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A Validated Stability Indicating RP-HPLC Method for Estimation of Canagliflozin in Dosage Form.

A Suneetha[1]*, and D Sharmila[2].

[1]Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Guntur, Andhra pradesh, India.
[2]Department of Pharmaceutical Analysis, V.V institute of Pharmaceutical Sciences, Gudllavaleru, Andhra Pradesh, India.

ABSTRACT

A simple, specific, precise, and accurate RP-HPLC method has been developed and validated for the estimation of Canagliflozin in bulk and tablet dosage form. Chromatographic separation was achieved on Hypersil BDS, C18 100 x 4.6 mm, 5 µ column using 0.1% ortho phosphoric buffer and acetonitrile (53:47) as mobile phase, water and aetonitrile (50:50) as diluent in isocratic mode. Flow rate of 1.1ml/min was optimized with detection wavelength at 240 nm. The retention time (Rt) was around 3.3±0.2 min. The method was validated with respect to specificity, selectivity, linearity, accuracy, precision, and robustness as per ICH gudieliness. The assay method was observed linear in the concentration range of 75-450 µg/ml with a Correlation coefficient (r^2) 0.9999. The percentage recovery of active pharmaceutical ingredient from tablet dosage form ranged from 99.83-100.27%. The Limit of Detection and Limit of Quantification were found to be 0.23µg/ml and 0.7µg/ml, respectively. Stress conditions of degradation in acidic, alkaline, peroxide, thermal and UV radiation were studied and found Canagliflozin is sensitive to alkali degradation comparative to other stress conditions.

Keywords: Canagliflozin, RP-HPLC, validation, stress studies.

Corresponding author

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INTRODUCTION

Canagliflozin is chemically (1S)-1, 5-anhydro-1-[3-[[5-(4-fluorophenyl)-2-thienyl] methyl]-4-methylphenyl]-D-glucitol (Figure 1) and belongs to the class of SGLT2 inhibitors. It is used in the treatment of type-2 diabetes (1). Canagliflozin inhibits the reabsorption of glucose from kidneys and lowers the renal glucose threshold by inhibiting sodium-glucose transport protein (SGLT2) (2,3,4). Canagliflozincan be used as monothreapy or multi therapy in the treatment of type-2 diabetes (5,6). It has an empirical formula of $C_{24}H_{25}FO_5S$ with a molecular weight 444.52. It is freely soluble in DMSO, ethanol and practically insoluble in water (1). It is marketed as oral tablet and has bioavailability of 65% and is rapidly absorbed in the gastrointestinal (GI) tract. It has a relative oral bioavailability of 65% and reaches peak concentrations within 1 to 2 hours. Canagliflozin is highly protein-bound, mostly to albumin at 99% (7). The present study was aimed for establishing a simple, accurate, and rapid RP-HPLC method for determination of Canagliflozin in presence of its degradation products or other pharmaceutical excipients. The method was validated following analytical performance parameters suggested by ICH guidelines (8).

EXPERMINENTAL

Chemicals and reagents

The working standard of Canagliflozin was procured from CHEMSCENE, NJ USA. The marketed formulation Invokana (300mg) tablets were procured from US market. HPLC grade acetonitrile, and methanol were purchased from Merck specialty Pvt., Mumbai, India. HPLC grade water from Rankem Pvt, Gujarat, India. Orthophosphoric acid, hydrochloric acid and sodium hydroxide of AR grade were obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

Preparation of standard solutions

Standard solution containing a combination of Canagliflozin (300 µg/mL) was prepared in water and acetonitrile (ACN) (50:50). Appropriate dilutions of the standard solution were made in diluent containing water and ACN (50:50) to produce solutions in the range 75 -450 µg/mL. Samples for the determination of recovery, precision and accuracy were also prepared by spiking control in appropriate concentrations (i.e., 50, 100, and 150 %).

