DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING HPLC METHOD FOR THE ESTIMATION OF CANAGLIFLOZIN

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Abstract:
The objective of the study was to develop and validate a simple, specific, precise and accurate HPLC method for the estimation of Canagliflozin in bulk and tablet dosage form. Chromatographic separation was achieved by using Acetonitrile: Water (pH adjusted to 4.5 using 0.1% v/v Ortho -phosphoric acid) as mobile phase in a ratio of 50:50 v/v. Chromatographic separation was achieved using Neosphere C18 column (150×4.6mm i.d. 3.5µm) at 1ml/min as flow rate. The detection was carried out at 291nm using PDA detector. The retention time was observed to be 4.7 ±0.05min. This drug was subjected to various stress degradation conditions as per ICH Q1A (R2). Linearity was found to be in the concentration range of 10-50µg/ml with R^2 =0.996 .The suitability of this HPLC method for quantitative estimation of Canagliflozin was proved by validation as per ICH Q2A(R1) guidelines and can be used for the routine analysis of Canagliflozin in bulk and tablet dosage form.

Keywords- Canagliflozin, HPLC, stress degradation, Stability indicating.

I. INTRODUCTION

Canagliflozin is an anti-diabetic agent used to improve glycemic control in people with type-2diabetes. [1] Chemically it is (2S,3R,4R,5S,6R)-2-(3-{[5-(4-fluorophenyl)thiophen-2-yl]methyl}-4-methylphenyl)-6-(hydroxymethyl)oxane-3,4,5-triol with molecular formula $C_{24}H_{25}FO_5S$. It is white to off-white solid, soluble in organic solvents like methanol, acetonitrile but insoluble in aqueous media. [2] Canagliflozin is an inhibitor of subtype 2 sodium-glucose transport proteins (SGLT2) which is responsible for at least 90% of renal glucose re-absorption. Canagliflozin is effective in reducing blood glucose levels and has lowered blood glucose levels more significantly than DPP-4 inhibitors in clinical trials. The anti-diabetic drug causes the body to pass out glucose from the blood via the urine, which means that calories in the glucose are excreted. This action helps support weight loss when the drug is used in combination with a healthy diet and regular physical activity. It was developed by Mitsubishi Tanabe Pharma and is marketed under license by Janssen, a division of Johnson & Johnson.[3] As per the literature survey it is revealed that the drug has been estimated by Modified HPLC Quantification Analytical Technique for Canagliflozin and Metformin Hydrochloride in Bulk and Tablets.[4] RP-HPLC Method Development and Validation for the Determination of Canagliflozin in Human Plasma. [5] Development and validation of a Stability-Indicating High Performance Thin Layer Chromatography analysis has been reported for the estimation in bulk and pharmaceutical dosage form. (Ishpreet Kaur et al., 2015). [6] Development and Validation of a Stability- Indicating Reverse Phase HPLC-PDA Method for Determination of Canagliflozin in Bulk and Pharmaceutical Dosage Form. [7] SIM RP-HPLC Method for Estimation of Canagliflozin in Dosage Form .[8] Thus, there are only three stability indicating High Performance Liquid Chromatography analysis has been reported for the estimation of Canagliflozin in bulk and pharmaceutical dosage forms. ICH conditions for photostability has not been used and it has been reported that the drug is prone to photolysis in one paper whereas it has also been reported that the drug is prone to alkaline degradation and is photostable in other reference [6][7][8] Though, the photostability results and ICH Conditions has not been used in the reported literature , therefore, the aim of the present work is to develop and validate a simple, stability indicating

HPLC-PDA method for the determination of Canagliflozin in bulk and pharmaceutical dosage form to confirm susceptibility to stress conditions and validate the method as per ICH Q2A (R1) guidelines.

