ORIGINAL ARTICLE

A High Performance Liquid Chromatography Method Development and Validation for the Estimation of Canagliflozin in Bulk and Marketed Dosage Form

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Abstract

Aim: An efficient and simple high-performance liquid chromatography (HPLC) method has been developed and validated for the determination of canagliflozin in marketed formulations containing canagliflozin. **Materials and Methods:** The mobile phase used for the chromatographic runs consisted of 5 mMammoniumformate in water: Methanol (25:75, v/v) The separation was achieved on a Symmetry, Waters C 18 (100 mm × 4.6 mm × 3.5 µm) using isocratic mode. **Results and Discussion:** Drug peaks were well separated and were detected by a UV detector at 290 nm. The method was linear at the concentration range of 1–10 µg/ml for both the formulations. The method has been validated according to the International Conference of Harmonisation guidelines concerning precision, accuracy, and forced degradation. Canagliflozin limit of detection and limit of quantification (LOQ) were 0.0026 ng/ml and 0.008 ng/ml, respectively. **Conclusion:** The current research epitomizes the report that deals with the development of a stability-indicating HPLC method for the determination of Canagliflozin in two different brands. Canagliflozin is very sensitive so it is unstable in alkaline, oxidative and thermal conditions but stable in UV light or acid conditions.

Key words: Assay, canagliflozin, high-performance liquid chromatography, International Conference of Harmonisation guideline, method development, method validation

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both. According to the International Diabetes Federation, approximately 415 million adults between the ages of 20–79 years had DM in 2015. DM is proving to be a global public health burden as this number is expected to rise to another 200 million by 2040.[1] The prevalence of Type 2 DM has doubled over the past three decades and is likely to affect a half a billion people in the next three decades. The sodium-glucose co-transporter 2 (SGLT2) inhibitors have recently emerged as important new treatments for DM.[2] These are a new class of antihyperglycemic agents that lower blood glucose levels in patients with type 2 diabetes. SGLT2 inhibitors have an insulin-independent mechanism of action, acting to inhibit the reabsorption of glucose in the kidney, which leads to increases in urinary glucose excretion in individuals with elevated blood glucose levels.[3,4] Canagliflozin is a C-glycosyl compound, a member of thiophenes, and an organofluorine compound.Its IUPAC name is (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[3-[[5-(4-fluorophenyl)thiophen-2-yl]methyl]-4-methylphenyl]-6-(hydroxymethyl)oxane-3,4,5-triol (C24H25FO5S) and its structure is shown in Figure 1.[5]

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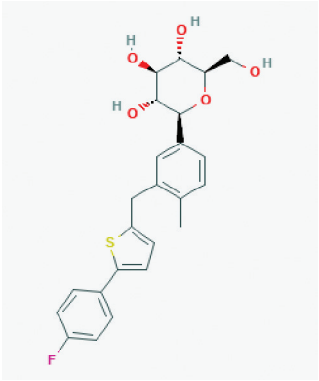

Figure 1: Structure of canagliflozin (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[3-[[5-(4-fluorophenyl)thiophen-2-yl]methyl]-4-methylphenyl]-6-(hydroxymethyl)oxane-3,4,5-triol

Analytical method validation ensures that various high-performance liquid chromatography (HPLC) analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the International Conference of Harmonisation (ICH) guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines.[6-8]

The present study is aiming to develop and validate a simple, sensitive, rapid, economic, and isocratic HPLC method for the determination of canagliflozin in marketed formulations containing canagliflozin alone and in combination.

EXPERIMENTAL

Materials, reagents and pharmaceutical products

Canagliflozin Brand Tablet Invokana 100 mg (Johnson & Johnson LTD), Tablet Sulisent 100 mg (USV LTD), and Tablet Prominad (Cipla LTD) were obtained from the local pharmacy. Ammonium formate and HPLC grade methanol were obtained from Sigma-Aldrich. A Millipore Milli-Q water ultra-pure water system (Millipore, Australia) was used to obtain distilled water.