Apparatus and chromatographic conditions

The analytical technique was developed using Waters HPLC equipment, Model- HPLC-269, fitted with a Hypersil BDS C18 column (100 mm x 4.6 mm, 5 µ). The mobile phase consisted of a mixture of 0.1% ortho phosphoric buffer and ACN in the ratio 53:47. The mobile phase was filtered through a 0.22-mm nylon filter and degassed using ultrasonic bath sonicator for 30 min before running the experiment. All experiments conducted on the HPLC were carried out in isocratic mode. Injection volume was 10 µL with a flow rate of 1.1 mL/min. The column temperature was maintained at 30^{o}C and elution was monitored at 240 nm using a Photo diode array detector. All chromatographic data were acquired and processed with the Empower 2 software.

Validation of the analytical method

The developed method was validated as per the ICH guidelines for linearity, accuracy and precision and specificity. Limit of detection (LOD) and limit of quantification (LOQ) were determined using the serial dilution method.

Linearity

Different aliquots of standard solution of Canagliflozin was transferred into set of 10 ml volumetric flasks and diluted up to the mark with diluent such that the final concentrations of Canagliflozin were in the linearity range of 75-450µg/ml. Evaluation of the drug was performed with PDA detector at 240 nm and peak area was recorded. The response for the drug was linear and the regression equation was found to be y=5012x+1202 and correlation coefficient value of Canagliflozin was found to be 0.9999. The results showed that an excellent correlation exists between peak area and concentration of drug within the specified range.

Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Canagliflozin in the drug product. The study was carried out in triplicate at 50, 100 and 150 %. The percentage recovery in each case was calculated. The percentage recovery ranges from 99.20-100.85% and the mean recovery of Canagliflozin was 99.95% that shows there is no interference from excipients and the lower values of %RSD of assay indicates the method is more accurate.

Precision

The precision was determined for Canagliflozin in terms of system and inter-day precision. For system precision evaluation, a standard solution of fixed concentration was injected six times at different time intervals and %RSD for Canagliflozin was 1.17% (limit %RSD < 2.0%). In addition, the inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the %RSD for Canagliflozin was 0.58% (limit %RSD < 2.0%).

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method for Canagliflozin were found to be 0.23µg/ml and 0.7µg/ml respectively.

System suitability

System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions like flow rate, temperature and mobile phase composition. It was observed that there were no marked changes in the chromatograms, which demonstrated that the developed HPLC method is more robust.

Solution stability

The stability of solution under study was established by keeping the solution at room temperature for 24 hrs. The result showed no significant change in concentration and thus confirms the stability of the drug in the solvent used for the analysis.

Analysis of the marketed formulations

The proposed method was applied to the determination of Canagliflozin in pharmaceutical formulation. 10µl of each standard and sample solution were injected separately and from the peak area of Canagliflozin, amount of drug in samples were computed. The result of assay undertaken yielded 99.55% of label claim of Canagliflozin. The assay obtained is more than 99% and no interference of impurity peak observed.

Forced degradation studies

The forced degradation studies were performed to establish the stability indicating nature and specificity of the assay method and to observe any degraded compounds. The stress studies are carried out by using stock solution of 3mg/ml concentration.

The acid, alkaline and oxidative stress degradation is carried out by using 2N Hydrochloric acid, 2N Sodium Hydroxide, 20% hydrogen peroxide and by refluxing for 30min at 60^0C. Thermal stress testing was carried out by placing the drug solution in oven at 105^0C for 6hrs. Photostability studies were performed by placing the drug solution in UV chamber for 7 days. The resultants in all cases were further diluted with diluent to give final concentration of 300µg/ml.

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RESULTS AND DISCUSSIONS

In the present work a simple reverse phase high performance liquid chromatographic method has been developed, optimized and validated for the estimation of Canagliflozin in pharmaceutical formulations. The work is carried out on Hypersil BDS, C18 (100mm x 4.6 mm, 5µ.) in the isocratic mode using a mobile phase consisting of buffer and ACN taken in the ratio 53:47v/v. The analysis performed at ambient temperature using a flow rate of 1.1 ml/min with a run time of 8 min. The eluent was monitored using PAD at a wavelength of 240 nm. The results of optimized HPLC conditions were shown in Table 1.