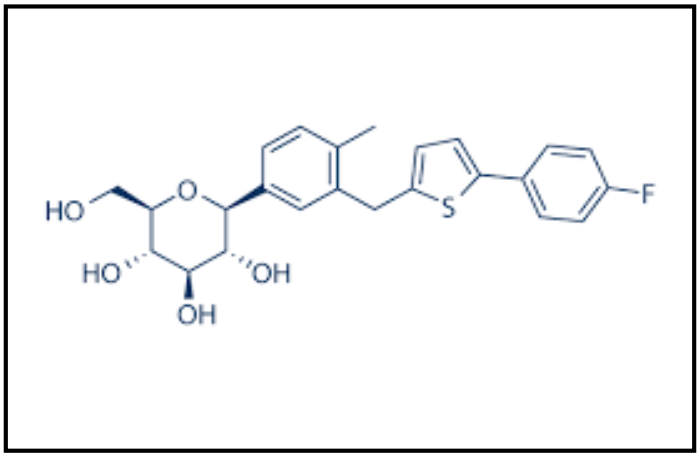

Figure I. Structure of Canagliflozin

II. MATERIALS AND METHODS

2.1 Reagents and Chemicals

The working standard of Canagliflozin was provided by Sanofi- Synthelabo India Pvt Ltd, Verna (Goa, India). The reagents for present study are as follows Acetonitrile HPLC grade (ACN), Distilled water, Hydrochloric acid (HCl), Sodium hydroxide(NaOH), 6%v/v Hydrogen peroxide (H_2O_2), were of analytical grade from LOBA CHEMIE PVT. LTD., Mumbai, India.

2.2 Instrumentation

The method development and validation of RP-HPLC method was performed on JASCO HPLC system comprising of model PU 2080 Plus pump, Rheodyne sample injection port with 20µl loop, using Neosphere C18 column with MD 2010 PDA detector. The chromatogram was recorded with Borwin-PDA software (version1.5). Shimadzu (Model AY-120) balance was used for weighing. Other instruments used were UV-Visible double beam spectrophotometer make JASCO MODEL V-730, ELGA Lab water purification system (PURELAB UHQ-II), Hot Air Oven (Kumar Laboratory Oven), Photo stability chamber (Make Newtronic., Model IC DAC version 1.2).

2.3 Preparation of stock solution

Standard stock solution of Canagliflozin was prepared by dissolving 10mg of drug in 10ml of Acetonitrile to get concentration of 1000µg/ml. It was further diluted with mobile phase to get concentration of solution 500µg/ml., and was ultra sonicated for 10 min. Working standard solution was prepared containing linearity range 10-50µg/ml using mobile phase as solvent.

2.4 Preparation of mobile phase

The mobile phase was prepared by using Acetonitrile: Water (pH adjusted to 4.5 using 0.1%v/v Ortho-phosphoric acid) in the ratio of 50:50 v/v. The mobile phase was filtered through 0.45mm membrane filter and degassed before use.

2.5 Selection of detection wavelength:

From standard stock solution, appropriate dilution was made using Acetonitrile, and scanned over range of 200-400nm.

2.6 Stress degradation Studies of Bulk Drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The API was subjected to hydrolysis under different pH, oxidative, thermal and photolytic conditions. Optimization of conditions was done by changing strength of reagent and duration of exposure to achieve degradation in 10-30%range**.** [9]

2.7Acid Hydrolysis

1ml working standard solution of Canagliflozin (100µg/ml) was mixed with 1ml of 2N Hydrochloric acid (HCl) and 8ml of mobile phase to get final concentration of 10µg/ml and solution was kept for 4hrs at room temperature.

2.8 Alkali Hydrolysis

1ml working standard solution of Canagliflozin (100µg/ml) was mixed with 1ml of 2N Sodium hydroxide (NaOH) and 8ml of mobile phase to get final concentration of 10µg/ml and solution was kept for 4hrs at room temperature.

2.9 Oxidative Hydrolysis

1ml working standard solution of Canagliflozin (100µg/ml) was mixed with 1ml of 6% Hydrogen peroxide (H_2O_2) and 8ml of mobile phase to get final concentration of 10µg/ml and solution was kept overnight at room temperature.

2.10 Thermal Degradation

Thermal degradation was performed by keeping drug in oven at 60ºC for a period of 6 hrs.
A sample was withdrawn, weighed and dissolved in mobile phase to get solution of 10µg/ml.

