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Original Article

Development and Validation of Stability indicating UV Spectroscopic Method for Determination of Canagliflozin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Objective: To develop and validate simple, definite, stability indicating UV spectroscopic method for determination of Canagliflozin in bulk and pharmaceutical formulations as per ICH Q2 R1 Guidelines. **Methods**: Canagliflozin was subjected to different stress conditions as per ICH guidelineQ1A (R2). A stability-indicating UV Spectrophotometric method has been developed for analysis of the drug in the presence of the degradation products and is validated with different parameters such as Linearity, Precision, Repeatability, Limit of Detection (LOD), Limit of Quantification (LOQ), Accuracy, Robustness and Ruggedness. It involved a 2-h study in which methanol and distilled water were used as solvents. **Results**: Canagliflozin in methanol shows maximum absorbance at 290 nm. Beer's law was obeyed in the concentration range of 5-10 mcg/mL. The LOD and LOQ were found to be 0.084 mcg/ml and 0.255 mcg/ml respectively. A recovery of Canagliflozin in tablet formulation was observed in the range of 80.00-120.00%. Percentage assay of Canagliflozin tablets (INVOKANA®) was found to be more than 99%. Degradation of Canagliflozin was found to occur in acid, alkaline, hydrogen peroxide and photolytic conditions where as it was found to be thermally stable. The amount of degraded drug was calculated by taking absorbance at 290 nm. **Conclusion**: The proposed method is definite, meticulous, reproducible and can be used for routine analysis of Canagliflozin in bulk and pharmaceutical dosage form.

Key words: Canagliflozin, Method development, Validation, Ultraviolet Spectroscopy, Forced degradation.

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INTRODUCTION

Canagliflozin is an oral selective Sodium-Glucose co-transporter 2 (SGLT2) inhibitor used for the management of type 2 Diabetes Mellitus[1]. 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 $C_{24}H_{25}FO_5S$ (Figure 1). It is white to off white solid with melting point of 95-105°C.[2-4] 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.[4,5] It is a product of Mitsubishi Tanabe Pharma and Janssen Pharmaceuticals, a division of Johnson and Johnson and marketed with the brand name of INVOKANA® in strengths of 100 and 300 mg respectively.[6,7]

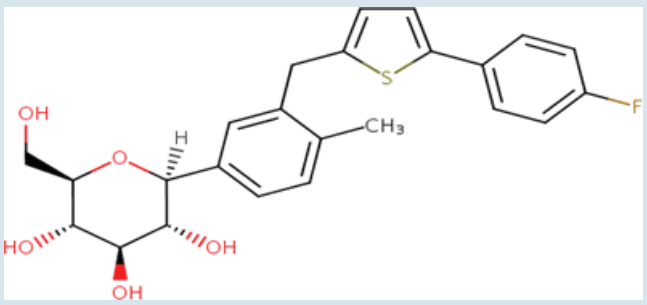

Figure 1: Chemical Structure of Canagliflozin.[5]

As per the Literature Survey, it is revealed that the drug has been estimated by Liquid chromatography[8,9] and Ultra High Performance Liquid Chromatography-Mass Spectroscopy(UHPLC-MS)[10] in biological fluids like human and rat plasma. But no UV-Spectroscopic method and Liquid Chromatography analysis has been reported for the estimation in bulk and pharmaceutical dosage forms.

The aim and objective of the present work was to develop and validate a simple, precise, sensitive spectroscopy method for Canagliflozin in its bulk and tablet dosage form.

MATERIALS AND METHODS

Instrument

A double beam UV-visible spectrophotometer (INCARP-SICAN 2301) consisting of two matched quartz cells with 1 cm light path and loaded with UV Solutions software (version 1.1) was used for recording and measuring of spectra and absorbance. An electronic analytical weighing balance (0.1 mg sensitivity, Shimadzu AU 220) and a sonicator (Sonica, model 2200 MH) were used in this study.

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 and Johnson, New Delhi, India with labelled claim of 100 mg. Methanol and Water was obtained from Merck Millipore, Germany.

