DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CANAGLIFLOZIN IN BULK & PHARMACEUTICAL FORM

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ABSTRACT

To develop and validate a simple, precise and cost-effective UV- visible spectrophotometric method for the estimation of Canagliflozin. All the parameters of the analysis were chosen and validated as per ICH Q2 (R1) guideline. Canagliflozin solution was scanned over entire UV-visible range for its wavelength of maximum absorbance. Various calibration standards of Canagliflozin were prepared and absorbance was recorded at its wavelength. Calibration curve of concentration vs. absorbance was plotted and linearity and range were calculated. Various analytical method validation parameters viz. accuracy, precision, LOD, LOQ, robustness and ruggedness were calculated using QC standards. The maximum wavelength of Canagliflozin was found to be 288 nm. The correlation coefficient over the concentration range of 1-25 µg/ml was found to be 0.9998. Developed UV method was found to be precise during the intra-day and inter-day study and showed percent relative standard deviation in the range of 0.34 to 1.44 & 0.072 to 1.44 respectively. The total percent recovery of canagliflozin was found to be 99.48 to 100.52 %. Developed method was found to be robust and rugged for the intended use. Canagliflozin content of marketed formulation was successfully calculated using developed UV-Visible method.**:** A simple, precise and cost-effective UV- visible spectrometric method for the estimation of canagliflozin was developed. The said method was developed using economical percentage of organic phase in aqueous media as solvent. Said validated UV- visible method can be efficiently used for the estimation of canagliflozin in bulk as well as formulation.

Keywords

UV- visible spectrometry, canagliflozin, method development, Validation, Dissolution,

I. INTRODUCTION

Canagliflozin is an antidiabetic drug and is chemically (2S,3R,4R,5S,6R)-2-{3-[5-(4-fluorophenyl)-thiophen-2-ylmethyl]-4-methyl phenyl} 6hydroxymethyltetrahydro-pyran-3,4,5-triol (Figure 1) [1-4]. Canagliflozin belongs to class a of drugs known as sodium-glucose co-transporter 2 (SGLT2) inhibitors which improves glycemic control in patients with type 2 diabetes. Canagliflozin shows its therapeutic property by lowering blood glucose level by simultaneously acting on kidney to decrease the renal threshold for glucose (RTG) and increased urinary glucose excretion (UGE). [5-10]

Literature review revealed that there are several analytical methods like HPLC, LS-MS, HPTLC and RP-HPLC and only few UV-Spectroscopic methods were reported for the estimation of canagliflozin in bulk and pharmaceutical dosage forms. Hence the present work aimed at the development and validation of a simple, precise, sensitive UV spectrophotometric method for the estimation of Canagliflozin in its bulk and pharmaceutical dosage form. [11-19]

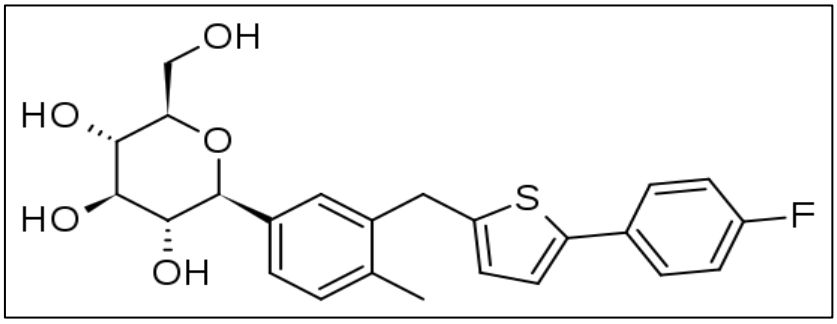

Fig1: Chemical structure of Canagliflozin

II.MATERIAL AND METHODS

Canagliflozin was purchased from TCI chemicals (India) Pvt. Ltd, Chennai. Methanol was purchase from Merck. All the chemicals of analytical grade were used for the proposed study.

Instrument used

A double beam UV-visible spectrometer (UV-530, Jasco) with spectra manager software was used for the analysis. Quartz cells having a 3 cm length with 1 cm path length were used for spectral measurement. Weighing balance (Essae, Vibra HT) with internal calibration mode was used for the accurate weighing purpose.