2.11 Photolytic Degradation

Canagliflozin was exposed to UV light (200watt hours/square meter) and cool white fluorescent light (1.2million lux hours). Sample was weighed, dissolved in mobile phase and diluted to concentration of 10µg/ml.

III. RESULTS AND DISCUSSION

Selection of analytical wavelength:

It was observed that Canagliflozin showed considerable absorbance in 291nm, so it was selected as detection wavelength.

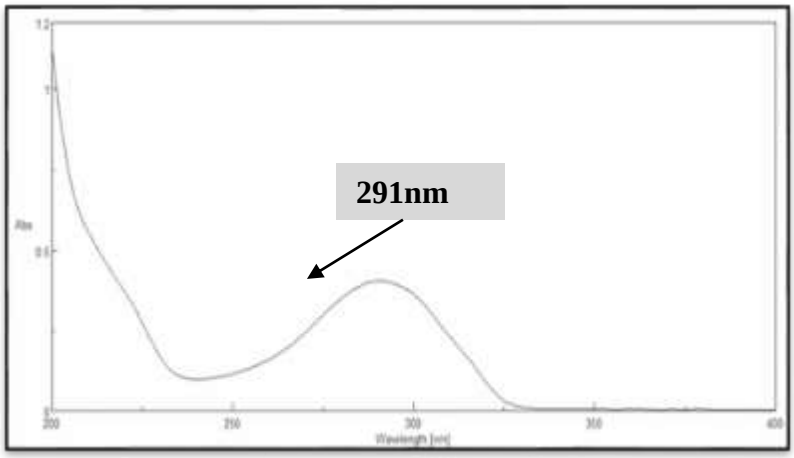

Figure II. UV spectrum of Canagliflozin (10ppm)

Table I. Optimization of chromatographic conditions

Sr. No.	Parameter	Conditions used for Analysis
1.	Column	Neosphere C18 column (150×4.6mm i.d. 3.5µm)
2.	Mobile phase	Acetonitrile: Water (pH adjusted to 4.5 using 0.1%v/v Ortho-phosphoric acid) : 50:50v/v
3.	Flow rate	1ml/min
4.	Detection wavelength	291nm
5.	Sample volume	20µl

6.	Column temperature	Room temperature

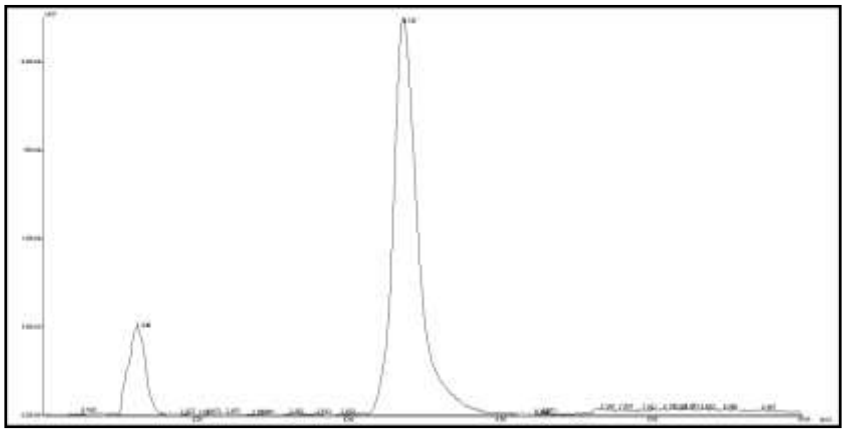

Figure III. Chromatogram of standard Canagliflozin (10ppm)

Table II. Chromatogram and system suitability parameter of drug

Name	RT (min)	Conc. (µg/ml)	Area	Asymmetry	Resolution	Theoretical Plates
Canagliflozin	4.7±0.05	10	506608.274	0.95	3.24	2287.73

3.1 Result of forced degradation studies

Degradation was observed in all the stress conditions and in oxidative condition the degradation is comparatively more as compared to other stress conditions for pure sample of Canagliflozin in HPLC. Also there is no separate peak of degradation observed in any condition and it was confirmed with multi wavelength analysis peak purity comparison. These degradation studies indicated that Canagliflozin was susceptible to all the stress conditions.