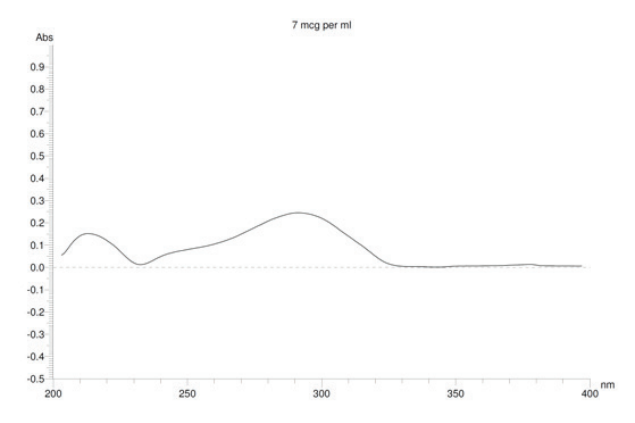

Figure 2: UV spectrum of the standard Canagliflozin.

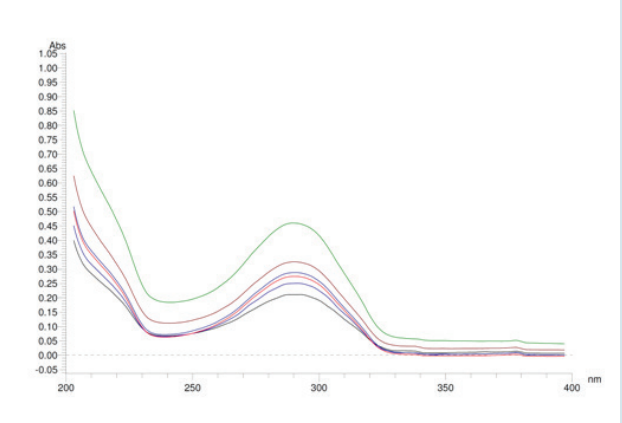

Figure 3: Overlay spectrum of the standard Canagliflozin at different concentrations.

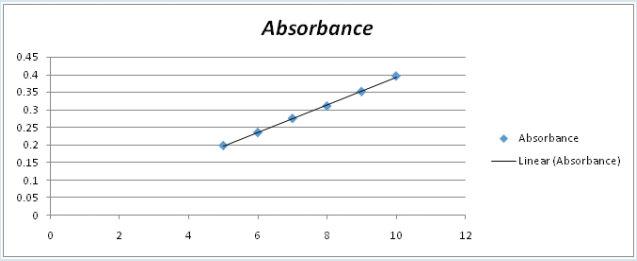

Figure 4: Linearity graph of Canagliflozin.

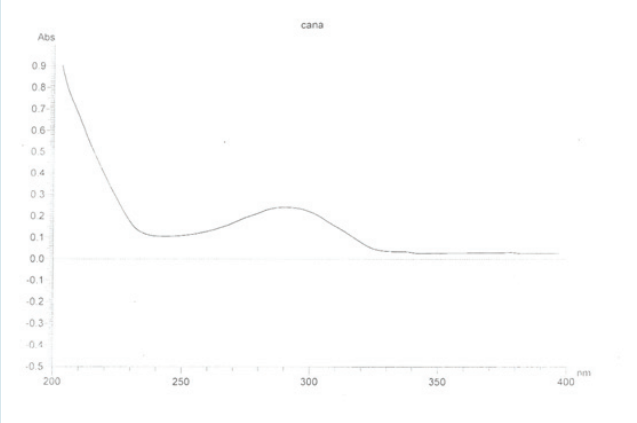

Figure 5: Degradation in acidic condition.

Selection of Wavelength[11]

Canagliflozin is soluble in organic solvents like Methanol and Dimethyl sulfoxide (DMSO) so Methanol was selected throughout the study. Canagliflozin 7 µg/ml of working standard solution was scanned in between 200 nm to 400 nm and showed maximum absorption at 290 nm by UV spectrophotometer (Figure 2).

Preparation of stock and working standard solution[11,12]

10 mg of Canagliflozin was accurately weighed and taken in 10 ml clean and dry volumetric flask. Drug was dissolved and diluted up to the mark using methanol. This was considered as the standard stock solution (1000 µg/ml). 10 ml of the stock solution was pipette out and made up to 100 ml to get a concentration 100 µg/ml and was treated as the working standard.

