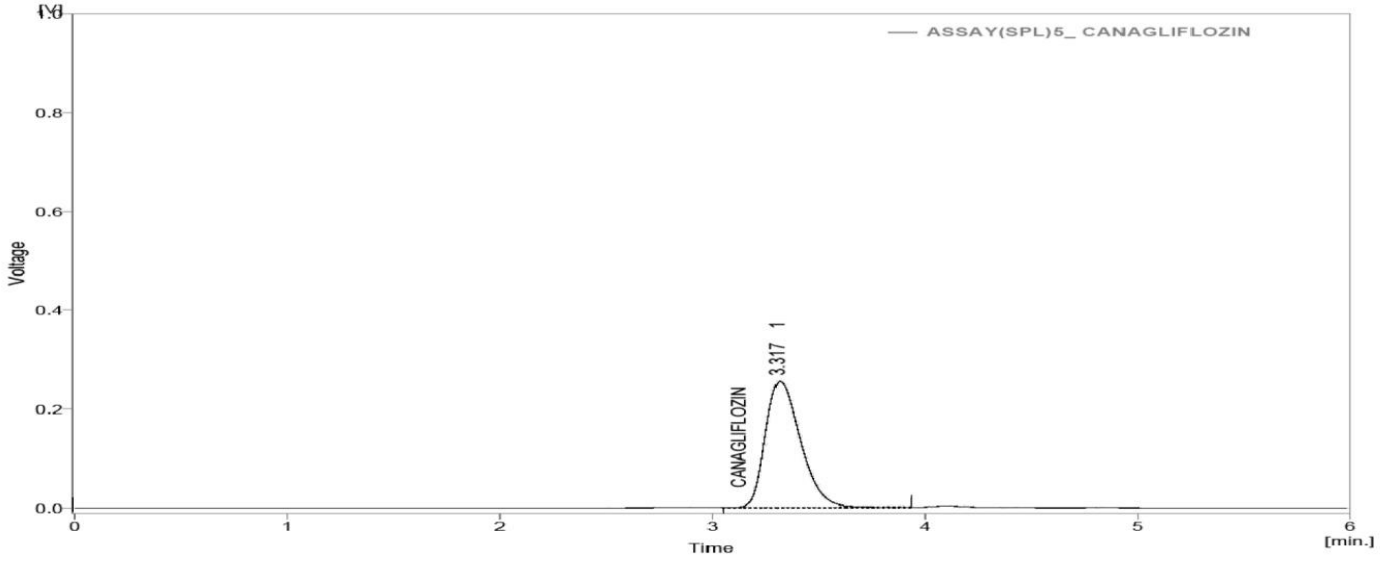
5 tablet (each tablet contains 100mg of Canagliflozin) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 400µg/ml were prepared by dissolving weight equivalent to 10mg of Canagliflozin dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 25ml with mobile phase. Further dilutions are prepared in 5 replicates of 40µg/ml of Canagliflozin was made by adding 1ml of stock solution to 10 ml of mobile phase.

RESULTS AND DISCUSSION

The amount of Canagliflozin present in the taken dosage form was found to be 99.52% (Table-1 and Fig-1).

Table-1 Assay Results

Canagliflozin		
	Standard Area	**Sample Area**
Injection-1	2929.483	2915.223
Injection-2	2925.543	2928.592
Injection-3	2946.561	2945.457
Injection-4	2925.890	2923.218
Injection-5	2900.370	2915.166
Average Area	2933.862	2925.531
Assay(%purity)	99.52%	



Result Table (Uncal - ASSAY (SPL) 5_ CANAGLIFLOZIN

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.317	2915.166	255.620	100.00	100.0	0.18
	Total	2915.166	255.620	100.00	100.0	

Column Performance Table (From 50% - ASSAY (SPL) 5_ CANAGLIFLOZIN

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	3.317	0.180	1.676	0.00	1881	18809	-

Fig-1 Chromatogram of Assay sample preparation

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of Canagliflozin is 0.999. The relationship between the concentration of Canagliflozin and area of Canagliflozin is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Table-2 and Fig-2).

Table-2 Linearity of Canagliflozin

S.No.	Conc.(µg/ml)	Area
1	20	1438.77
2	30	2197.92
3	40	2882.59
4	50	3550.79
5	60	4255.54

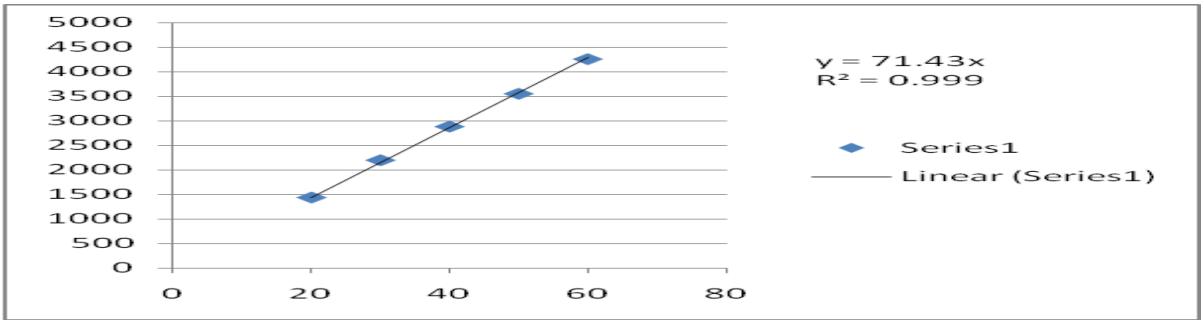


Fig-2 Linearity graph of Canagliflozin

The percentage mean recovery of Canagliflozin is 99.89%. Test results for Canagliflozin are showing that the %RSD of Assay results are within limits. The results were shown in table-3.

Table-3 Results for Method precision of Canagliflozin

Canagliflozin		
S.No	**Rt**	**Area**
1	3.367	2912.410
2	3.367	2932.566
3	3.533	2946.873
4	3.333	2920.975
5	3.777	2911.577
avg	3.3474	2924.978
stdev	0.0220	14.819
%RSD	0.66	0.51

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Canagliflozin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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