Degradation Kinetics and Characterization of Canagliflozin Under Solution Stress by LC and LC-MS/MS Method

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ABSTRACT

Objectives: Using the RP-HPLC method, this work investigates the degradation behavior of canagliflozin and its commercial formulation under ICH-recommended stress conditions. It also validates the suggested method and analyzes degraded samples' solution state using LC-MS/MS. Materials and Methods: The solution-state degradation study of canagliflozin in tablet dosage form was performed using the Shimadzu-HPLC series 1100. The acetonitrile (50:50) v/v mobile phase, ammonium acetate buffer pH 4.5 and an ACE BDS C18 column (150×2.5 mm, 4.6 mm) were utilized in the analysis. The wavelength chosen for the study was 291 nm, at which the drug displayed a strong peak and methanol was employed as diluent. An Agilent 1110-LC system was connected to a Shimadzu LC-MS/MS-8040 outfitted with a PDA-SPD M20A and an Electrospray Ionization (ESI) source interface for LC-MS analysis. Canagliflozin was exposed for 180 min at 50°C in solution state degradation experiments using acidic, basic, neutral and peroxide conditions. Results: HPLC was used to detect the degradation products and HPLC-Mass Spectroscopy (LC/ MS/MS) was used to characterize them. Major peaks at 293.0, 281.70, 224.90, 221.50, 180.90, 145.90 and 104.90 were visible in the LC-MS data. It was found that the canagliflozin formulation which was marketed was labile under stress conditions which included neutral, oxidative, alkaline and acidic. Conclusion: The deteriorated samples were analyzed using the LC-MS/MS technique and potential structures were allocated in accordance with the drug's known reactivity.

Keywords: Canagliflozin, Degradation, Kinetics, LC-MS/MS, Stability.

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INTRODUCTION

The family of Sodium Glucose-Transport protein (SGLT2) inhibitors includes canagliflozin. The treatment of type-2 diabetes involves its usage. By blocking SGLT2, canagliflozin reduces the renal glucose threshold and prevents the kidneys from reabsorbing glucose. When treating type-2 diabetes, canagliflozin can be used as monotherapy or in combination with other medications. Canagliflozin's IUPAC name is (2S, 3R, 4R, 5S, 6R). 2-{3-[5-thiophen-2-ylmethyl]-(4-fluoro-phenyl) 4-Methylphenyl-6-hydroxymethyltetrahydro-pyran-3,4,5-triol, with a molecular weight of 444.52 g/mol and the formula $C_{24}H_{35}FO_5S$.

According to Martindale (2017),¹ and Nomura S *et al.*, (2010),² it is a white, crystalline powder that is soluble in methanol, acetonitrile, ethanol and dimethyl sulfoxide but insoluble in water. The various methods are reported for estimation of selected drug alone or in combination in bulk, formulation and plasma by Ultra-Violet spectrophotometry, High-Performance

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Liquid Chromatography and High-Performance Thin Layer Chromatography.³⁻⁶ Some of the HPLC methods reported for estimation of canagliflozin in combination with other drugs.⁷⁻⁹ The work published by Suma et al., utilizes mobile phases which are not compatible with MS system as MS requires volatile buffer during the operation preferably ammonium buffers and UV method reported by Kaur et al., is not capable of differentiating the degradants from parent nucleus. Focus on Canagliflozin over other SGLT2 inhibitors due to its well-established efficacy and safety profile in various clinical trials. For patients with Type 2 diabetes, heart failure and Chronic Kidney Disease (CKD), canagliflozin has important effects, particularly for individuals who are at risk for cardiovascular events or the advancement of renal disease. Its combined benefits of controlling blood glucose levels and enhancing heart and kidney outcomes are linked to its pharmacological relevance.10

The reported methods are stability indicating but no information is available on degradation kinetics at various hydrolytic conditions. Further, no methods have reported characterization of degraded samples using hyphenated techniques. The main objective of the current study is,

1. To develop stability-indicating RP-HPLC method for estimation of drug.

2. Evaluation of degradation kinetics and identification of the degraded product and their characterization using LC/MS/MS.

