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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CANAGLIFLOZIN IN TABLET DOSAGE FORM

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ABSTRACT

In this study, we describe a simple and sensitive reversed-phase high performance liquid chromatography (HPLC) method for the determination of Canagliflozin in pharmaceutical dosage form. The chromatographic separation was achieved on ODS column (4.6 x150mm, 5µ particle size) column. The mobile phase, water and acetonitrile (55:45v/v), were delivered at a flow rate of 1.0 ml/min. The eluent was monitored using PDA detection at 214 nm. Canagliflozin was detected at 2.8 minutes. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. This method can be employed for routine quality control of Canagliflozin tablets in quality control laboratories and pharmaceutical industries.

Key words: Canagliflozin, hplc, Method validation, ICH guidelines

INTRODUCTION

Canagliflozin is a drug for the treatment of type 2 diabetes. It was developed by Mitsubishi Tanabe Pharma and is marketed under license by Janssen, a division of Johnson & Johnson. Canagliflozin inhibits Na+- dependent 14C-AMG uptake in a concentration-dependent fashion. It is a novel C-glucoside with thiophene ring. 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..

Following single and multiple oral doses of canagliflozin to patients with type 2 diabetes, dose-dependent decreases in the renal threshold for glucose (RTG) and increases in urinary glucose excretion were observed. From a starting value of RTG of approximately 240 mg/dL, canagliflozin at 100 mg and 300 mg once daily suppressed RTG throughout the 24-hour period. The mean absolute oral bioavailability of canagliflozin is approximately 65%. The mean steady-state volume of distribution of canagliflozin following a single intravenous infusion in healthy subjects was 119 L, suggesting extensive tissue distribution. Canagliflozin is extensively bound to proteins in plasma (99%), mainly to albumin. O-glucuronidation is the major metabolic elimination pathway for canagliflozin, which is mainly glucuronidated by UGT1A9 and UGT2B4 to two inactive O-glucuronide metabolites.

Various UV Spectroscopy[2-7], Spectrofluorometric[8], GC[9], UPLC-MS[10], HPLC with ampherometry[11] and Raman spectroscopic[12] assay methods are reported in the literature for the estimation of Canagliflozin. According to literature survey there is no official method for the estimation of Canagliflozin by RP-HPLC in tablet dosage forms. Hence, an attempt has been made to develop new method for the estimation and validation of Canagliflozin in tablet formulation in accordance with the ICH guidelines[13].

EXPERIMENTAL

Instrumentation: Chromatography was performed with Alliance waters 2965 HPLC provided with high

speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and convenient for LC with class Empower-2 software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Canagliflozin solutions.

Reagents and chemicals: The reference sample of Canagliflozin was provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade Acetonitrile, HPLC grade Methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets (NIMOTOP-30mg) were purchased from the local pharmacy.

Chromatographic condition: The mobile phase consisted of water and acetonitrile was taken in ratio of 55:45 at a flow rate of 1.0 mL/min. ODS column (4.6 x150mm, 5µ particle size) was used as the stationary phase. 214 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution: Accurately Weighed and transferred 10mg Canagliflozin working Standard into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution, 1 ml (100µg/ml) was pipeted out in to a 10ml Volumetric flask and then make up to the final volume with diluent.

***Preparation of Working Standard Solutions*:** Aliquot of 0.3, 0.6, 0.9, 1.2, 1.5 & 1.8 mL were pipette out from stock solution into 10 mL volumetric flask and volume was made up to 10 mL with diluent. This gives the solutions of 6, 12, 18, 24, 30 and 36µg/mL for Canagliflozin.

***Preparation of phosphate Buffer*:** Accurately weighed and transferred 1.36gm of Potassium dihydrogen Orthophosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and degassed to sonicate and finally make up the volume with water, then pH adjusted to 3.6 with dil. Ortho phosphoric acid solution.

Sample preparation: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 ml volumetric flask, 5ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Method validation: Parameters such as systems suitability, Linearity, accuracy, specificity, LOD & LOQ and robustness were performed according to the ICH guidelines.

RESULTS AND DISCUSSION:

Method development:

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Phosphate buffer and Acetonitrile as mobile phases, in which the drug did not responded properly. The organic content of mobile phase was also investigated to optimize the elution of the drug. To improve the tailing factor, the pH of mobile phase becomes important factor. Thereafter, Acetonitrile and water were taken in isocratic ratio: 55: 45 and with flow rate of 1.0 mL/min was employed. ODS column (4.6 x150mm, 5µ particle size) was selected as the stationary phase to reduce the tailing of the peak. 214 nm was selected as the detection wavelength for PDA detector. The retention time was found to about 2.8 min and the results were shown in Table 1 and Figure 2.

Method Validation:

System suitability: A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, tailing of the peak) before the validation runs. The results of system suitability parameters were given in Table 3. The analytical method validation was carried out as per ICH method validation guidelines.

Linearity: The linearity range was found in the range of 25-150 ppm. The response for the drug was linear and the regression equation was found to be y=30445x + 1063 and correlation coefficient was found to be 0.9999 and the results are given in Table 2 and Figure 3.

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. Precision of the method was performed as Repeatability and intermediate precision.

Repeatability: To study the Repeatability, Six working sample solutions of 100ppm are injected and the percent relative standard deviation (%RSD) was

calculated and it was found to be 0.83 which are well within the acceptable criteria of not more than 2.0.