Table III. Results of the stress degradation studies for standard Canagliflozin hemi-hydrate

Sr. No.	Parameters	Conditions	% Recovery
1.	Initial	No treatment	100
2.	Acid hydrolysis	2N HCl 4hrs at RT	82.22
3.	Alkali hydrolysis	2N NaOH 4hrs at RT	70.10
4.	Oxidative hydrolysis	6%H_2O_2 overnight at RT	53.22
5.	Thermal	60ºC, 6hrs	78.44
6.	Photolytic degradation	UV light(200 watt hours/square meter	83.48
6.1	a. UV light		
6.2	b. Fluorescent light	Cool white fluorescent light(1.2million lux hours)	67.74

3.2 VALIDATION OF THE METHOD

The method was validated for various parameters in accordance with ICH guidelines. [10]

3.2.1 Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 990 indicating the non-interference of any other peak of degradation product or impurity.

3.2.2 Linearity

Calibration curve was obtained in the range of 10-50µg/ml, peak area were recorded. Standard calibration curve was plotted of peak area Vs concentration injected. The equation of calibration curve was found to be Y= 40263 x + 63630 having coefficient of correlation (R^2) = 0.997
shown in Figure V.

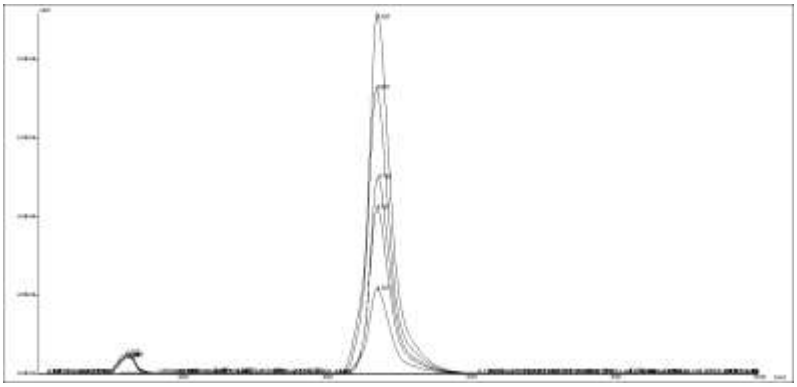

Figure IV. Chromatogram of Calibration curve of Canagliflozin (10-50µg/ml)

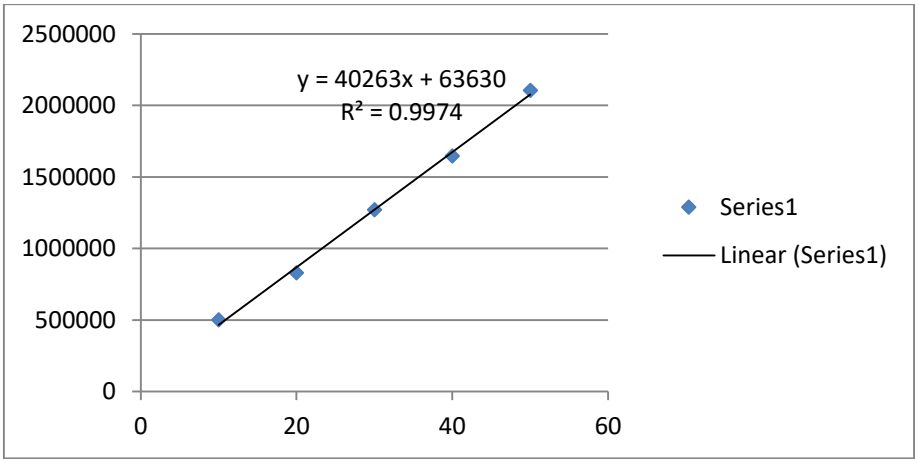

Figure V. Calibration curve for Canagliflozin at 291nm

3.2.3 Range

The linearity range was found to be 10-50µg/ml of Canagliflozin.