MATERIALS AND METHODS

Chemicals and Reagents

Pure standard of Canagliflozin (Assigned purity 99.98%) was obtained as a gift sample from Indoco Remedies Ltd., The gift samples were used as standard without further purification. The purity of drug was ascertained by checking the physical constant melting point of drug and Lambda max determination. HPLC grade water, Methanol and Acetonitrile (HPLC Grade), Ortho Phosphoric acid (HPLC Grade), Ammonium Acetate, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (GR Grade) was used throughout the experiment. Tablet formulation was procured from a local pharmacy with the labeled claim of 100 mg was used in the analysis.

Instruments

The UV-visible spectrophotometer used was Jasco V-630, Chromatographic studies were performed on Shimadzu HPLC 1100 series equipped with isocratic pump LC-10ADVP, PDA-SPD M20A detector set at 291 nm. Lab Solutions software (Version) was used for data acquisition and system suitability calculations. Shimadzu LC-MS/MS-8040 equipped with PDA-SPD M20A used to analysis of degraded samples. An electronic analytical weighing balance (0.1 mg sensitivity, Shimadzu AU 220 and Analytical CAS-44) and a sonicator (PCI Mumbai, model No. 3.5L 100H) were used during the overall study. Calibrated glassware was used for the whole experimental work.

Chromatographic Conditions

High-performance liquid chromatography was employed, together with a UV-vis detector SPD10A-10A VP and a pump LC-10AT VP (Shimadzu). On an ACE C18 column (150×4.6 mm, 5 μ m), an isocratic elution of the mobile phase containing acetonitrile and ammonium acetate buffer pH-4.5 in a 50:50% (v/v) ratio was carried out at a flow rate of 1.0 mL/min. At 291 nm, the effluent was detected. Canagliflozin showed a retention period of 4.633 min. 20 μ L of injection volume was used and the column temperature was kept at room temperature (28-30°C). The column was equilibrated with the mobile phase for 30-40 min prior to the injection of an analyte.

LC-MS/MS conditions

An Agilent 1110-LC system was connected to a Shimadzu LC-MS/MS-8040 outfitted with a PDA-SPD M20A and an Electrospray Ionization (ESI) source interface for LC-MS analysis. The LC gradient program and the mobile phase were identical to the HPLC chromatographic conditions. In order to let 0.2 mL of eluent into the mass spectrometer, the flow rate was split at the

column exit and kept at 1 mL/min. The MS was operated with the following parameters and it was recorded in both positive and negative electrospray ionization modes: 10 min for data capture; the scan starts at 100 m/z and ends at 1000 m/z.

Preparation of Standard solutions

A standard stock solution of 100 μ g/mL of canagliflozin was prepared by dissolving in methanol. The standard stock solution was appropriately diluted with methanol to get the final concentration of 30 μ g/mL of CANA.

Preparation of Mobile phase

The mobile phase was prepared by mixing Ammonium Acetate buffer (pH-4.5) and Acetonitrile in ratio 50:50 v/v. Each mobile phase was sonicated.

Stressed Degradation Studies By HPLC

Solution state analysis

The canagliflozin sample was studied for its acidic hydrolysis, alkaline hydrolysis, oxidative hydrolysis and neutral hydrolysis at 50°C.

General procedure for preparation of sample

The required quantity of CANA was transferred to different volumetric flasks. Acidic reagent (1N hydrochloric acid), alkaline reagent (1M sodium hydroxide), 10% hydrogen peroxide and neutral distilled water were used to degrade it. The oven was set to 50°C and samples were taken out of the flasks every 30 to 180 min. The solution was produced with diluent and had a final concentration of 40 µg/mL of CANA (as stated on the label).

Kinetics of Solution State Degradation Studies

A specific rate of degradation under particular circumstances (temperature, humidity, exposure to light), which could help ascertain how rapidly the drug may lose its effectiveness or turn unsafe over time.^{11,12}

In order to ensure that the drug stays effective for as long as feasible, manufacturers and regulatory agencies can establish suitable expiration dates and storage conditions (such as suggested temperatures or light protection) by having a thorough understanding of the kinetics.

