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Development and Validation of a Stability-Indicating High Performance Thin Layer Chromatography (HPTLC) Method for estimation of Canagliflozin in bulk and Pharmaceutical Dosage Form

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ABSTRACT

The objective of the study was to develop and validate simple, authentic and stability indicating high performance thin-layer chromatographic method for determination of Canagliflozin in bulk and pharmaceutical formulations as per ICHQ2 R1 Guidelines. HPTLC aluminium plates Precoated with Silica Gel $60F_{254}$ using Toluene: Ethyl acetate: Methanol (2:2:1, v/v/v) as mobile phase were used for the chromatographic separation and it was validated with different parameters such as Linearity, Precision, Accuracy, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ). Also, Forced degradation study was carried out in different mediums. The densitometric analysis of the spots was performed at 290 nm. A Linear data over the range of 10-500ng/spot with a good correlation coefficient of 0.9988 unfolds linear relationship between area and concentration in calibration curve. The LOD and LOQ were found to be 0.39 and 1.19 respectively. A recovery of Canagliflozin in tablet formulation was observed in the range of 99.04-99.82%. Percentage assay of Canagliflozin tablets (INVOKANA®) was found to be 99.8%. Forced degradation studies of canagliflozin showed the degradation in acidic, alkaline, photolytic and oxidation but were most stable in thermal degradation. The proposed method is definite, meticulous and reproducible and can be used for routine analysis of Canagliflozin in bulk and pharmaceutical dosage form.

INTRODUCTION

Canagliflozin is an oral selective Sodium-Glucose co-transporter 2 (SGLT2) inhibitor used for the management of type 2 Diabetes Mellitus (Elkinson *et al*., 2013). The chemical name (IUPAC) of Canagliflozin is (2S,3R,4R,5S,6R)-2-{3-[5-(4-fluoro-phenyl)-thiophen-2-ylmethyl]-4-methyl-phenyl} -6-hydroxymethyltetrahydro-pyran-3,4,5-triol with molecular formula $C_{24}H_{25}FO_5S$. It is white to off white solid with melting point of 95-105ºC. It is soluble in many organic solvents (methanol, Dimethyl sulfoxide) but insoluble in aqueous media. It curbs the transporter protein SGLT2 present in the proximal tubules of the kidney which curtails renal glucose absorption, thereby increasing urinary glucose excretion and lowering blood glucose levels.

It is a product of Mitsubishi Tanabe Pharma and Janssen Pharmaceuticals, a division of Johnson and Johnson (Neumiller *et al*., 2010; Singh *et al*., 2015; Song *et al*., 2014). As per the Literature Survey, it is revealed that the drug has been estimated by Liquid chromatography (Iqbal *et al*., 2015) and Ultra High Performance Liquid Chromatography-Mass Spectroscopy (UHPLC-MS) (Iqbal *et al*., 2015) in biological fluids like human and rat plasma. Bulk drug and its marketed formulation has been analyzed by Ultraviolet Spectroscopic but no High Performance thin layer Chromatography analysis has been reported for the estimation in bulk and pharmaceutical dosage forms (Kaur *et al*., 2015) .

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The aim and objective of the present work was to develop and validate a stability indicating simple, precise, sensitive high performance thin layer chromatography method for Canagliflozin in its bulk and tablet dosage form and validate as per International Conference on Harmonization (ICH) Q2 (R2) guidelines. (Validation of Analytical Procedures: Text and Methodology Q2 (R1), Geneva, 2005)

MATERIALS AND METHODS

Instrumentation

The HPTLC system (CAMAG, Switzerland) consisted of Linomat V auto sampler connected to a nitrogen cylinder, a twin trough chamber (20 × 10cm), a derivatisation chamber, a plate heater, TLC Scanner IV (Camag Muttenz, Switzerland), UV cabinet with dual wave length UV lamps and win CATS software were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose.

Chemicals and reagents

Analytically pure sample of Canagliflozin was obtained from Xi'an Kingsmart Group Co. Limited, Xi'an City, China and tablet formulation (INVOKANA®) was procured from Johnson & Johnson, New Delhi, India with labelled claim of 100 mg. Methanol was obtained from MERCK, Germany. Analytical reagent grade of Toluene and Ethyl acetate were purchased from Rankem, Mumbai, India.