Instrumentation

The HPLC system used for the method development and validation consisted of the Agilent LC1260 series, with VWD detector. Analysis and separation have been done on Symmetry, Waters C 18 (100 mm × 4.6 mm × 3.5µm) at 290 nm in an air-conditioned lab (temperature maintained at 25°C throughout all chromatographic runs).[9,10] The mobile phase used for the chromatographic runs consisted of 5 mM ammonium formate in water: methanol at ratio (25:75, v/v), the flow rate was set at 1 ml/min in an isocratic mode and the injection volume was set at 1 µl for all samples.

Preparation of the buffer solution

A 20 mM buffer solution was prepared by dissolving 1.26 g ammonium formate in 1000 mL Milli-Q water and the final pH adjusted to 3.5 using formic acid. The buffer solution was then filtered through (0.45 Nylon NY membrane filter) and degassed in a sonicator for 10 min.[11,12]

Preparation of standard stock solution

About 100 mg of standard canagliflozin was accurately weighed and transferred into a 100 ml volumetric flask and 20 mL of the mobile phase mixture was added to it and sonicated for 10 min, the final volume was made up to 100 mL using the mobile phase mixture. This gave a standard stock solution of 1000 µg/ml. The standard stock solution was further diluted to get the desired concentrations.[13-15]

Preparation of working solution

It was prepared by taking 1 ml of the stock solution into a 10 ml volumetric flask and the final volume was made up with diluent (100 µg/ml). The solution was filtered and then diluted immediately before use to appropriate concentration levels by using the mobile phase.[16,17]

Preparation of Pharmaceutical sample

Brand 1

Twenty tablets of Canagliflozin® Invokana were weighed and crushed. 100 mg powder equivalent to one Canagliflozin® tablet (100 mg canagliflozin) was placed in a 100 ml volumetric flask and sonicated for 10 min and the final volume was made up to the mark with mobile phase mixture followed by 5 min shaking. The solution was filtered and 10 ml of the filtrate was transferred into 20 ml volumetric flasks and the final volume was made to the mark with the mobile phase mixture. An aliquot of 2 ml from the above solution was transferred into a 20 ml volumetric flask and the mobile phase was added to the mark to produce a final concentration of 50 µg/ml canagliflozin.[18]

Brand 2

Twenty tablets of Canagliflozin® Prominad were weighed and crushed. 50 mg powder equivalent to one Canagliflozin® tablet (50 mg canagliflozin) was placed in a 50 ml volumetric flask and sonicated for 10 min and the final volume was made up to the mark with mobile phase mixture followed by 5 min shaking. The solution was filtered and 5 ml of the filtrate was transferred into 10 ml volumetric flasks and the final volume was made to the mark with the mobile phase mixture. An aliquot of 1 ml from the above solution was transferred into a 10 ml

volumetric flask and the mobile phase was added to the mark to produce a final concentration of 50 µg/ml canagliflozin.[19,20]

Method development and optimization

The suitability of the column and the mobile phase used in the optimized method has been decided based upon the basis of the selectivity, sensitivity as well as acceptable chromatographic parameters of the produced peaks. We used the mobile phase as a solvent for all samples to ensure minimum noise and to eliminate any unwanted solvent peaks.

Selection of UV wavelength

Canagliflozin has a λ_{max} at 290 nm in methanol.[21] An acceptable response was obtained upon the detection of both the brands of the drug at 290 nm [Figure 2].

The optimized HPLC condition is depicted in Table 1.

Method validation

The method has been validated as per the ICH guidelines Q2 (R1)[7] for evaluating system suitability, precision, accuracy, linearity, the limit of detection (LOD), the limit of quantitation (LOQ), and forced degradation studies.[22,23]

System suitability

System suitability parameters concerning tailing factor, number of theoretical plates, and retention time of canagliflozin peak were assessed by injecting a blank mobile phase followed by six replicates of canagliflozin (50 µg/ml).