The findings may also have an impact on how the drug is packaged, stored, or administered to patients and healthcare professionals in order to preserve its effectiveness and safety.

The kinetics of the degraded sample were evaluated for all hydrolytic conditions. The plot of the Regression coefficient (r) obtained and the best fit observed indicates the order of degradation reaction.

Under the different conditions, where the degradation of marketed formulation occurred, the kinetics of degradation fitted graphical

models of zero; first and second orders were constructed. After having submitted to various hydrolytic conditions like acidic, alkali, oxidative and neutral at 50°C, a reduction of drug concentration in a marketed formulation according to the time of exposure to stress could also be observed.

Assay

Preparation of sample

After weighing, twenty tablets were pulverized. Powder containing 10.0 mg of CANA was weighed and then transferred to 100.0 mL of a volumetric flask. Enough diluent (methanol) was added to the flask and the volume was adjusted with the diluent after 15 min of sonication. The flask's contents were filtered using 0.45 μm filter paper. Using diluent (30 $\mu g/mL$), a 3.0 mL portion of the filtered material was further diluted to 10.0 mL. Chromatograms were collected following the injection of each of the 5 sample solutions after the stationary phase had equilibrated.

Validation of proposed method

Accuracy

Based on recovery studies carried out using the standard addition method, the accuracy of the suggested methodology was determined.¹³ The assay drug was mixed with the standard drug at three different concentrations, ranging from 50 to 150%, as suggested in analytical method validation guidelines and the sample solution was prepared using the same methodology.¹⁴

The amount of drug recovered was calculated from the expected total amount of drug.

Precision/Intermediate precision

Replicate analysis of homogeneous tablet samples was used to determine the accuracy of the CANA estimation provided by the proposed method. The methodology was used to carry out drug estimation in the samples both within and between days.

Linearity and Range

The Linearity studies show linearity of Area under the Curve of drug between 80 and 120% of the tablets' label claim. The percent label claim vs area under the curve Plot for the drug in formulation reveals a linear relationship with a correlation coefficient that is extremely near to 1.15

Ruggedness

The tablet samples were analysed using the proposed method by two different analysts and the sample preparation was done as described earlier under assay.

Detection Limit and Quantification Limit

The Limit of Detection (DL) and Limit of Quantification (QL) of the method were evaluated for Canagliflozin by evaluating

the standard deviation and slope data from the calibration curve parameter. DL and QL were found to be 1.54 μg and 4.68 μg respectively.

RESULTS

Method development and optimization of chromatographic conditions

A mobile phase consisting of 65:35% v/v of acetonitrile and ammonium acetate buffer pH 4.0 at a flow rate of 1 mL/min was tried on Ace 100-5-C18 column. It was observed that the peak eluted with low intensity and poor resolution at high concentration in this mobile phase. Next, a mobile phase of 65:35% v/v acetonitrile and ammonium acetate buffer pH 4.5 was tested on the same column at a flow rate of 1 mL/min. The peak was eluted Peak, widening was noted. A mobile phase of 50:50% v/v acetonitrile and ammonium acetate buffer pH 4.5 was attempted at a flow rate of 1 mL/min to increase resolution. In an isocratic mode of elution, good, sharp peaks with a reasonable retention time were obtained.

The chromatographic conditions mentioned above were used to perform the system suitability analysis and the findings were found to be within the limitations. In the concentration range of 10-50 μ g/mL, the standard drug showed linearity with a 0.9973 regression coefficient. The results of the recovery study are shown in Table 1.

The assay results are shown in Table 2. The method was validated for the parameters accuracy, precision intermediate precision, ruggedness, linearity and range of the sample as per the guidelines and the results are recorded in Table 2.

Linearity and Range

The data recorded using the HPLC analysis was plotted as Percent of Label claim against the AUC at each level and regression coefficient was estimated. The correlation efficient was found to be 0.9997 which passes the test as per ICH guidelines (r²>0.999).