Chromatographic conditions

Stationary phase: Precoated silica gel 60 F_{254} HPTLC aluminium plates (20 × 10 cm, 0.2mm thick).
Mobile phase: Toluene: Ethyl acetate: Methanol (2:2:1).
Saturation time: 30 minutes.
Wavelength: 290 nm.
Lamp: Deuterium

The HPTLC analysis was performed on Pre-coated Silica Gel 60 F_{254} HPTLC plates (20 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany). HPTLC plates were pre-washed with 10 mL of methanol and activated at 80ºC for 5 min before application of sample. The standard and formulation samples of Canagliflozin were spotted using a Linomat 5 auto sampler fitted with a 100 μL Hamilton syringe (CAMAG, Muttenz, Switzerland) and operated with settings of a band length of 3.5 mm; band distance of 7.2 mm; distance from the side of plate of 10mm; and distance from the bottom of the plate of 10 mm. The plates were developed to a distance of 70mm in a mobile phase consisting of Toluene: Ethyl acetate: Methanol (2:2:1 v/v/v) and development was carried out in twin trough chamber (20 x 10 cm) presaturated with the mobile phase. The developed HPTLC plates were air dried and densitometric scanning was performed on CAMAG TLC scanner III in absorbance mode equipped with WINCATS planar chromatography manager (version 1.4.6) software. The spots were analyzed at a wavelength of 290nm. The scanning of the spots was done at a rate of 20mm/s. Evaluation was performed using linear regression analysis via peak areas.

Preparation of solutions

Preparation of stock solution

Accurately weighed quantity of Canagliflozin (10 mg) was transferred to a 10 ml volumetric flask, dissolved and diluted up to the mark with methanol (Concentration:1000 µg/ml)(Skoog DA *et al*.,2007).

Preparation of standard working solution

It was prepared by taking 0.1 ml of stock solution into 1 ml volumetric flask and the final volume was made up with 0.9 ml of methanol (100 µg/ml) (Skoog DA *et al*.,2007).

Preparation of mobile phase

The mobile phase was prepared by mixing Toluene, Ethyl acetate and Methanol in the ratio of 2:2:1 v/v/v.

Preparation of sample solution for Force degradation studies

To assess the stability indicating property of the developed HPTLC method, stress studies were carried out under ICH recommended conditions. Forced degradation of Canagliflozin was carried 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 presence of its degradation products (Rajput DK *et al*., 2013).

Acid and alkali hydrolysis

Aliquot of 1 ml of Canagliflozin solution (10 mg dissolved in 10 ml i.e. 1 mg/ml) was transferred to a small round bottom flask and it was mixed with 9 ml of 0.1N hydrochloric acid or 0.1N sodium hydroxide. The prepared solutions were subjected to reflux for 2 h in a boiling water bath. The samples were cooled to room temperature (25ºC), neutralized with an amount of acid or base equivalent to that of the previously added. From the resulting neutral solution, 5µl/spot was applied at TLC plate with the help of applicator.

Oxidation

Aliquot of 1 ml of Canagliflozin solution (10 mg dissolved in 10 ml i.e. 1 mg/ml) was transferred to round bottom flask and it was mixed with 9 ml of 30% hydrogen peroxide solution, the reaction mixture was allowed to proceed at room temperature (25ºC) for 2 h with intermittent shaking. A volume of 5µl/spot was applied at TLC plate with the help of applicator.

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 10 ml water. The solution was claimed to have concentration of 1 mg/ml. A volume of 5µl/spot was applied at TLC plate with the

help of applicator. As well as, an aqueous solution of Canagliflozin (1 mg/ml) was exposed to UV light (254 nm) for 48 h, and after that a volume of 5µl/spot was applied at TLC plate with the help of applicator.

Thermal degradation

A sample powder of Canagliflozin (10 mg) was exposed to a temperature of 70ºC for 48 h in hot air oven. The material was dissolved in 10 ml methanol. The solution was claimed to have concentration of 1 mg/ml. A volume of 5µl/spot was applied at TLC plate with the help of applicator.As well as, an aqueous solution of Canagliflozin (1 mg/ml) was exposed to a temperature of 70ºC for 48 h, and after that a volume of 5µl/spot was applied at TLC plate with the help of applicator.