The developed chromatographic method was validated for specificity, linearity, precision, accuracy, sensitivity, robustness, and system suitability. The method was linear in the range of 75-450µg/ml for Canagliflozin with correlation coefficient of 0.9999 (Figure.3) and the results were shown in Table 2. The % recoveries of Canagliflozin were found in the range of 99.20-100.85% and the mean recovery was found to be 99.94% for Canagliflozin, which indicated that the method is accurate. The results of recovery studies were incorporated in Table 3. The %RSD for method precision and inter-day precision for Canagliflozin were found to be 1.17 and 0.58, respectively, which indicate the method is precise. The results of precision studies were shown in Table 4.

The retention time of Canagliflozin was 3.3 min, cuts down on overall time of sample analysis and the method was more cost effective as it utilizes very less quantity of mobile phase. The number of theoretical plates was 2567 and tailing factor was 1.25 for Canagliflozin, which indicates efficient performance of the column. Selectivity of the method was demonstrated by the absence of any interfering peaks from other coexisting excipient substances at the retention time of the drug. The limit of detection and limit of quantification for Canagliflozin were found to be 0.23µg/ml and 0.7µg/ml, which indicate the sensitivity of the method. A system suitability test was performed to evaluate the chromatographic parameters and the summary of system suitability parameters were shown in Table 5. Validated method was applied for the determination of Canagliflozin in commercial formulations. The % assay was found to be 99.55% for Canagliflozin and the assay results were shown in Table 6 (Figure. 4).

The typical chromatograms of degradation behavior of Canagliflozin in different stress conditions are shown in Figure 5 to Figure 9. During the acidic and alkaline degradation, 7.61% and 6.61% of drug was decomposed respectively. Canagliflozin has undergone oxidative, thermal and photo stability degradation slightly that is less than 6%. The results of analysis are given in Table 8.

Table 1: Optimized chromatographic conditions of Canagliflozin.

S. No.	Parameter	Condition
1	Mobile phase	Buffer:Acetonitrile (53:47)
2	Diluent	Water: Acetonitrile(50:50)
3	Column, make	GL Sciences Inc
4	Column temperature	30^0C
5	Flow rate	1.1ml/min
5	Wave length	240nm
6	Injection volume	10 µL
8	Run time	8mins

Table 2: Linearity dataof Canagliflozin.

Linearty range (µg/ml)	75 - 450
Slope	5012
y- intercept	1202
Correlation coefficient (r^2)	0.999

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Table 3: Recovery results of Canagliflozin.

Level	Concentration added (ppm)	Concentration found (ppm)	% Recovery* (Mean±SD)
50%	150	149.7386	99.82575 ± 0.64
100%	300	299.2321	99.74404±0.31
150%	450	451.2354	100.2745±0.56
Mean ± SD			99.94±0.51
%RSD			0.51

*- Mean of three determinations

Table 4: Precision results of Canagliflozin.

Precision	%assay of Canagliflozin(Mean ±SD)	%RSD
Method Precision	99.55 ± 0.641	0.644
Intermediate Precision	**99.90 ± 1.166**	**1.167**

Table 5: Summary of system suitability parameters of Canagliflozin.

S. No.	System suitability	Results
1	Linearity range (µg/mL)	75-450 µg/mL
2	Correlation coefficient	0.999
3	Theoretical plates (N)	2567
4	Tailing factor	1.25
5	LOD (µg/mL)	0.23 µg/mL
6	LOQ (µg/mL)	0.7 µg/mL
7	Regression Equation	y=5012x+1202

Table 6: Assay results.

S. No.	Formulation	Label claim	Amount found	%Assay
1	INVOKANA	300 mg	298.65mg	99.55%

Table 7: Degradation studies of Canagliflozin.