Preparation of calibration curve

From this stock solution, appropriate dilutions were made to get final concentration of 5, 6, 7, 8, 9 and 10 µg/ml and absorbance was taken at λ_{max} 290 nm (Table 1). Averages of such 5 sets of values were taken for standard calibration curve, and the calibration curve was plotted. Figure 3 illustrates overlay spectrum of canagliflozin at different concentrations.

Force degradation studies

To assess the stability indicating 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 presence of its degradation products.[16,17]

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. The solution was mixed with 9 ml of 0.1 N 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, 2 ml was taken in cuvette and absorbance was recorded.

Oxidation

One milliliter of Canagliflozin solution (10 mg dissolved in 10 ml i.e. 1 mg/ml) was transferred to 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 2 ml was taken in cuvette and absorbance was recorded.

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

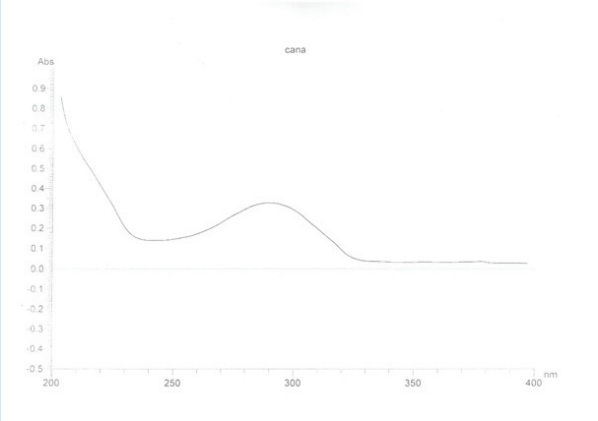

Figure 6: Degradation in basic condition.

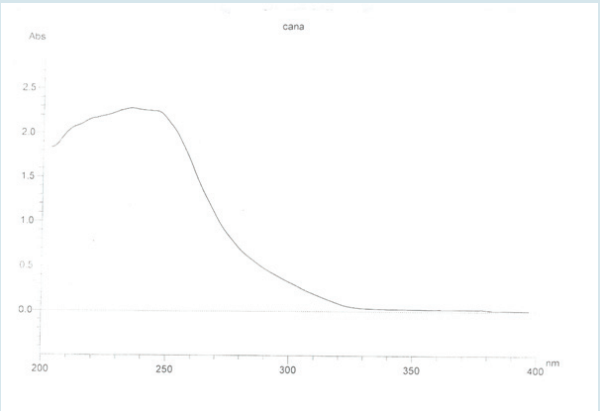

Figure 7: Degradation in hydrogen peroxide.

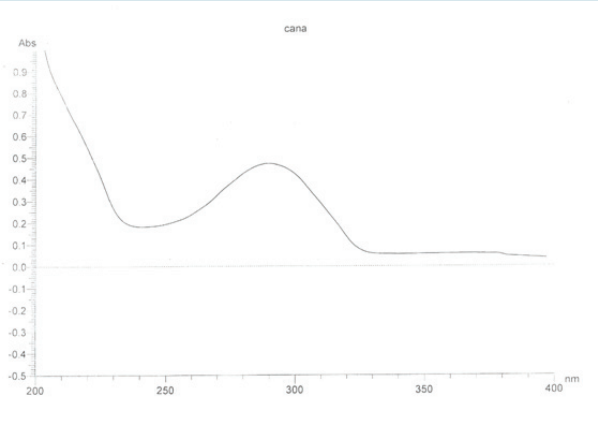

Figure 8: Degradation in thermal conditions.

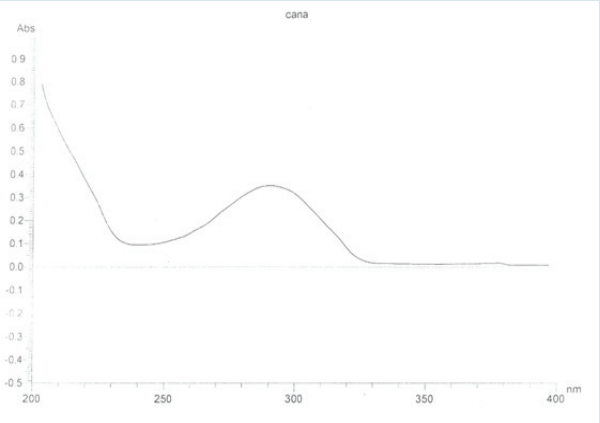

Figure 9: Degradation in sun light.

was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was suitably diluted and a volume of 2 ml was taken in cuvette and absorbance was recorded. As well, an aqueous solution of Canagliflozin (1 mg/ml) was exposed to UV light (254 nm) for 48 h, and after diluting, a volume of 2 ml was taken in cuvette and absorbance was recorded.