Table 1: HPLC conditions

System	Agilent 1260 series
Mobile Phase	5 mM Ammonium formate: MeOH (25:75 v/v)
Flow rate	1 ml/min
Column	C-18, 100×4.6 mm, 3.5 µm (Symmetry, Waters)
Oven Temperature	40°C
Wavelength of detection	290 nm
Back Pressure generated	134–136 bars
Total Run time	6 min
Average Retention time	2.64 In

Linearity

Linear regression data over the range of 1 to 10 µg/mL for Canagliflozin with a correlation coefficient of 0.999 unfolds a good linear relationship between area and concentration in the calibration curve.[24]

Precision, repeatability (intra-day precision) and intermediate (inter-day precision)

System and method precision were assessed by injecting 5 independent samples of canagliflozin (50 µg/ml each) on the same day under the same operating conditions.

Intermediate or inter-day precision was assessed by comparing the results of 5 independent determinations on 3 different days.

Accuracy study and recovery

Accuracy of the method was resolved by the standard addition method in which standard addition of pure API at three different concentration levels of 70%, 100%, and 130% was performed in triplicate. The accuracy of the method is calculated in the terms of % recovery of the API.

LOD and LOQ

LOD and LOQ for canagliflozin were calculated from the linear regression equation based on the standard deviation of the intercept and the slope using the formula.

LOD = 3.3 Q/S and LOQ = 10 Q/S

where Q: The standard deviation of the intercept, S: the slope of the calibration curve.

Forced degradation studies

To assess the stability-indicating a property of the developed HPLC method stress studies were carried out under ICH recommended conditions. Forced degradation of Canagliflozin was carried out by exposing the bulk sample to acidic, alkaline, oxidative, photolytic, dry heat, and neutral conditions. The aim was to study the ability of the proposed method to measure the analyte response in the presence of its degradation products.[25]

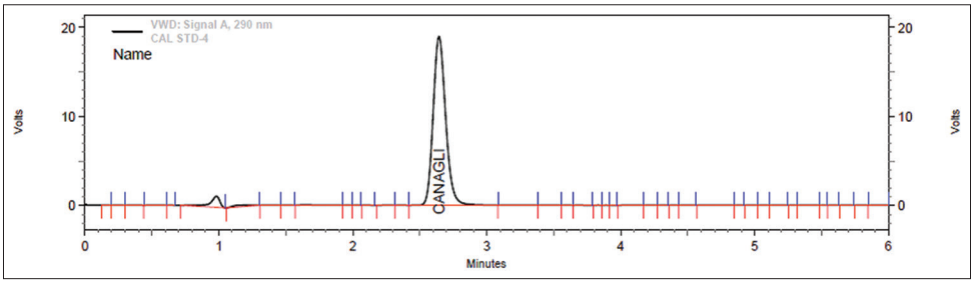

Figure 2: Canagliflozin λ_{max} at 290 nm in methanol

Acid and alkali hydrolysis Aliquot of 1 ml of Canagliflozin solution (1 mg/ml) was transferred to a small round bottom flask. The solution was mixed with 9 ml of 0.1N hydrochloric acid or 0.1 N sodium hydroxide. The prepared solutions were subjected to reflux for 2 h in a boiling water bath. The samples were cooled to room temperature (25°C), neutralized with an amount of acid or base equivalent to that of the previously added. From the resulting neutral solution, 20 µl of each was injected into the HPLC system.

Oxidation One milliliter of Canagliflozin solution (1 mg/ml) was transferred to a round bottom flask. The contents were then mixed with 9 ml of 30% hydrogen peroxide solution, and the reaction mixture was allowed to proceed at room temperature (25°C) for 2 h with intermittent shaking. A volume of 20 µl was injected into the HPLC system. Irradiation with ultraviolet light A sample powder of Canagliflozin (10 mg) was exposed to UV light (254 nm) for 48 h. The material was dissolved in 5 ml water. The solution was filtered with a syringe filtration disk claimed a concentration of 1 mg/ml. It was suitably diluted and a volume of 20 µl was injected into the HPLC system. As well, an aqueous solution of Canagliflozin (1 mg/ml) was exposed to UV light (254 nm) for 48 h, and after diluting 20 µl was injected into the HPLC system.

Thermal degradation Canagliflozin (10 mg) was exposed to a temperature of 70°C for 48 h in a hot air oven. The material was dissolved in 5 ml water. The solution was filtered with a syringe filtration disk claimed a concentration of 1 mg/ml. It was suitably diluted and a volume of 20 µl was injected into the HPLC system. As well, an aqueous solution of Canagliflozin (1 mg/ml) was exposed to a temperature of 70ºC for 48 h, and after diluting 20 µl was injected into the HPLC system.