Stress Conditions Degradation	% Drug recovered	% Drug decomposed
Standard	100	100
Acid	92.38853	7.61147
Base	93.38325	6.61675
Oxidative	94.76119	5.23881
Thermal	95.49324	4.50676
Photo stability	99.25612	0.74388

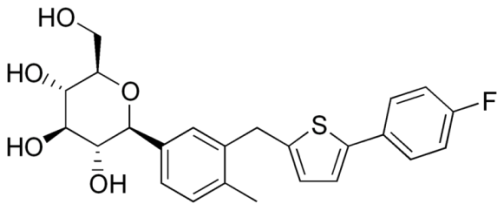

Figure 1: Structure of Canagliflozin.

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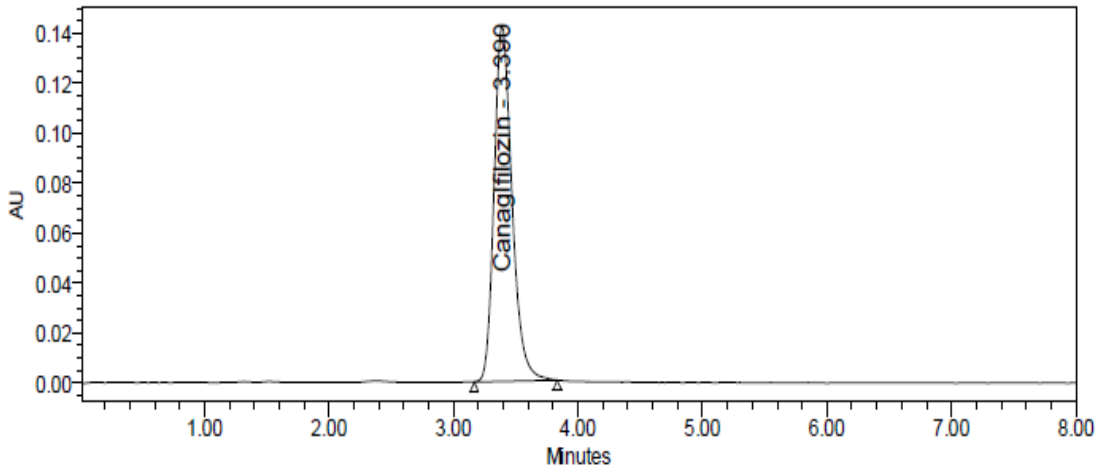

Figure 2: Typical standard chromatogram of Canagliflozin.

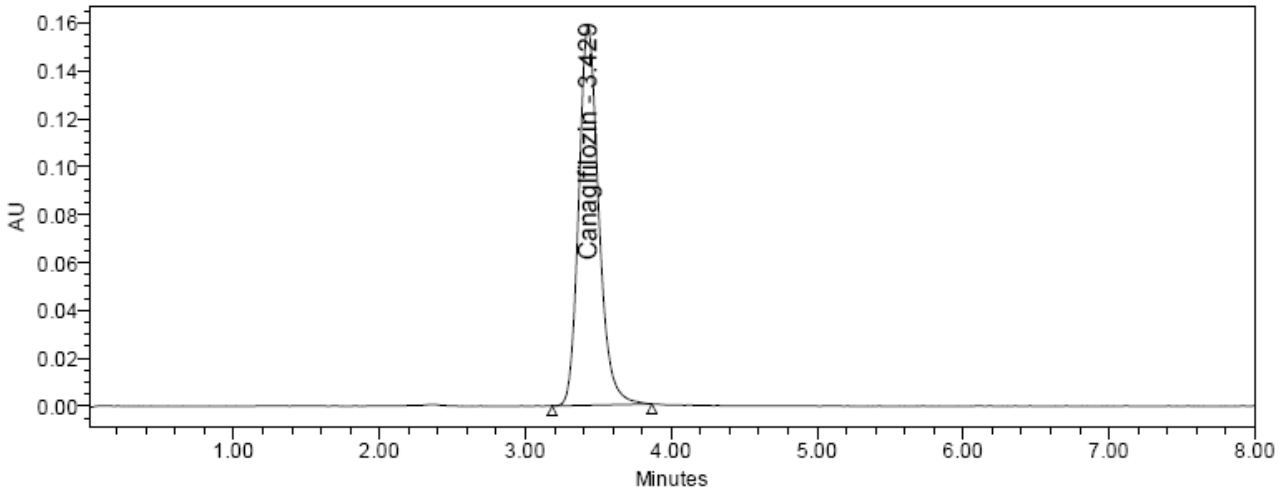

Figure 3: Typical sample chromatogram of Canagliflozin.