Thermal degradation

A sample powder of Canagliflozin (10 mg) was exposed to a temperature of 70°C for 48 h in hot air oven. The material was dissolved in 10 ml water. The solution was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was suitably diluted and a volume of 2 ml was taken in cuvette and absorbance was recorded. As well, an aqueous solution of Canagliflozin (1 mg/ml) was exposed to a temperature of 70°C for 48 h, and after diluting, a volume of 2 ml was taken in cuvette and absorbance was recorded.

RESULT AND DISCUSSION

Method development & Validation[11-14]

Solvents were analysed including Ethanol, DMSO, and Methanol at 1 mg/ml concentration. However, Canagliflozin was found to be soluble and stable for minimum of 1 h at room temperature using methanol and water. Therefore, this solvent was used for the determination of suitable

Figure 10: Primary and Secondary Package of Canagliflozin Tablet Dosage Form (INVOKANA®).

detection wavelength and working concentration of standard. In order to test the appropriateness of the developed method to the pharmaceutical formulation, an assay of INVOKANA® tablets 100 mg was performed at working concentration. Assay for working concentration of sample at 290 nm was in limits of acceptance (98-102%) using the solvent with the sonication method for 15 min. Hence, the determined method was optimized. Figure 4 represents linearity graph of canagliflozin.

Table 1: Absorbance at different concentrations

Concentration (µg/ml)	Absorbance
5	0.198
6	0.235
7	0.275
8	0.310
9	0.351
10	0.395
R^2=0.9989	
y= 0.0391x + 0.0009	

Table 2: Results of System Precision

n	Absorbance
1	0.391
2	0.393
3	0.395
4	0.396
5	0.397
6	0.398
Average	0.395
Standard Deviation	0.002
% Relative Standard Deviation (RSD)	0.66

Table 3: Results of Method Precision (Intraday)

Concentration (µg/ml)	Sample Absorbance	Mean Absorbance ± S.D.	% RSD
5	0.195 0.196 0.192	0.194 ± 0.002	1.07
6	0.237 0.232 0.234	0.234 ± 0.002	1.07
7	0.271 0.274 0.273	0.272 ± 0.001	0.56

Table 4: Results of Method Precision (Interday)

Concentration (µg/ml)	Sample Absorbance	Mean Absorbance ± S.D.	% RSD
5	0.193 0.195 0.191	0.193 ± 0.002	1.30
6	0.236 0.233 0.234	0.234 ± 0.001	0.65
7	0.272 0.275 0.274	0.273 ± 0.001	0.55

Table 5: Results of Accuracy

Level (%)	Absorbance	% Recovery	Mean % Recovery	% RSD
80	0.244	98.57	98.77	0.62
80	0.243	98.25		
80	0.246	99.50		
100	0.273	99.41	99.90	0.55
100	0.274	99.78		
100	0.276	100.51		
120	0.301	102.33	102.21	0.50
120	0.302	102.67		
120	0.299	101.65		

Table 6: Results of Ruggedness

Analyst	Sample Absorbance	Mean Absorbance ± S.D.	% RSD
Analyst 1	0.391 0.394 0.395	0.393 ± 0.002	0.52
Analyst 2	0.397 0.399 0.396	0.397 ± 0.001	0.38

Table 7: Results of Robustness

Wavelength (in nm)	Sample Absorbance	Standard Absorbance	Mean Absorbance ± S.D.	% RSD
289	0.410 0.408 0.413	0.395	0.410 ± 0.002	0.61
291	0.420 0.418 0.415	0.397	0.417 ± 0.001	0.60