RESULTS AND DISCUSSION

Linearity studies

The analytical calibration curve constructed for canagliflozin was linear in the specified ranges, indicated by the closeness of the correlation coefficient R^2 to 1 (R^2 = 0.9999). The linear regression equation for the drug is ($Y = 2.08081 \times 10^{-5} \times +0.570271$, $R^2 = 0.9999$).

Precision studies

The %RSD values of intra-day and inter-day for Canagliflozin are less than 2% which reveal that the proposed method is precise and is shown in Table 4 and 5.

Accuracy and recovery studies

The accuracy experiments were carried out by the standard addition method. The high value of recoveries obtained for Canagliflozin indicates that method is accurate as shown in Table 6.

LOD and LOQ

The calculated LOD and LOQ were 0.002669 ng and 0.008007 ng for canagliflozin.

Forced degradation studies

From the forced degradation, it was clear that there was no effect of photolytic degradation on the drug as it was completely recovered (Figure 6). Moreover, the acid stability of Canagliflozin was also appreciable as it was degraded to a negligible amount (Figure 2). However, in the case of alkaline hydrolysis, thermal and oxidation degradation, complete degradation of the drug was seen. In the case of acid hydrolysis, alkaline hydrolysis and oxidation degradation were observed and are shown in the respective chromatograms (Figure 3-5). Nonetheless, the method was able to isolate completely the degradation products from the intact Canagliflozin.

This confirmed stability-indicating the property of the proposed method. The concentration of the produced degradation products analogous to the intact Canagliflozin was calculated and is shown in table 7.

The HPLC chromatogram of standard Canagliflozin and that of Brand 1 and 2 are shown in figure 8-10.

DISCUSSION

Various mobile phases of different compositions were tested to develop an optimum mobile phase to achieve a satisfactory separation and good peak symmetry for Canagliflozin. A mobile phase consisting of 5 mM Ammonium formate: MeOH (25:75 v/v) was developed. The analysis was carried out based on peak area with UV detection at 290 nm [Figure 2]. The retention time obtained for Canagliflozin was at 2.64 min. The detector response was linear in the concentration range of 1–10 µg/ml.

Validation of the proposed method

System suitability

The obtained results of 6 replicate injections showed that the parameters tested were within the acceptable range. Canagliflozin

Table 2: System suitability parameters

Parameter	Results
Retention time	2.647
Tailing factor	1.22
Theoretical plates	4640
% RSD	2.02918

was repeatedly retained at 2.64 min with RSD% of the recorded retention 2.02918 to indicate good repeatability of replicate injections on the integral HPLC system used, the tailing factor never exceeded 1.24 in all peaks indicating good peak symmetry (acceptance limit is < 2) and the number of theoretical plates was always >2000 in all chromatographic runs to ensure good column efficacy throughout the developed separation process. The results of system suitability are given in Table 2.

Linearity

A linear correlation was attained between peak area used absorbance vs concentration of Canagliflozin in the range of 1-10 mcg/ml. The linearity of the calibration curve was validated by the high value of the correlation coefficient of regression as shown in Figure 3 and the results are shown in Table 3.

Accuracy

The accuracy experiments were carried out by the standard addition method. The high value of recoveries obtained for Canagliflozin indicates that the method is accurate as shown in Table 4.

Precision

The %RSD values of intra-day and inter-day for Canagliflozin are less than 2% which reveals that the proposed method is precise and is shown in Tables 5 and 6.

LOD and LOQ

The LOD and LOQ of Canagliflozin were found 0.002669 ng and 0.008007 ng, respectively.