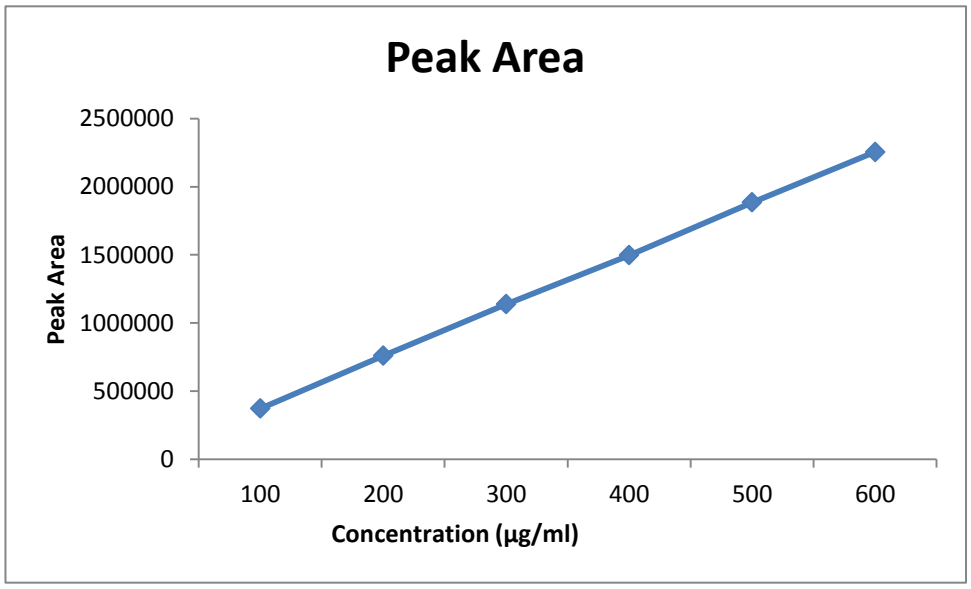

Figure 4: Calibration curve of Canagliflozin.

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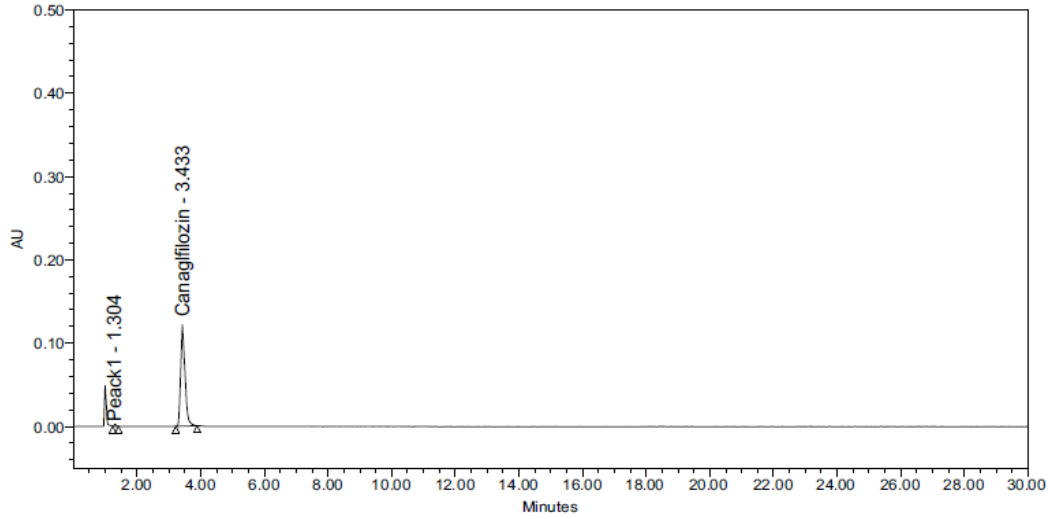

Figure 5: Acid degradation chromatogram of Canagliflozin.