3.2.4 Assay

The assay was carried out using marketed formulation of Canagliflozin which is available as Sulisent tablets 100mg equivalent powder was weighed and dissolved in 20 ml of Acetonitrile, sonicated for 10min and volume was made up to 25ml (filtrate A) , centrifuged the mixture and filtered through membrane filter paper. Then further dilution was made using 0.5ml (filtrate A) and diluted with mobile phase to 10ml to get the final concentration of 20µg/ml. Assay was found to be 97.57 % for Canagliflozin.

3.2.5 Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to blank blend at three different levels 80, 100 and 120%. The results of the recovery studies indicated that the method is accurate for estimation of drug in the blend. The results obtained are shown in table IV.

Table IV. Recovery studies for Canagliflozin

Level (%)	Amount after spiking (µg/ml)	Amount recovered (µg/ml)	%Recovery
80	18	17.83	99.05
100	20	19.46	97.31
120	22	21.72	98.75

3.2.6 Precision

The intra-day and inter-day precision of HPLC method is shown in Table V. Results expressed in terms of % RSD, which describes intra-day and inter-day variation of Canagliflozin at concentration of 10µg/ml (n=6).

Table V. Intra-day and Inter-day precision

Sr. No.	Amount(µg/ml)	Intra-day	SD	%RSD	Inter-day	SD	%RSD
1.	10	503214.8	3338.663	0.66	503780.502	3112.517	0.61
2.	10	503331.3			503359.124		
3.	10	502314.4			503346.585		
4.	10	509098.2			509100.524		
5.	10	509808.5			509810.869		
6.	10	503318.1			503325.841		

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

The limit of detection and limit of quantification is the lowest concentration of analyte in a sample which can be detected and quantified with acceptable accuracy and precision. The LOD and LOQ of the developed method were calculated using the formula as given below.

Limit of detection=3.3*σ /S
Limit of Quantification = 10*σ / S

Where, σ = standard deviation of Y-intercept, S= slope of the calibration curve.
LOD and LOQ were found to be 1.13 µg/ml and 3.45 µg/ml respectively, which shows the sufficient sensitivity of the method.

3.2.8 Robustness

Robustness of the method was determined by making slight deliberate changes in the chromatographic conditions like flow rate, change in wavelength and mobile phase composition. It was observed that there were no marked changes in the chromatograms, which shows that the HPLC method is robust and within the limit of 2.

Table VI. Summary of validation study

Sr. No.	Validation parameters	Canagliflozin
1.	Linearity equation (r^2)	Y= 40263 x + 63630, R^2 =0.997
2.	Range	10-50µg/ml
3.	Precision (%RSD) a)Intra-day b)Inter-day	 0.66 0.61
4.	Accuracy a)80% b)100% c)120%	 99.05 97.31 98.75
5.	Limit of detection(µg/ml)	1.13
6.	Limit of quantification(µg/ml)	3.45
7.	Specificity	Specific
8.	Robustness	Robust

3.3 Discussion

The method was developed using isocratic system with retention time of 4.7±0.05 min. As per the literature survey it is revealed that the drug has been estimated by High performance thin layer chromatography analysis for the estimation in bulk and pharmaceutical dosage forms. (Ishpreet Kaur et al., 2015).There are only three stability indicating High Performance Liquid Chromatography analysis has been reported for the estimation of Canagliflozin in bulk and pharmaceutical dosage forms, and ICH conditions for photostability has not been used and it has been reported that the drug is prone to photolysis ., it has also been reported that the drug is prone to alkaline degradation and is photostable. Though, the photostability results and ICH Conditions has not been used in the reported literature , therefore, the aim of the present work is to develop and validate a simple, precise, accurate, specific and reproducible stability indicating HPLC-PDA method for the determination of Canagliflozin in bulk and pharmaceutical dosage form and validate as per ICH Q2A (R1) guidelines and the results are quiet comparable to that reported by Ishpreet Kaur et al. which shows that the drug is susceptible to all the stress conditions and due care should be taken during formulation and packaging.

IV. CONCLUSION

The developed stability-indicating method is simple, rapid and it was validated as per ICH guidelines and can be applied for the determination of Canagliflozin tablets routinely.

V. ACKNOWLEDGEMENTS

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