Stability indicating study

The ICH guideline characterized stability testing of drug substances and products require stress testing to be carried out to enlighten the inherent stability characteristics of the active substance and also to produce a rapid identification of differences that might result from changes in the manufacturing processes

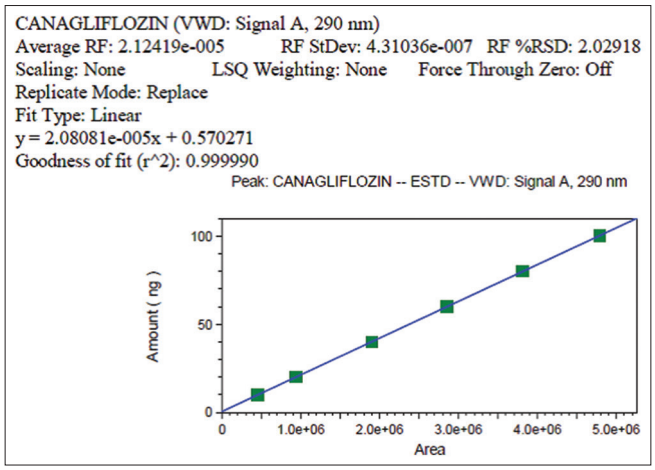

Figure 3: Calibration curve of canagliflozin

Table 3: Linearity of canagliflozin

Parameter	Result
Linearity range	1–10 mcg/ml
Slope	2.08081×10^{-5}
Intercept	0.570271
Coefficient of correlation	0.9999

Table 4: Accuracy studies of canagliflozin

Parameter	Results		
Amount of sample taken (μg/ml)	2	2	2
Amount of standard added (μg/ml)	1.5	2.4	2
Percentage of standard added	70	100	130
% Recovery	99.5	99.8	99.6
Relative standard deviation	0.15	0.14	0.08

*Average of three determinations (*n*=3)

Table 5: Intra-day precision studies of canagliflozin

Day 1	Area (mAU)
Morning (ng/ml)	
15	720236.2
50	2371504
95	4654963
Afternoon (ng/ml)	
15	713629.6
50	2377222
95	4693435.8
Evening (ng/ml)	
15	710425
50	2389567.4
95	4742636.2

Table 6: Inter-day precision studies of canagliflozin

Day 1 Conc	Area (mAU)
Day 1 (ng/ml)	
15	714763.6
50	2379431.0
95	4697011.6
Day 2 (ng/ml)	
15	720260.7
50	2439207.0
95	4919060.6
Day 3 (ng/ml)	
15	720098.0
50	2522346.3
95	5201346.6

or source sample. Vulnerability to oxidation, hydrolytic, photolytic, and thermal stabilities are the required tests. Ideal stability-indicating the method is one that not only evaluates the standard drug alone but also resolves its degradation products.

Table 7: Forced degradation studies of canagliflozin

Sample	Concentration used (ng/ml)	Concentration left after degradation (ng/ml)	% Recovery
Acid hydrolysis	25	0.009	0.036
Alkaline hydrolysis	25	0	0
Photolytic	25	25	100
Oxidation	25	0	0
Thermal	25	0	0