Preparation of sample solution for assay of marketed formulations

Twenty INVOKANA® tablets were weighed, average weight was calculated, and were triturated to fine powder. A powder proportionate to 10mg was taken in a 10ml volumetric flask to which meager amount of methanol was added. The flask is then ultra-sonicated for 15 minutes and volume is made up with methanol. The tablet solution is then filtered through whatman filter paper (No.41) and from the above solution, 1µl of solution containing 1000ng/band was applied on the HPTLC plate with the help of Linomat V auto sprayer in 3 replicates. The plate was developed and studied under earlier expressed chromatographic conditions.

Analysis of forced degradation samples

Analysis of the degraded sample solutions was done by applying 5µl with the help of auto sampler to same TLC plate in duplicate. The plate was developed, dried and analyzed as described earlier in chromatographic conditions.

Preparation of Calibration Curve

Aliquots of 0.1, 0.2, 0.4, 0.5,1,2,4 and 5µl of standard working solution of Canagliflozin were spotted on HPTLC plate using 100µL Hamilton syringe with the help of Linomat V auto sprayer to obtain a concentration of 10-500 ng/spot. The plate was developed, dried and analyzed at 290nm with the help of TLC Scanner 3 using Win-CATS software.

Sets of calibration curves were constructed and the area report was recorded. A plot of concentration versus area under curve was established (10-500 ng/spot).

RESULTS AND DISCUSSION

Analytical Method Validation

The developed method was validated for different parameters like linearity, precision, accuracy, specificity, ruggedness, robustness, LOD and LOQ as per ICH Q2A and Q2B guidelines (Validation of Analytical Procedures: Text and Methodology Q2 (R1), Geneva, 2005).

Linearity

Linear regression data over the range of 10-500ng/spot for Canagliflozin with a correlation coefficient of 0.9988 unfolds good linear relationship between area and concentration in calibration curve. Figure 1 presents calibration curve of Canagliflozin whereas Figure 2 and 3 displays Chromatograms of different Aliquots and overlay chromatograms of different aliquots at 290nm.

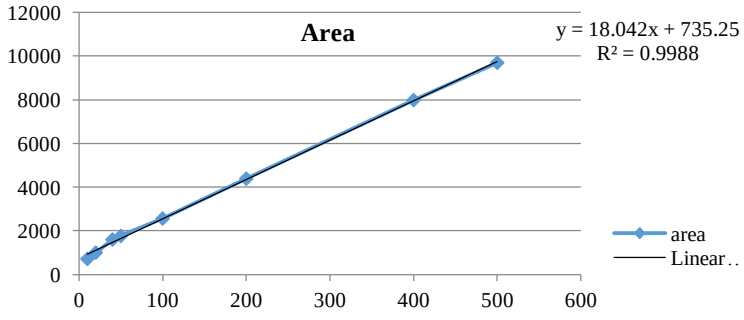
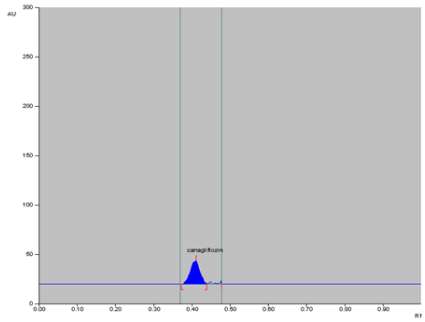
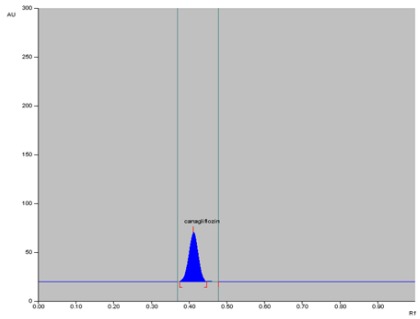


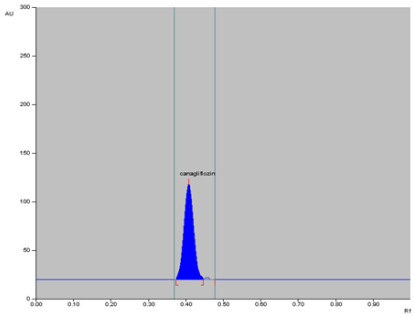
Fig. 1: Calibration Curve of Canagliflozin .



