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Original Article

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

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ABSTRACT

Objective: To develop and validate simple, sensitive, precise, rapid and cost effective method for determination of Canagliflozin in bulk and pharmaceutical formulations as per ICH Guidelines. **Methods:** A simple double beam UV Spectrophotometric method has been developed and validated with different parameters such as Linearity, Precision, Repeatability, Limit of Detection (LOD), Limit of Quantification (LOQ), Accuracy, Robustness and Ruggedness. **Results:** Canagliflozin in methanol shows maximum absorbance at 290 nm. Beer's law was obeyed in the concentration range of 5-10 mcg mL-1, 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%. **Conclusion:** The proposed method is precise, accurate and reproducible and can be used for routine analysis of Canagliflozin in bulk and pharmaceutical dosage form.

Keywords: Canagliflozin, Method development, Validation, Ultraviolet Spectroscopy.

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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 300mg respectively.[6,7]

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.

METHOD AND MATERIALS

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

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 290nm by UV spectrophotometer (Figure 2). To confirm the following analysis, an overlay spectrum using different concentrations was plotted (Figure 3).

Preparation of stock and working standard solution

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.[11,12]

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

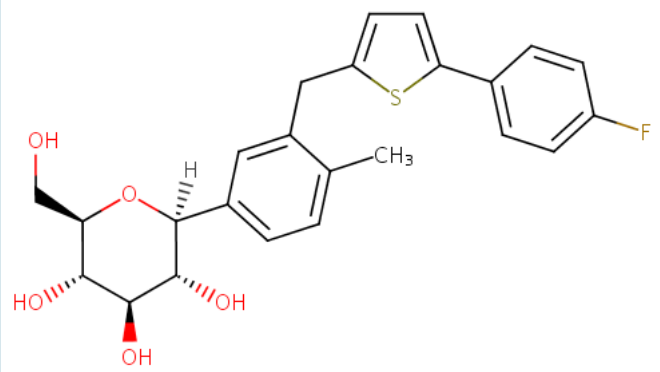

Figure 1: Chemical Structure of Canagliflozin[5]

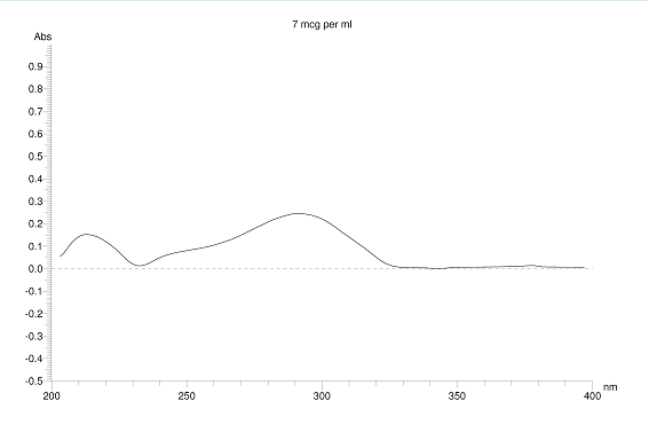

Figure 2: UV spectrum of the standard Canagliflozin

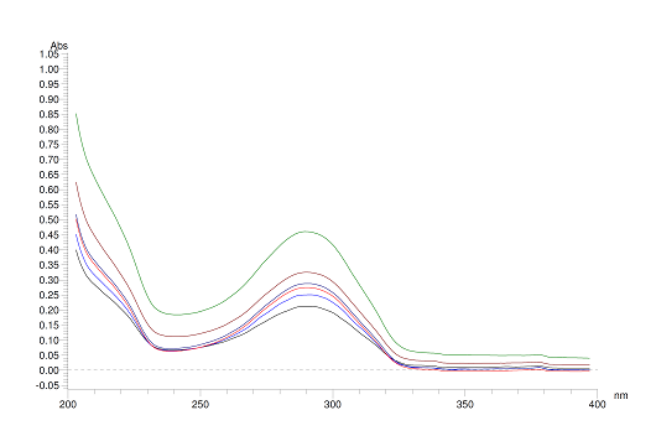

Figure 3: Overlay spectrum of the standard Canagliflozin at different concentrations

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.