Solution state degradation Studies of Canagliflozin

The drug was found to be susceptible to acidic conditions at 50°C temperature, with no substantial degradation in the standard drug and 17.88% in the marketed formulation, according to acid hydrolysis (Figure 1a). The alkali hydrolysis (1N NaOH), was done at 50°C, was found that the drug, in their marketed formulation, was 18.72% susceptible to alkali conditions (Figure 1b). In the case of oxidative hydrolysis, it was found that the drug was susceptible to degradation around 13.14% in the marketed formulation at 50°C (Figure 1c). The drug was found to be susceptible to degradation in neutral conditions at 50°C temperature to around 15.04% in the marketed formulation, according to the chromatogram (Figure 1d). The observations for degradation studies are depicted in Table 3.

Table 1: Observation and Results of Recovery study.

SI. No.	Recovery level (%)	Weight of Tablet taken (mg)	AUC (μV) Sample	Total Amount Recovered (mg)	% Recovery
1.	50	21.2	976256	5.01	100.02
2.	100	21.3	1296058	9.92	99.20
3.	150	21.2	1648222	15.35	100.0
Mean					99.74
±SD				0.382	
% RSD					0.384

Table 2: Results of Assay and validation parameters.

Parameters	Mean*	±SD	%RSD
System Suitability (AUC)	658553	0.99	1.01
Assay (%)	100.42	0.36	0.36
Intermediate Precision	99.90	0.51	0.51
Intraday (%)	100.24	0.62	0.62
Interday (%)	99.32	0.61	
Ruggedness	99.95	0.64	0.62
Analyst to analyst (%)			0.64

^{*}each is a mean of five observations.

Characterization of Degradation Product by LC-MS/MS Method

In acid stressed sample at 50° C for 180 min, the LC-MS chromatogram recorded for the degradation peaks is shown in Figure $2a.^{16}$ The fragments recorded at m/z values were 378.05, 293.0, 221.50 and 104.90 at a retention time of degradation peak in positive ionization scan shown in Figure $3a.^{16-19}$

In the alkali stressed sample at 50°C for 180 min, the LC-MS chromatogram recorded for the degradation peaks is shown in Figure 2b. The fragments recorded at m/z values were 401.0, 293.0, 266.05, 224.90, 145.90 and 104.85 at a retention time of degradation peak in positive ionization scan shown in Figure 3b.

In the oxidative stressed sample at 50°C for 180 min, the LC-MS chromatogram recorded for the degradation peaks is shown in Figure 2c. The fragments recorded at m/z values were 354.05, 338.0, 293.0, 281.70, 221.85 and 105.1 at the retention time of degradation peaks in the positive ionization scan shown in (Figure 3c).

In a neutral stressed sample at 50°C temperature for 180 min, the LC-MS chromatogram recorded for the degradation peaks is shown in Figure 2d. The fragments recorded at m/z values were 400.70, 354.05, 293.10, 274.90, 237.65, 224.70 and 104.85 at a retention time of degradation in positive ionization scan shown in (Figure 3d).

The retention times of degradation products obtained under various stress conditions are shown in Table 4.

The predicted degradation pathway for acidic, alkaline, oxidative and neutral hydrolysis is depicted in Figures 4a-d.

From the observation and results of degradation of various hydrolysis at 50°C in the oven, the graphs were plotted, regression coefficients were noted, and the different order of kinetics were obtained. The order of degradation kinetics was decided based on the best fit model. The rate constant and half-life of the drugs were also calculated from the obtained data using the formulas which correlate rate of reaction with half-life and shelf life. The results are shown in Table 5.