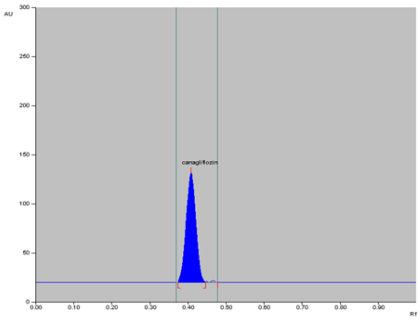
A-Chromatogram showing 10ng/spot at 290nm



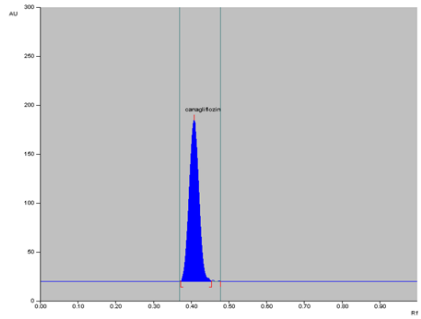
B- showing 20ng/spot at 290nm



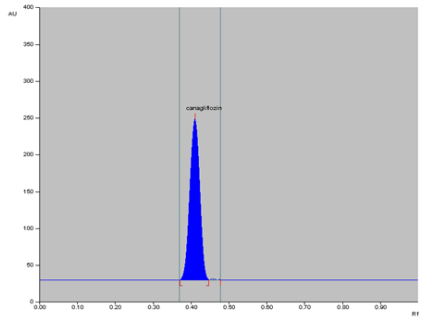
C-Chromatogram showing 40ng/spot at 290nm



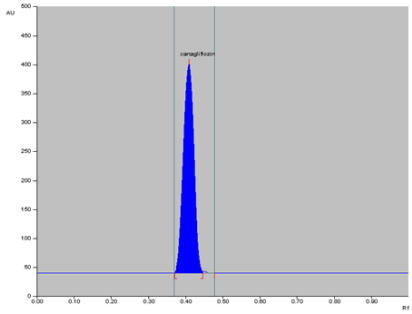
D-Chromatogram showing 50ng/spot at 290nm



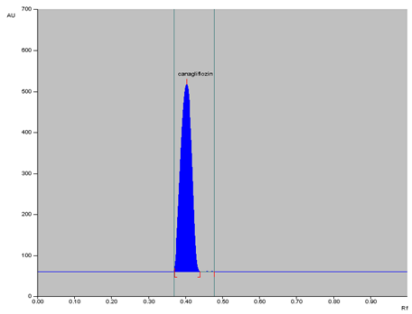
E-Chromatogram showing 100ng/spot at 290nm



F-Chromatogram showing 200ng/spot at 290nm



G-Chromatogram showing 400ng/spot at 290nm



H-Chromatogram showing 500ng/spot at 290nm

Fig. 2: Chromatograms (A-H) of different Aliquots at 290nm.

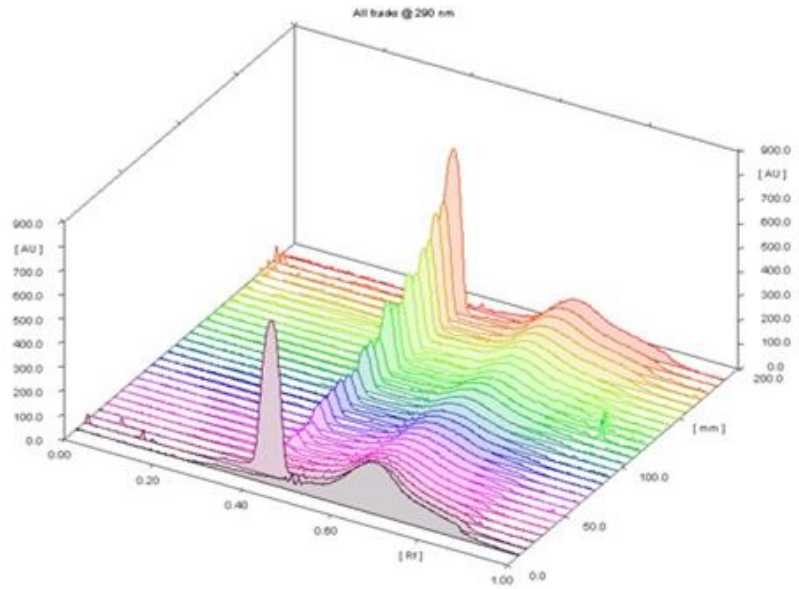


Fig. 3: Overlay Chromatograms of different aliquots at 290nm.