Preparation of standard stock solution

Accurately weighed 5 mg of canagliflozin was transferred into the calibrated volumetric flask and dissolved in 5 ml of Methanol to achieve a stock solution of 1000 µg/ml Primary Stock (Stock-I). Further, Stock-I was diluted suitably with a co-solvent system of Methanol: water (50:50) to get the working stock of 100 µg/ml (Stock -II).

Determination of wavelength of maximum absorbance (λ_{max})

Stock-II solution was scanned using full scan mode for the entire range of UV and visible i.e., 800 to 200 nm with a co-solvent system of Methanol and Water as a blank. After obtaining the spectrum, λ_{max} was identified with the help of software. To achieve reproducible results, the above method was repeated five times.

Preparation of calibration curve

The calibration curve was prepared by diluting the stock-II solution to achieve the seven different calibration standards representing CAL STD 1 –(1µg/ml), CAL STD 2 – (2µg/ml), CAL STD 3 – (4µg/ml), CAL STD 4 –(8µg/ml), CAL STD 5 – (12µg/ml), CAL STD 6 – (20µg/ml) & CAL STD 7 – (25µg/ml) strength. The absorbance of each calibration standard was measured at pre-identified λ_{max} 288 nm using fixed wavelength measurement mode. The calibration curve representing concentration vs. absorbance was plotted. Above mentioned procedure was repeated five times so as to ensure reproducibility.

Method Validation

Developed UV method for canagliflozin estimation was validated as per the ICH guideline. Different validation parameters like linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantitation (LOQ) for the proposed method were evaluated. [20-21]

Linearity and Range

The linearity of the proposed UV method was established using seven different calibration standards. Based on the analysis of calibration standards, calibration curves in terms of absorbance vs. concentration plots were developed and subjected to linear least square regression analysis. The correlation coefficient value was considered to be an important factor for establishing the linearity of the proposed method. The interval between the upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Accuracy:

The accuracy of the proposed UV method was evaluated using recovery studies after standard addition of analyte of interest. To the predefined concentrations, different amounts of canagliflozin were added (standard addition method) so as to achieve level of 80%, 100% and 120% of canagliflozin and the accuracy was calculated on the basis of percent recovery. For calculating the percent recovery following formula was used.

$$\% RC = (SPS-S/SP) \times 100$$

Where,

SPS = Amount found in the spiked sample

S = Amount found in the sample

SP = Amount added to the sample

% RC = Percent recovery

Precision:

The precision of the proposed UV method was established by performing intra- and inter-day UV analysis of QC standards. The study was performed at three concentration levels. Intraday precision study was carried out by preparing solutions of QC STD 1 – (1.5µg/ml), QC STD 2 –(12µg/ml), QC STD 3– (24.5µg/ml) strength of canagliflozin (3 solutions of each concentration) and analyzing the same at morning, afternoon and evening time of same day. Deviation in the results was calculated in terms of % Relative Standard Deviation (% RSD). Similarly, inter-day precision study was carried out by analyzing the above-mentioned solutions at three consecutive days.

Robustness:

Robustness of proposed method was determined by changing the working wavelength of canagliflozin (288 nm) by ±1nm and ratio of methanol: water (50:50) by ±1ml. Middle level quality control sample of canagliflozin (12 µg/ml) was prepared and analysed at ±1 nm wavelength working wavelengths. The results were defined in terms of % RSD.

Ruggedness:

Ruggedness study of the method was carried out by analyzing triplicate samples of canagliflozin (12 µg/ml) using two different instruments namely V-530, Jasco and BA-UV-2600, Bioage located at SAIF and Herbal Drug Technology laboratories of Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. In order to establish the extensive ruggedness of the proposed method, same study was carried out by two different analysts. Results were denoted in terms of % RSD.