Table 8: Summary of Optical Characteristics and Validation Parameters

Parameters	Result
Detection wavelength (nm)	290
Beer's Law limits (µg/ml)	5-10
Regression equation (y=mx+c)	0.0391x+0.0009
Correlation coefficient (r^2)	0.9989
Slope (m)	0.0391
Intercept (c)	0.0009
Precision (% RSD)	
Intra-day (n=9)	0.56-1.07
Inter-day (n=9)	0.55-1.30
Accuracy (% Mean Recovery)	
80 % Level	98.77
100 % Level	99.70
Ruggedness 2 Analysts (% RSD)	≤ 2
Robustness Wavelength (±2 nm) (% RSD)	≤ 2

Table 9: Stability studies of Canagliflozin

Sample	Concentration used (µg/ml)	Concentration left after degradation (µg/ml)	% Recovery
Acid Hydrolysis	360	280.26	77.85
Alkaline Hydrolysis	500	371.65	74.33
Oxidation	830	594.94	71.68
Photolytic	100	70.95	70.95
Thermal	100	83.76	83.76

Table 10: Result of Assay of Pharmaceutical Formulation (INVOKANA®)

Concentration (µg/ml)	Absorbance ± S.D.	% RSD	% Recovery* (Amount found)
7	0.274 ± 0.001	0.55	99.7

*mean of three determinations

International Conference on Harmonization (ICH) has provided guidelines i.e. Q2 (R1) for validation of analytical method which defines this process as characteristic performance that is established by laboratory studies.[12] Also, this process meets the requirements for intended analytical application. UV spectrophotometric method developed was validated according to guidelines for validation of analytical procedures. The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/intermediate precision, ruggedness and robustness.[15]

Precision

System precision

Six replicate recording of absorbance at 290 nm of 10 µg/ml concentration standard solution showed % RSD (Relative Standard Deviation) less than 2, which indicates acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 2.

Method precision

Method precision was determined by performing assay of sample under the tests of (i) repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision) performed during 2 consecutive days by two different analysts, at different working concentrations. Results of Intraday and Interday precision are shown in Table 3 and 4.

Accuracy

Accuracy was determined by performing recovery experiments in which determination of % mean recovery of sample by percentage method at three different levels (80-120%, *viz* 6.3, 7, 7.7 µg/ml). 80 to 120% of the sample solutions were prepared as per the procedure given in the methods from the dilutions used for linearity (7 µg/ml).

At each level, three analyses were performed. Percent mean recovery was calculated as shown in Table 5**.** The accepted limits of recovery are 98%-102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Ruggedness

Ruggedness was determined by performing the same proposed method on different instrument. Also, method was carried out by two different analysts and by performing the method on different days to check the reproducibility which showed % RSD less than 2 and indicates that the method developed is rugged (Table 6).

Robustness

Robustness was determined by performing the same proposed method on different wavelengths. The analysis showed % RSD less than 2 and indicates that the method developed is robust (Table 7).

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

The LOD and LOQ were calculated based on the standard deviation of the response (y intercepts of regression lines) and the slope using 3 independent analytical curves, as defined by ICH. Canagliflozin LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of Y intercept (ICH guidelines) and S is the slope of the Canagliflozin calibration curve. The LOD and LOQ were found 0.084 µg/ml and 0.255 µg/ml respectively. Table 8 shows summary of the validation parameters.

Stability indicating study

The ICH guideline characterized stability testing of drug substances and products requires the 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 or source sample. Vulnerability to oxidation, hydrolytic, photolytic and thermal stability are the required tests. An ideal stability indicating method is one that not only evaluates the standard drug alone but also resolves its degradation products.

From the forced degradation, it was observed that in case of acid hydrolysis, alkaline hydrolysis and oxidation degradation was observed and is shown in the respective chromatograms (Figure 5-7) but in case of thermal stability canagliflozin was most stable under the employed stress conditions as shown in Figure 8. Maximum degradation was degradation was seen on irradiation with U.V light (Figure 9). Nonetheless, the method was able to isolate completely the degradation products from the intact Canagliflozin.

This confirmed stability indicating property of the proposed method. The concentration of the produced degradation products analogous to the intact Canagliflozin was calculated and found to be 22.15%, 25.65%, 28.32%, 29.05%, 16.24% in case of acid hydrolysis, alkaline hydrolysis, oxidation, photolytic and thermal stability respectively (Table 9).

Analysis of marketed formulation

The validated method was applied to the determination of Canagliflozin in Tablets. The validated method was applied to the determination of Canagliflozin in Tablets (Figure 10). Twenty tablets were assayed and the results are shown in (Table 10) indicating that the amount of drug in tablet samples was in good agreement with the label claim of the formulation as indicated by % recovery (99.70%).