Table 1: Accuracy Studies of Canagliflozin.

Amount of sample taken (ng/spot)	Amount of standard added (ng/spot)	Percentage of Standard added	% Recovery	% Relative Standard Deviation
100	50	50	99.04	0.06
100	100	100	99.82	0.05
100	150	150	99.49	0.04

*Average of three determinations (n=3)

Accuracy

Accuracy of the method was resolved by standard addition method in which standard addition of pure API at three different concentration levels of 50%, 100% and 150% was performed in triplicate. Table 1 presents accuracy of the method in the terms of % recovery of the API.

Precision

Precision of the method was determined by evaluating intraday and interday precision. Intra-day and Inter-day variation was analyzed by selecting three concentrations which were 20, 100 and 400ng from linearity range. Intraday analysis was carried on same day whereas Interday analysis was carried on three different days in replicates of three. The respective peak areas for different concentrations were reported .Table 2 & 3 express precision data for the method in terms of % RSD.

Table 2: Intra-day Precision Studies of Canagliflozin.

Amount of Standard taken (ng/spot)	Peak Area (mAU)	% Relative Standard Deviation
Day-1 (Morning)		
20	998	0.2
100	2562.43	0.08
400	7983.33	0.01
Day-1 (Afternoon)		
20	997	0.1
100	2562.8	0.08
400	7983.62	0.02
Day-1 (Evening)		
20	998.33	0.15
100	2562.15	0.08
400	7983.19	0.01

Table 3: Inter-day Precision Studies of Canagliflozin.

Amount of Standard taken (ng/spot)	Area (mAU)	% Relative Standard Deviation
Day-1		
20	997.60	0.2
100	2561.39	0.08
400	7982.46	0.01
Day-2		
20	996.18	0.10
100	2563.46	0.06
400	7983.33	0.01
Day-3		
20	995.47	0.09
100	2563.55	0.07
400	7983.86	0.02

Specificity

The specificity of the method was evaluated by separating the peaks of both tablet and API. The spot of

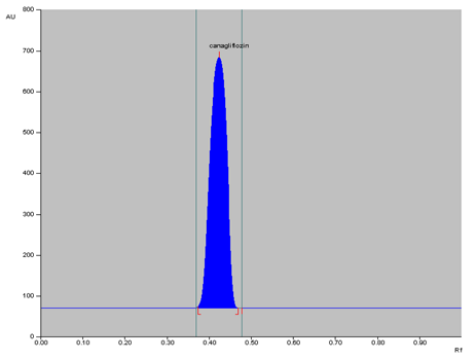


Fig. 4: Chromatogram of Canagliflozin Tablets (INVOKANA®) showing R_f = 0.44.

Canagliflozin in the sample was proved by matching the R_f and spectra of the standard spot. Therefore, the method was considered to be specific. Figure 4 shows the chromatogram of Canagliflozin tablets.

Robustness

Robustness of the method was ascertained by deliberately altering the chromatographic conditions. A change in the mobile phase composition and change in wavelength were selected as parameters and were varied separately whereas all other conditions were held constant as described in the method. No difference was observed in the peak area and R_f values. Table 4 indicates robustness of the method.

Table 4: Robustness studies of Canagliflozin.

Parameter	Area (mAU)	% Relative Standard Deviation
Change in Solvent system		
Toluene: Ethyl Acetate: Methanol (3:1:1)	2561.70	0.06
Toluene: Ethyl Acetate: Methanol (2:1:3)	2562.34	0.05
Change in Wavelength (±2nm)		
288nm	2564.32	0.05
292nm	2562.50	0.08

Ruggedness

Ruggedness of the method was performed by carrying out the method with the help of two different analysts. Table 5 explains the ruggedness of the method in terms of %RSD.