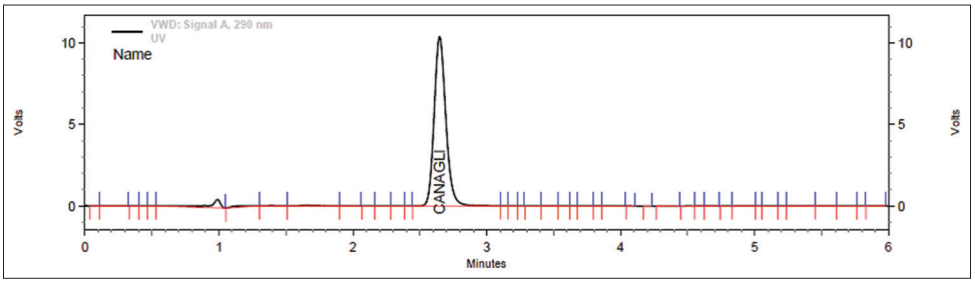

Figure 4: Photolytic degradation

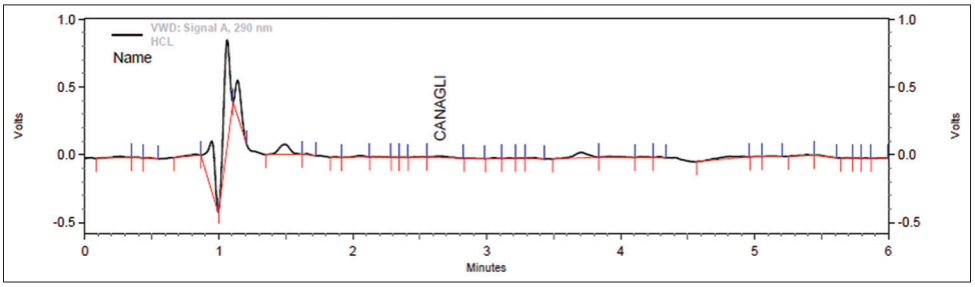

Figure 5: HCl mediated degradation

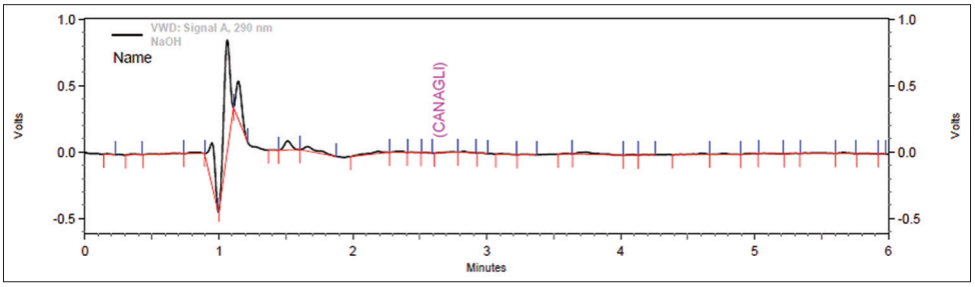

Figure 6: NaOH mediated degradation

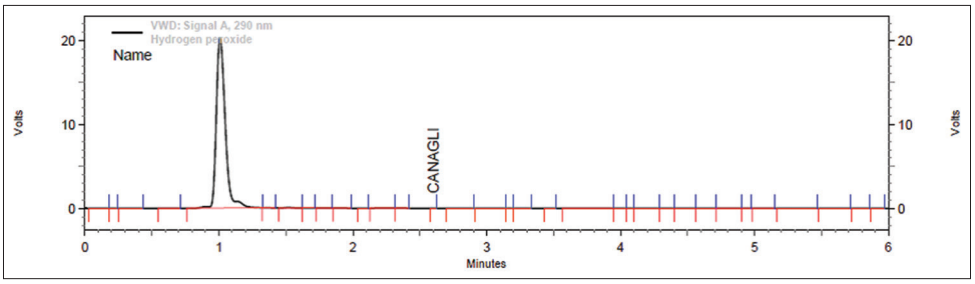

Figure 7: Hydrogen peroxide mediated degradation

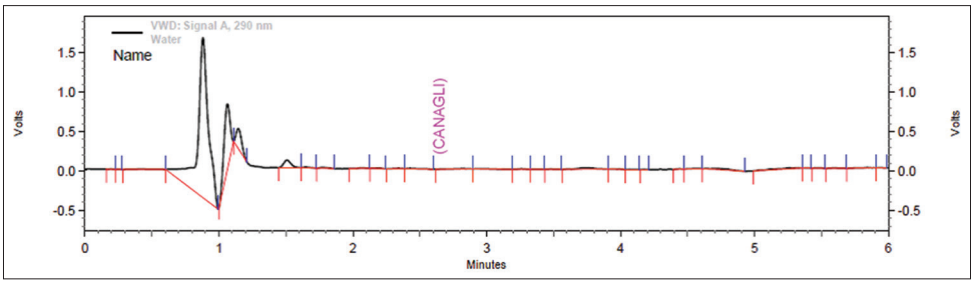

Figure 8: Thermal degradation

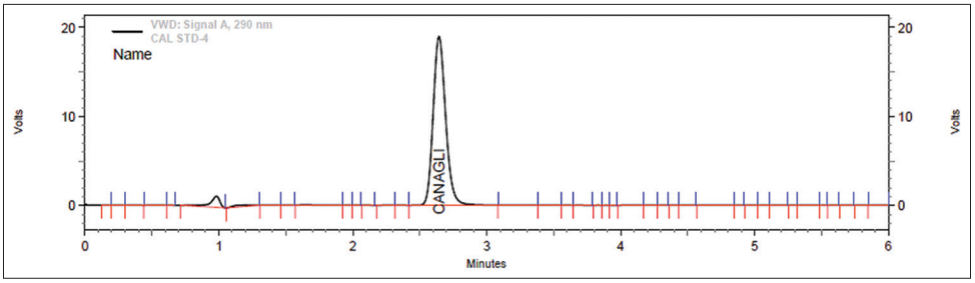

Figure 9: High performance liquid chromatography chromatogram of standard canagliflozin

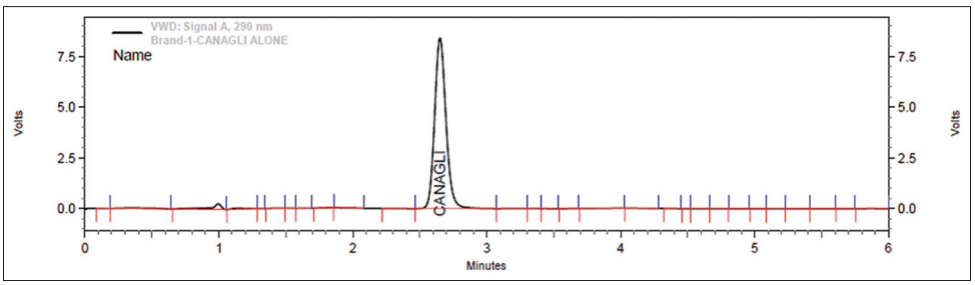

Figure 10: High performance liquid chromatography chromatogram of Brand 1

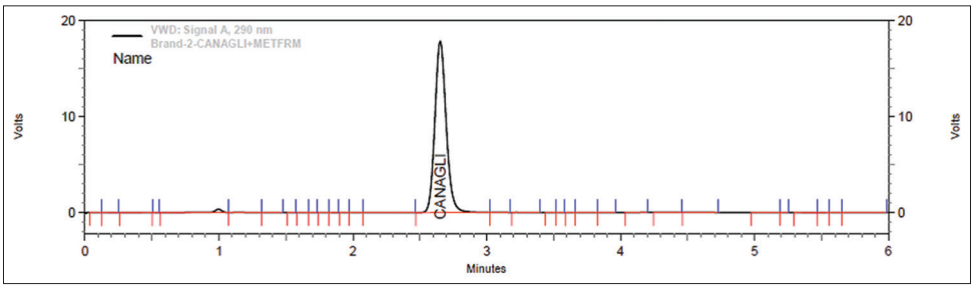

Figure 11: High performance liquid chromatography chromatogram of Brand 2

From the forced degradation, it was clear that there was no effect of photolytic degradation on the drug as it was completely recovered [Figure 4]. Moreover, the acid stability of Canagliflozin was also appreciable as it was degraded to a negligible amount [Figure 5]. However, in the case of alkaline hydrolysis, thermal and oxidation degradation, complete degradation of the drug was seen. In the case of acid hydrolysis, alkaline hydrolysis and oxidation degradation were observed and are shown in the respective chromatograms [Figures 6-8]. Nonetheless, the method was able to isolate completely the degradation products from the intact canagliflozin.

This confirmed stability-indicating the property of the proposed method. The concentration of the produced degradation products analogous to the intact canagliflozin was calculated and is shown in Table 7.

The HPLC chromatogram of standard canagliflozin and that of Brand 1 and 2 are shown in Figures 9-11.

CONCLUSION

The current research epitomizes the report that deals with the development of a stability-indicating HPLC method for the determination of canagliflozin in two different brands. The values of accuracy, precision, LOD, and LOQ were within the limits. Canagliflozin is very sensitive so it is unstable in alkaline, oxidative, and thermal conditions but stable in UV light or acid conditions. Statistical analysis for the results demonstrates that the method is suitable for the determination of canagliflozin in different marketed drugs without any interference from the degradation products, and it is endorsed for routine use in quality control industry laboratories.

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