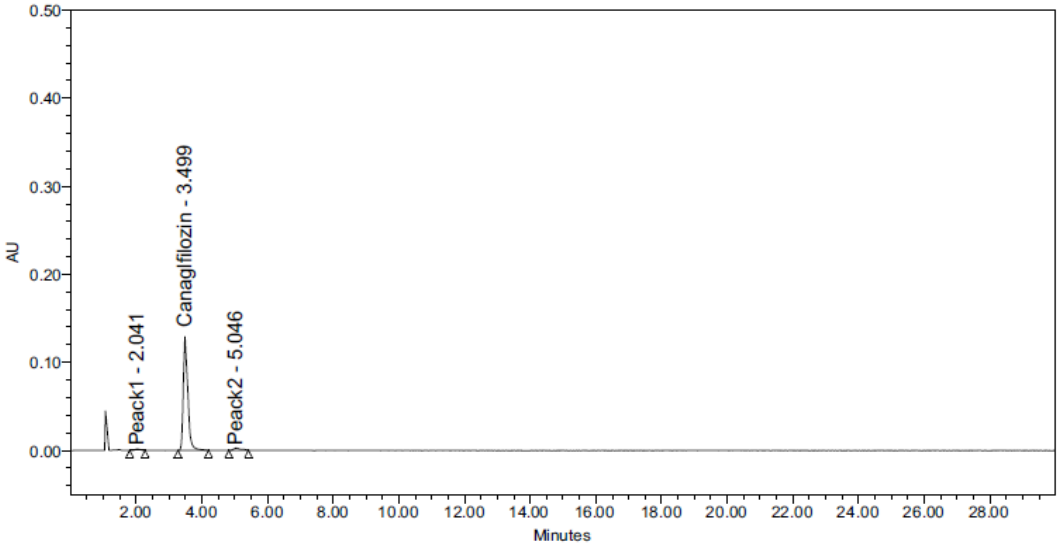

Figure 6: Alkali degradation chromatogram of Canagliflozin.

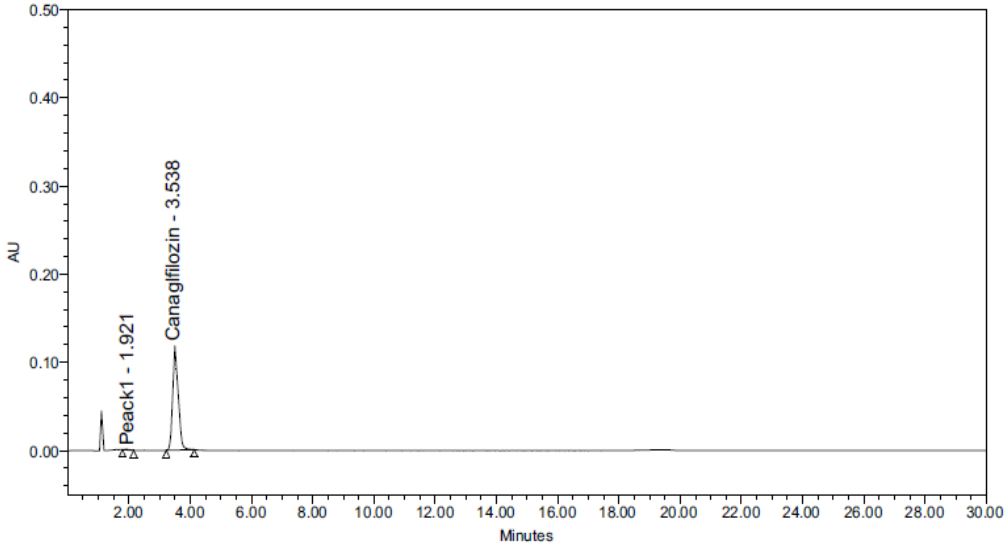

Figure 7: Oxidative degradation chromatogram of Canagliflozin.