Inter-day precision: To study the inter-day precision, Six working sample solutions of 100ppm are injected on the next day of the preparation of samples and the percent relative standard deviation (%RSD) was calculated and it was found to be 0.29 which are well within the acceptable criteria of not more than 2.0.

Specificity: The effect of wide range of excipients and other additives usually present in the formulation of Canagliflozin in the determinations under optimum conditions were investigated. Chromatographic parameters maintained are specific for Canagliflozin.

Limit of detection and limit of quantification: A calibration curve was prepared using concentrations in the linearity range (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined. The LOD and LOQ of Canagliflozin were 0.037 and 0.112 µg/ml, respectively (Table 3).

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed standard solution. The standard addition method was performed at 50%, 100% and 150% level of standard ppm. The solutions were analyzed in triplicate at each level as per the proposed method. Satisfactory recoveries ranging from 98% to 102% were obtained by the proposed method. This indicates that the proposed method was accurate.

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Tablet Analysis*:* The Content of Canagliflozin in the tablets was found by the proposed method. RSD values for Canagliflozin are found to be 0.83 and results were shown in table.4.

CONCLUSION:

A new precise accurate and simple HPLC method was developed and validated for the estimation of Canagliflozin in tablet dosage form. This method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of Canagliflozin tablets in QC laboratories and industries.

Table 1: Optimized chromatographic conditions

S. No.	Parameter	Condition
1	Mobile phase	Water : Acetonitrile (55:45)
2	pH	3.6(+/-0.5)
3	Column	ODS column (150 x 4.6 mm, 5µ)
4	Column temperature	30^0C
5	Wave length	214nm
6	Injection volume	10µl
7	Flow rate	1.0ml/min
8	Run time	7mins
9	Retention time	2.8mins

Table 2: Linearity results

S. No.	Concentration (ppm)	Area
1	25	761127.3
2	50	1547033
3	75	2280856
4	100	3008794
5	125	3807459
6	150	4585607

Table 3: Summary of validation parameters

S. No.	System suitability	Results
1	Linearity range (ppm)	25 - 150 ppm
2	Correlation coefficient	0.9999
3	Theoretical plates (N)	6843
4	Tailing factor	1.19
5	LOD (µg/mL)	0.037 µg/mL
6	LOQ (µg/mL)	0.112 µg/mL
7	Regression Equation	Y=30445x + 1063

Table 4: Assay results

S. No.	Formulation	Label claim	Amount found	%Assay
1	INVOKANA	2mg	2.003mg	100.30%

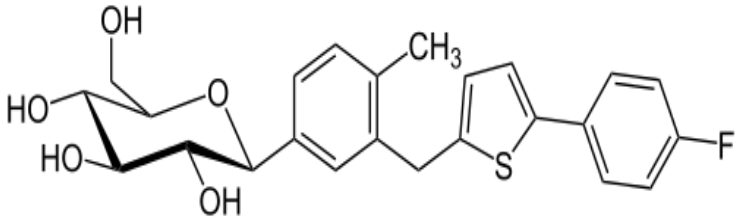

Figure 1: Structure of Canagliflozin

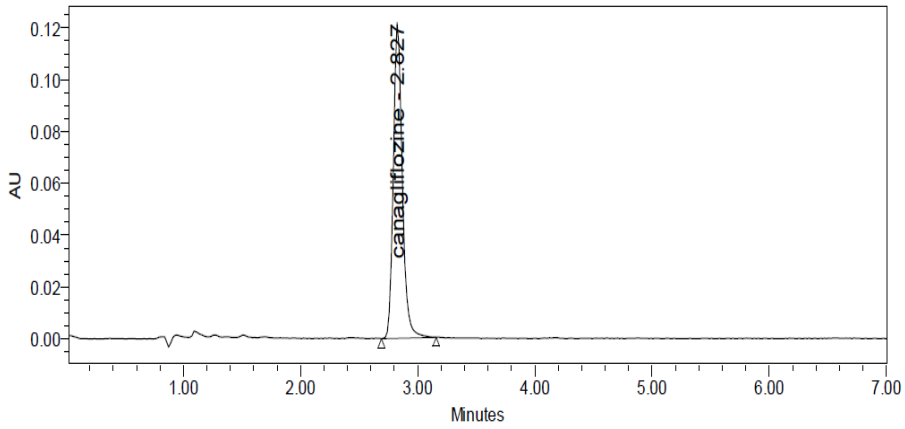

Figure 2. Chromatogram of Canagliflozin Standard

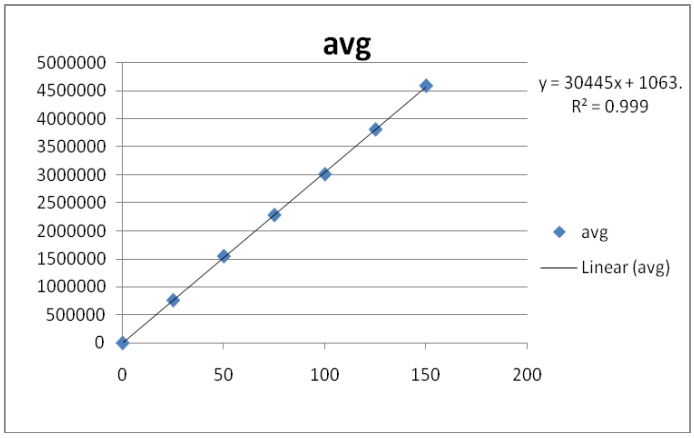

Figure: 3. Linearity curve

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