DISCUSSION

The system's suitability was performed using the above chromatographic conditions, the results were found to be within the limits. Both the upper and lower limits of analytical performance are intended to be tested with these concentrations. A method exhibits robustness and ensures that it is dependable all through a broad range of values, not just the nominal one, if it can measure accurately at 50% and 150%. Slight differences in intraday and interday precision would not have a substantial impact on the analysis's overall reliability, it's crucial to recognize the possibility of minor inaccuracies. It is crucial to maintain an eye on these differences in routine analysis to make sure they stay within acceptable limits, particularly when the same procedure is carried out repeatedly over time. Minor differences could be due operators' variability or instrument drift. Issues like repeatability problems, matrix interferences, or sensitivity, which they arise during method development were resolved with standard

Table 3: Observation for Degradation Studies.

Sl. No.	Stress Parameter	Stress Condition	Time	Temp	% Un-degraded
1.	Acidic	1N HCl	180 min		82.12
2.	Basic	1M NaOH			81.28
3.	Oxidative	$10\% \ H_2O_2$		50°C	86.86
4.	Neutral	Distilled water			84.69

Table 4: LC-MS/MS data of Retention time at 50°C.

Conditions	Drug Peak Retention time (in min)	Degradation Peak Retention time (in min)			
		DP-I	DP-II	DP-III	
Acidic	4.491	3.561	-	-	
Alkali	4.473	3.511	-	-	
Oxidative	4.519	3.539	6.468-6.685	9.216	
Neutral	4.715	7.245	7.606	-	

Table 5: Results of the order of kinetic, rate constant and Half-life estimation.

SI. No.	Condition	Order	Rate Constant	Half-Life
			(k)	(t _{1/2})
				(min)
		50°C	50°C	50°C
1	1 N HCl	First	4.3×10 ⁻⁵	1611.62
2	1 N NaOH	Zero	1.47×10 ⁻¹	339.02
3	Oxidative	First	3.8×10 ⁻⁵	1823.68
4	Neutral	First	1.8×10 ⁻⁵	3850.0

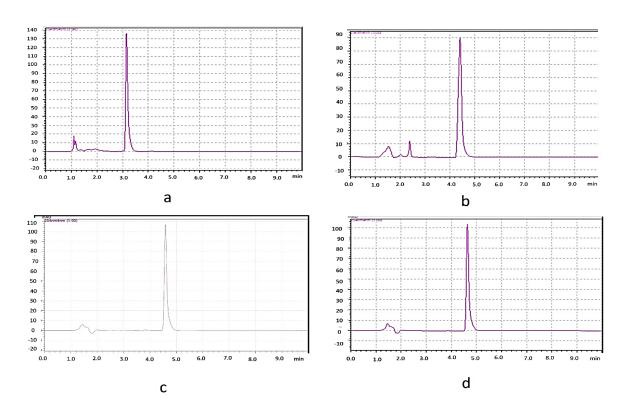


Figure 1: HPLC Chromatogram of sample canagliflozin under stress at 50°C. a) Acidic b) Alkaline c) Oxidative d) Neutral.

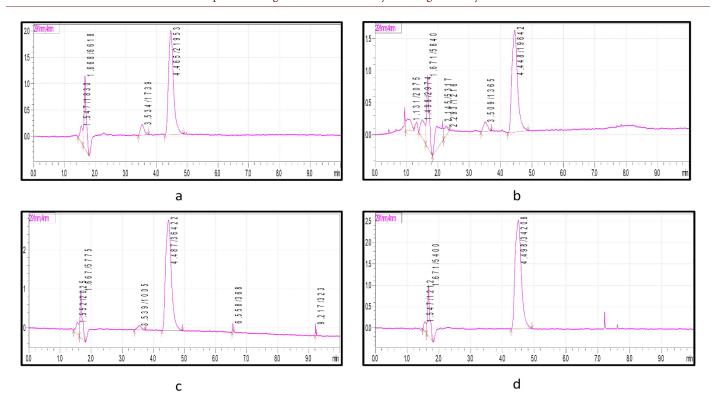


Figure 2: LC-MS Chromatogram of canagliflozin sample. a) Acidic b) Alkaline c) Oxidative d) Neutral.