RESULT AND DISCUSSION

Method development & Validation

Solvents were analysed including Ethanol, DMSO, and Methanol at 1 mg/ml concentration. However, canagliflozin was found to be soluble and stable

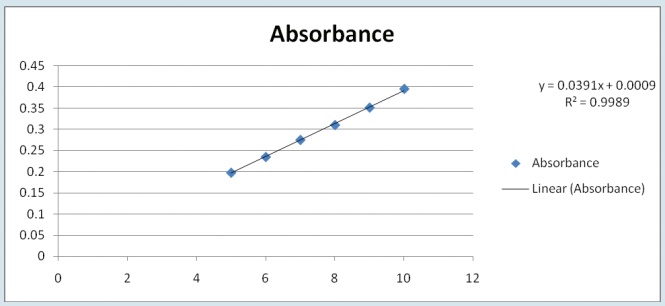

Figure 4: Linearity graph of Canagliflozin

Figure 5: Primary and Secondary Package of Canagliflozin Tablet Dosage Form (INVOKANA®)

for minimum of 1 hour at room temperature using methanol and water. Therefore, this solvent was used for the determination of suitable 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 100mg 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 minutes. Hence, the determined method was optimized. Figure 2 illustrates UV spectrum for the sample.

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.[11-14]

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) (Table 3) and (ii) Intermediate precision (Interday precision) (Table 4) performed during 2

consecutive days by two different analysts, at different working concentrations.

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).

Table 1: Absorbance at different aliquots

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		
100	0.274	99.78	99.90	0.55
100	0.276	100.51		
120	0.301	102.33		
120	0.302	102.67	102.21	0.50
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

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

Table 8: Summary of Optical Characteristics & 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)	
Indra-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: 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

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.

Analysis of marketed formulation

The validated method was applied to the determination of Canagliflozin in Tablets. Twenty tablets were assayed and the results are shown in (Table 9) 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 sensitive, accurate, precise, reproducible, and economical. The proposed method can be used for routine quality control analysis of Canagliflozin in bulk and pharmaceutical formulation.

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ABBREVIATION 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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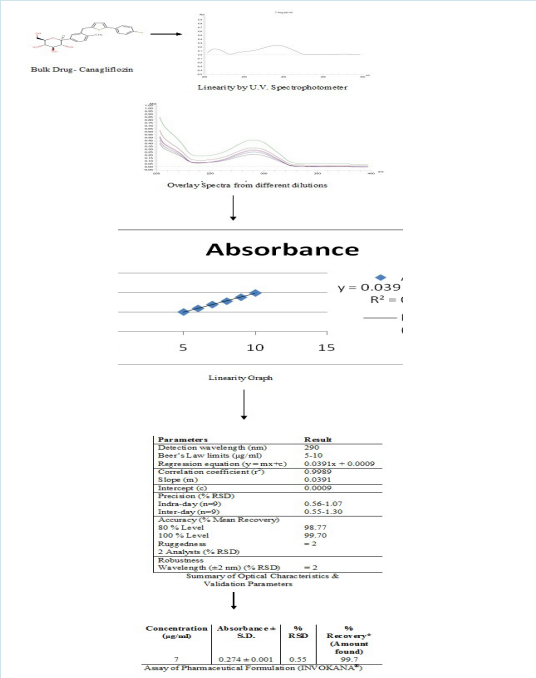
SUMMARY

- Canagliflozin is the novel SGLT2 inhibitor with excellent clinical results on humans.
- As per the literature review, there is no developed analytical method on the drug.
- An economical and easy U.V. spectrophotometric method is developed and validated as per ICH guidelines.
- The Absorbance of the prepared samples was analyzed at 290nm with excellent linearity.
- Analysis of Pharmaceutical Dosage form showed the percentage recoevery of 99.7%.

PICTORIAL ABSTRACT



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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.