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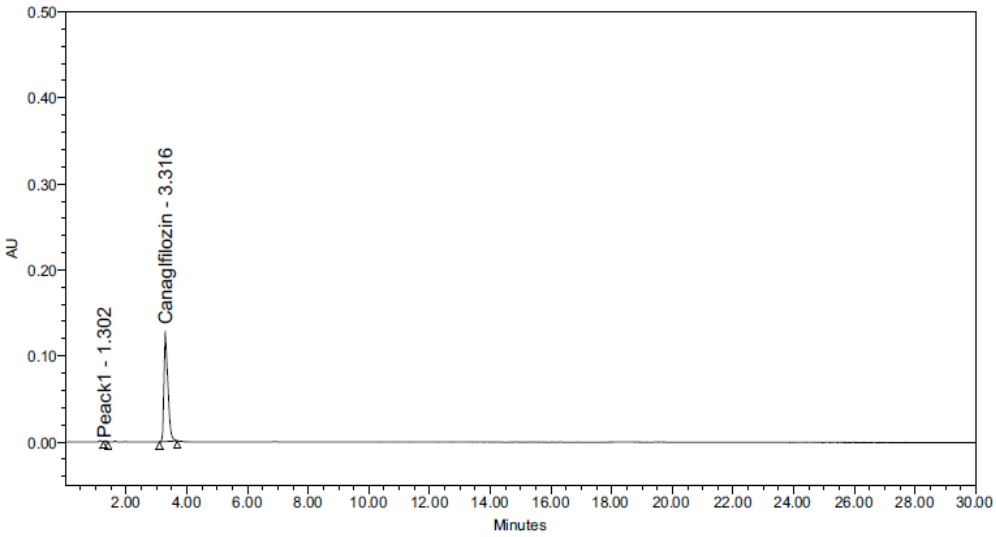

Figure 8: Thermal degradation chromatogram of Canagliflozin.

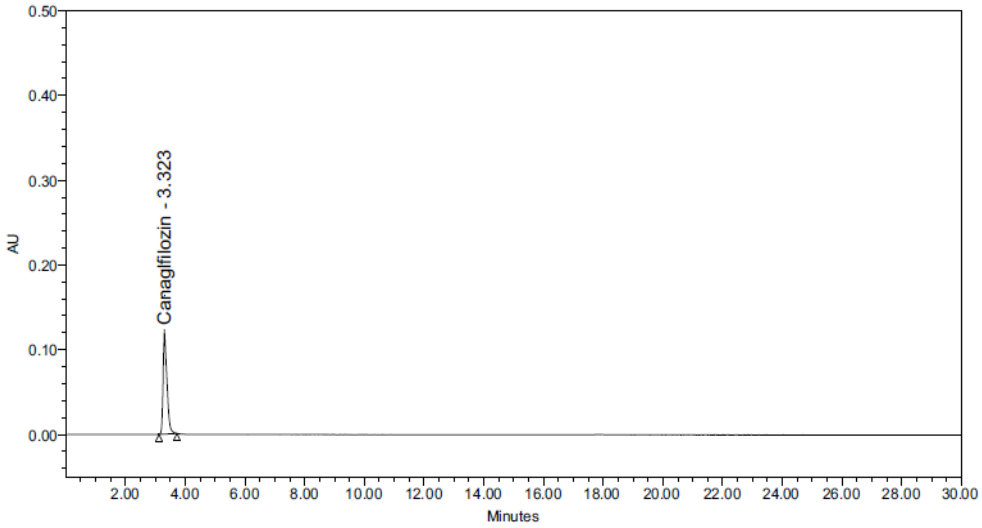

Figure 9: Photo stability degradation chromatogram of Canagliflozin.

CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and can be applied for the determination of Canagliflozin in Tablet dosage forms. The method was found to be accurate, precise, robust and specific as the drug peak did not interfere with the extra peaks aroused during the forced degradation studies. At the same time the chromatographic elution step is undertaken in a short time (< 5 min). No interference from any components of pharmaceutical dosage form can be successfully applied to perform the routine analysis of the drug Canagliflozin in pharmaceutical formulations.

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