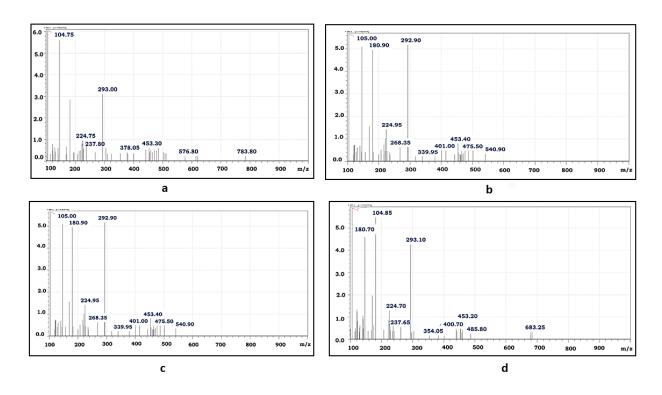


Figure 3: LC-MS/MS-ESI data of Canagliflozin and its degradation product under. a) Acid hydrolysis b) Alkaline hydrolysis C) Oxidative hydrolysis d) Neutral hydrolysis.

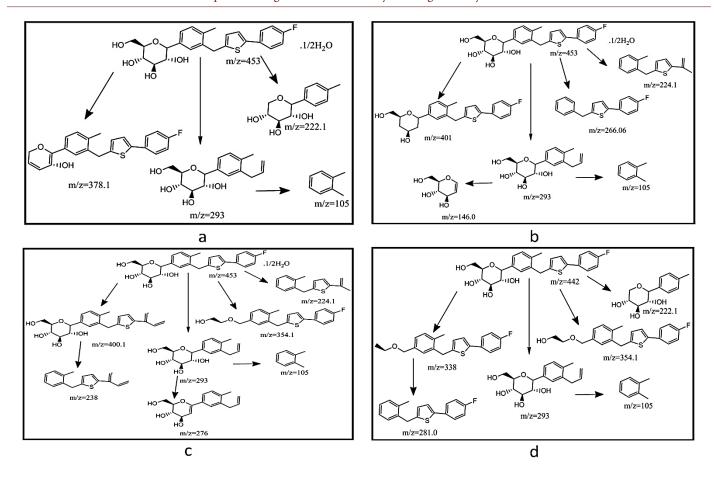


Figure 4: Fragmentation pattern depiction of canagliflozin under. a) Acidic hydrolysis b) Alkaline hydrolysis C) Oxidative hydrolysis d) Neutral hydrolysis.

techniques and modifications. The observed results should have confirmed that the degradation mechanisms were consistent with known chemical behaviours, as the predictions based on the drug's chemical understanding matched the fragmentation patterns and degradation pathways.

CONCLUSION

From the above results obtained by RP-HPLC and LC-MS/MS analysis of the samples, it can be concluded that the proposed method was successfully applied for its assay, degradation (stress testing) of the drug and degradation kinetics in the solution state. The method was found to be accurate, precise, rugged and robust. Further, the hyphenated technique was successful in identifying the degradants under the final chromatographic parameters selected and used during the experimentation. From the LC-MS/MS data, degradant structures were depicted. Hence the proposed method can be applied for an overall analysis of the selected drug.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None.

ABBREVIATIONS

CANA: Canagliflozin; **RP-HPLC:** Reverse Phase High Performance Liquid Chromatography; **LC-MS/MS:** Liquid Chromatography Mass Spectroscopy.

SUMMARY

A mobile phase comprised of 50:50% v/v acetonitrile and ammonium acetate buffer pH 4.5 for proposed HPLC method which was further extended in LC-MS/MS analysis as well. The stressed samples under acidic, alkaline, oxidative and neutral condition showed the presence of degraded product, which were well separated from the drug peak i.e. canagliflozin. The generated degraded peaks were further analysed by LC-MS/MS, to identify the products formed. Fragmentation pattern of the drug was depicted for various stress condition from the data obtained from degradation product ions in LC-MS/MS under different stress

conditions. The method was validated for different validation parameters for its accuracy, precision, linearity, ruggedness, robustness, LOD and LOQ, all the parameters were found to be with the acceptance criteria in terms of percent RSD values as per the guidelines.

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