Table 5: Ruggedness studies of Canagliflozin.

Analyst	Area (mAU)	% Relative Standard Deviation
Analyst 1	2563.79	0.06
Analyst 2	2563.62	0.07

Detection limit and Quantification Limit

A limit of detection (LOD) and a limit of quantification (LOQ) were calculated according to the formula:

LOD = 3.3 σ/s

LOQ = 10 σ/s

Where, 'σ' is the standard deviation of 'y' intercept of regression line and 's' is the slope of the calibration curve.

The LOD and LOQ values were found to be 0.39 and 1.19 respectively

Assay of the Pharmaceutical Dosage Form

Table 6 displays that the %Mean recovery in formulation is 99.8 and %relative standard deviation is less than 2% which is within the limits. Figure 5 represents chromatogram of INVOKANA®.

Table 6: Assay of Canagliflozin Tablets (INVOKANA®).

Formulation	Labelled claim	Amount found (%)*	%Relative Standard Deviation
INVOKANA®	100gm	99.8	0.02

*Average of three determinations

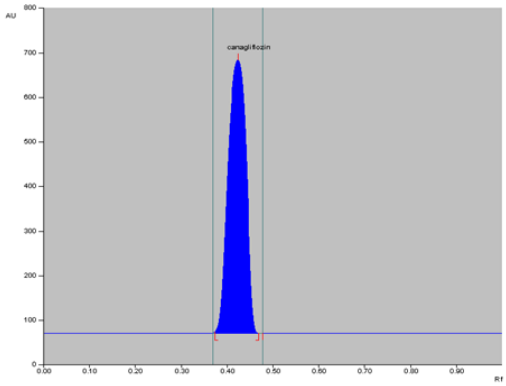


Fig. 5: Chromatogram of Canagliflozin Tablets (INVOKANA®).

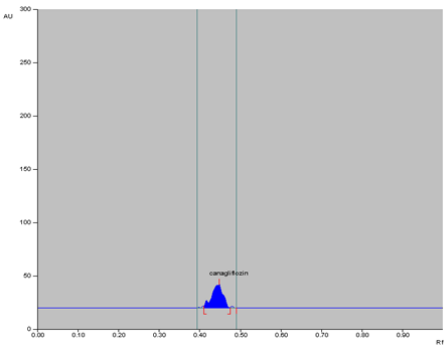
Analysis of forced degradation samples of Canagliflozin

From this forced degradation, it was clear that in case of thermal stability Canagliflozin was most stable under the employed stress conditions as shown in table 7. In case of acid hydrolysis, alkaline hydrolysis and oxidation degradation was observed and is shown in the respective chromatograms but maximum degradation was seen on irradiation with U.V. light. Nonetheless, the method was able to separate isolate the degradation products from the intact drug.

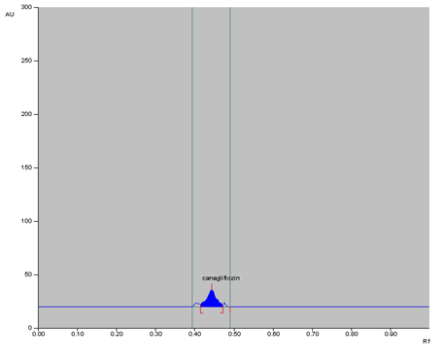
Table 7: Stability studies of Canagliflozin.

Sample	Concentration Used (µg/spot)	Concentration left after degradation (µg/spot)	% Recovery
Acid Hydrolysis	200	152.46	76.23
Alkaline Hydrolysis	200	159.68	79.84
Oxidation	200	143.78	71.89
Thermal	200	169.04	84.52
Photolytic	200	147.28	73.64

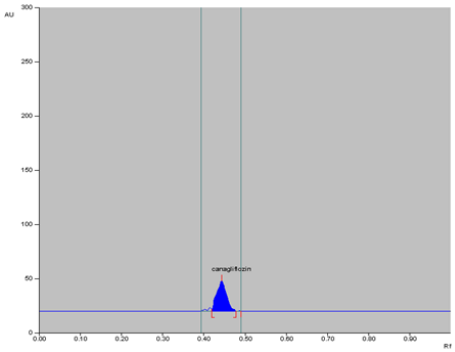
This confirmed the stability indicating property of the developed method. The concentration of the produced degradation products analogous to the intact Canagliflozin was determined and found to be 23.77%, 20.16%, 28.11%, 26.36%, 16.24% in case of acid hydrolysis, alkaline hydrolysis ,oxidation, photolytic and thermal stability respectively as shown in Table 7.Figure 6 presents chromatograms showing forced degradation of Canagliflozin.