CONCLUSION

It could be concluded that the developed method for estimation of Canagliflozin in pharmaceutical dosage form and in bulk is simple definite, reproducible, and economical. The values of accuracy, precision, robustness, ruggedness, LOD and LOQ were within the limits. Canagliflozin is very sensitive so it is unstable in alkaline, acidic, oxidative, photo light but is thermally stable. Statistical analysis for the results clearly demonstrate that the method is suitable for the determination of Canagliflozin in bulk and tablet forms without any interference from the degradation products, and it is endorsed for routine use in quality control industry laboratories.

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CONFLICT OF INTEREST

Authors report no conflicts of interest in this work.

ABBREVIATIONS USED

UV: Ultraviolet, **LOD:** Limit of Detection, **LOQ:** Limit of Quantification, **SGLT2:** Sodium Glucose co-transporter2, **UHPLC-MS:** Ultra High Performance Liquid Chromatography-Mass Spectroscopy, **DMSO:** Dimethyl sulfoxide, **µg:** microgram, **ICH:** International Conference on Harmonization, **RSD:** Relative Standard Deviation.

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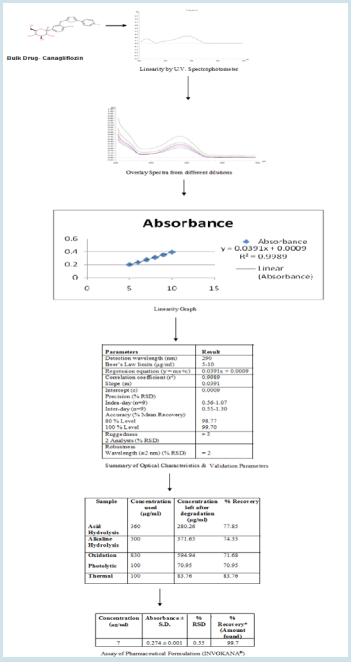
PICTORIAL ABSTRACT



SUMMARY

- Canagliflozin is the novel SGLT2 inhibitor with excellent clinical results on humans.
- As per the literature review, there is no stability indicating U.V. method on the drug except a developed U.V. Method by Kaur *et al*.
- A U.V. spectrophotometric method along with its stability with various agents is developed and validated as per ICH guidelines.
- The Absorbance of the prepared samples was analyzed at 29 0nm with excellent linearity.
- Analysis of Pharmaceutical Dosage form showed the percentage recovery of 99.7%.

ABOUT AUTHORS

Ishpreet Kaur: Is a post graduate student at the Department of Quality Assurance, Delhi Institute of Pharmaceutical Sciences & Research, New Delhi affiliated from University of Delhi. Her research focuses on Development of various analytical techniques for determination of novel SGLT2 inhibitor in bulk and dosage form. She has published more than 8 publications in international journals.

Harsharan Pal Singh: Has completed his B.Pharm from Amity University (India) in the year 2014. He had been working as a Quality Control Analyst and Research Associate in Formulation & Development Department of AIMIL Pharmaceuticals (I) Limited. Presently, he is pursuing his post-graduate studies in the field of Quality Assurance at Institute of Food Processing Technology, Conestoga College Institute of Technology and Advanced Learning, Kitchener, Canada. He also has core knowledge of Clinical Research and Pharmacovigilance. Moreover, he is certified with Professional Diploma in Clinical Research and Professional Certificate in Pharmacovigilance. He has published 10 papers in reputed journals and presented more than 20 posters as author and co-author in Conferences of International and National repute.

Dr. Sharad Wakode: Obtained his PhD degree in 2004 from Rajiv Gandhi Prodyogiki Vishwavidyalaya under the supervision of Prof. S.G.Kaskhedikar. Currently, he is positioned as Associate Professor at the Department of Pharmaceutical Chemistry, Delhi Institute of Pharmaceutical Sciences & Research (now known as Delhi Pharmaceutical & Research University), New Delhi. Dr. Wakode is working on various research projects in the field of pharmaceutical chemistry sponsored by esteemed agencies such as DST and AICTE. Also, he is a part of editorial board of several journals of international and national repute.