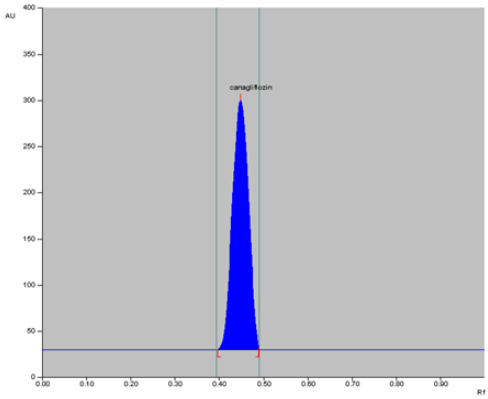
1-Chromatogram of Acid treated Canagliflozin.



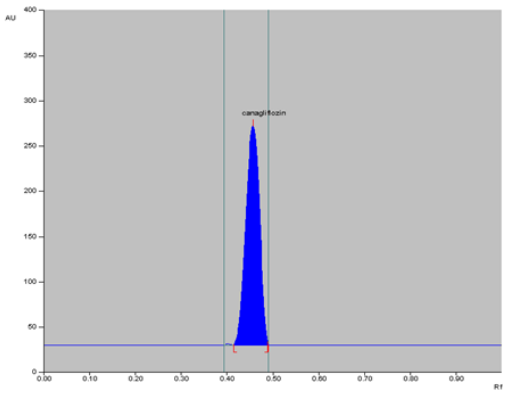
2-Chromatogram of Alkali treated Canagliflozin.



3-Chromatogram of Hydrogen Peroxide treated (Oxidative degradation) Canagliflozin.



4-Chromatogram of Thermally treated Canagliflozin.



5-Chromatogram of Photo treated Canagliflozin.

Fig. 6: Chromatograms (1-5) showing Forced Degradation of Canagliflozin

The method explained in the current work provides an agreeable, definite and meticulous way for analysis of Canagliflozin in bulk and pharmaceutical dosage form by high performance thin layer chromatography (Table 7). Development of TLC procedure was done for the determination of Canagliflozin. The mobile phase consisting of Toluene: Ethyl acetate: Methanol in the ratio of 2:2:1(v/v/v) culminated into favorable resolution, sharp and symmetrical peaks at R_f 0.41. Linear regression data over the range of 10-500ng/spot for Canagliflozin with a correlation coefficient of 0.9988 was unfolded by regression analysis. % RSD for all the parameters was found to be less than 2%. Percentage recovery for Canagliflozin was found within the range of 99.04-99.82%. The LOD and LOQ values were found to be 0.39 and 1.19 respectively. The assay for Canagliflozin was found to be 99.8 ± 0.02. Specificity of the proposed method was confirmed when the formulation was spotted on the HPTLC plates, developed and scanned and the excipients did not interfere with the sample peak. Table 8 portrays all the standard and validation parameters along with the results obtained.

Table 8: Summary of Standard & Validation Parameters.

Parameter	Result
Linearity Range	10-500 ng/spot
Slope	18.042 ± 0.001
Intercept	735.25 ± 0.01
Correlation coefficient	0.9988 ± 0.003
Intra-day Precision (n=3)	0.01-0.2
Interday Precision (n=3)	0.01-0.2
% Recovery (n=3)	99.04%-99.82%
Limit of Detection (LOD)	0.39
Limit of Quantification (LOQ)	1.19
Specificity	Specific

CONCLUSION

It is found that the developed HPTLC technique is quite simple, authentic, definite, reproducible, sensitive, favorable, specific and economical. It can become efficient analytical tool for routine quality control of Canagliflozin in bulk drug and its pharmaceutical dosage forms.

ACKNOWLEDGMENT

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