Limit of Detection (LOD):

The LOD of the developed UV method was calculated by using following formula

LOD=3.3×SD/S

Where, SD= Standard deviation of Y-intercepts

S= Slope

Limit of Quantitation (LOQ):

The LOQ of the developed UV method was calculated by using following formula

LOQ= 10×SD/S

Where, SD= Standard deviation of Y-intercepts

S= Slope

Estimation of canagliflozin content in marketed formulation:

The canagliflozin content in its marketed formulations (Brand 1- Invokana and Brand 2-vokanamet) was estimated using pre-validated UV-Visible spectrophotometric method. Tablet formulation contents (labeled strength: Brand 1-100µg/tablet and Brand 2- 100µg/tablet) were dissolved in 1 ml of co-solvent system to achieve a stock solution of 100 µg/ml (n=5) and obtained solution was filtered through 0.45 µm syringe filter. Said solution was suitably diluted with co-solvent system and analyzed for the canagliflozin content using proposed UV method.

In vitro drug release studies:

In vitro drug dissolution testing of marketed tablets of canagliflozin (formulation I with strength of 100 mg per tablet) and (formulation II with strength of 50 mg per tablet) was performed in phosphate buffer PH 6.8 as dissolution media (900ml) using USP type II apparatus at 50 RPM for formulation I and at 100

RPM for formulation II with sampling intervals of 5, 10, 15, 20, 25, 30, 45 and 60 min. The temperature of dissolution media was maintained at 37°C±0.5°C. An aliquot of 5ml was withdrawn at above metioned sampling time intervals and drug content was determined by UV-spectrometer at 288nm.

III. RESULTS AND DISCUSSION

Determination of wavelength of maximum absorbance:

Identification of wavelength of maximum absorbance is prerequisite for quantitative UV analysis. Solution representing absorbance value less than 1 is generally considered to be suitable for the determination of wavelength of maximum absorbance. Considering the prerequisite and the suitability, determination of maximum wavelength for canagliflozin solution was carried out using full scan mode of UV-Visible spectrophotometer. Full scan was processed using UV software and the λ_{max} was identified with the help of software. It was found to be 288 nm for canagliflozin (Fig.2).

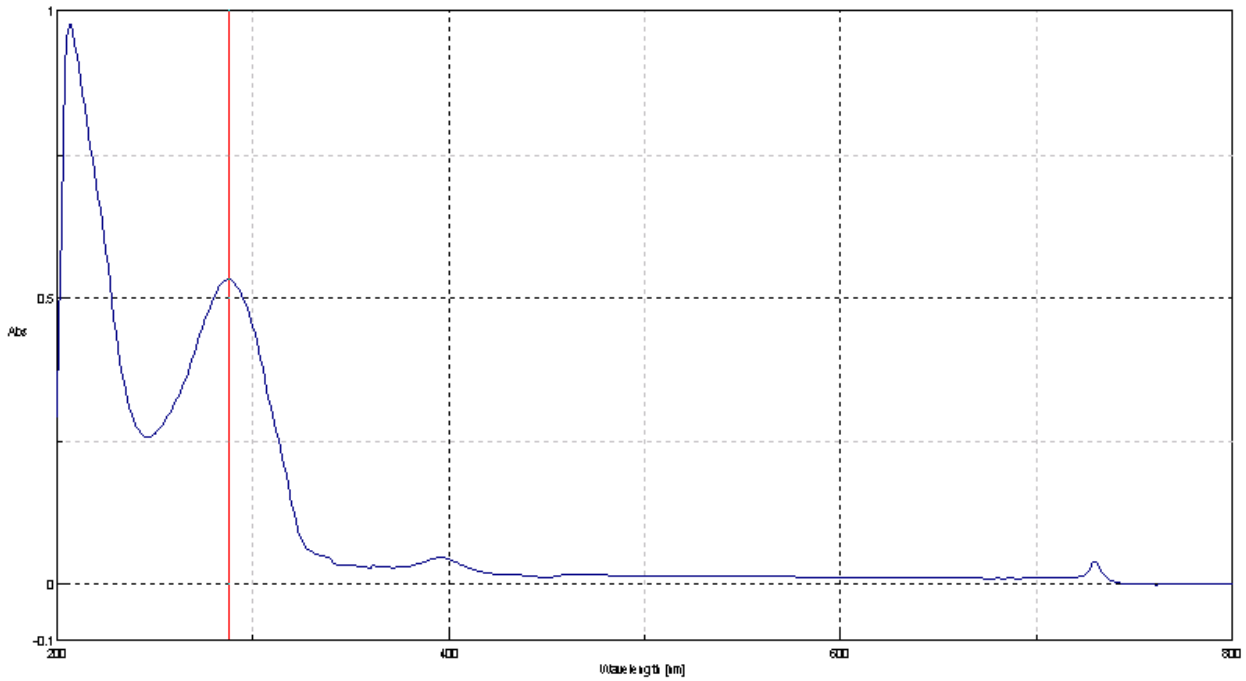

Fig. 2: UV-visible spectra of canagliflozin

Preparation of calibration curve:

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating correlation between concentration

and the response. As compare to graphical method, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of canagliflozin, calibration curve for canagliflozin was developed using seven different calibration standards. The absorbance of different calibration standards at 288 nm was recorded using fixed wavelength mode of UV-Visible spectrophotometer. Calibration curve was repeated five times and the mean values ± deviation was reported as shown in Table 1.

Table 1: Details of Calibration standard used for canagliflozin

Concentration (µg/ml)	**Absorbance**
1(µg/ml)	0.0897±0.0011
2(µg/ml)	0.1292±0.0006
4(µg/ml)	0.2124±0.0004
8(µg/ml)	0.3677±0.0007
12(µg/ml)	0.5145±0.0014
20(µg/ml)	0.8194±0.0016
25(µg/ml)	0.9994±0.0011

Method validation

Linearity and Range:

Linearity and range are the key parameters of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, seven-point calibration curve of canagliflozin covering a range of 1µg/ml to 25µg/ml was plotted. Details of concentrations and the respective mean absorbance values are depicted in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; $y = 0.0379x + 0.0575$ with correlation coefficient 0.9998 as shown in Figure 3.

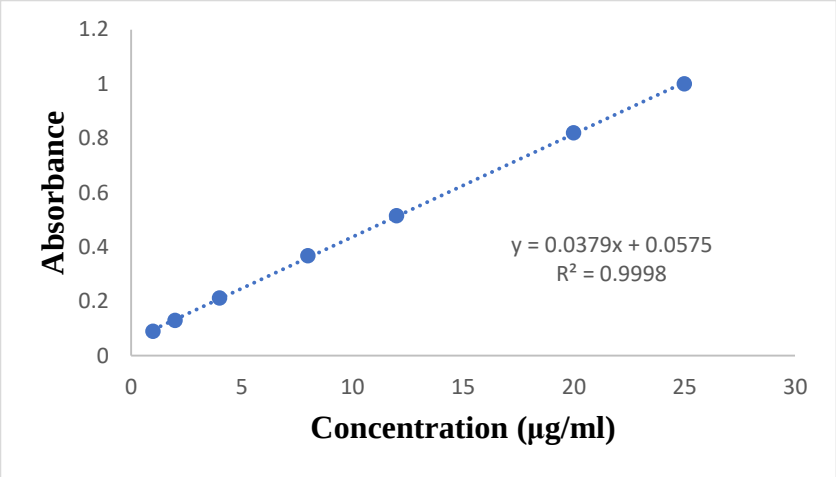

Fig. 3: Calibration curve for canaglilozin

From the linearity study, it was revealed that, developed UV method was linear in the pre-defined concentration range of calibration standards.

Accuracy:

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. In case of proposed UV method for canagliflozin, accuracy was established using recovery studies. At 80 % standard addition, mean recovery of canagliflozin was found to be 99.48% whereas at 100 and 120 % standard addition, it was found to be 100.07 and 100.52% respectively. % RSD was found to be less than 2 for the canagliflozin recovery studies as shown in Table 2.

Table 2: Accuracy data of UV method for canagliflozin.

Concentration (%)	Origin level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80	1.5	1.2	100.03	99.48	0.9789
	1.5	1.2	98.35		
	1.5	1.2	100.07		
100	12	12	100.14	100.07	0.4250
	12	12	99.61		
	12	12	100.46		
120	24.5	29.4	99.90	100.52	1.0659
	24.5	29.4	101.75		
	24.5	29.4	99.90		

From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 98 to 102% and the % RSD was well below 2%.

Precision:

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at, 1.5µg/ml, 12µg/ml and 24.5µg/ml levels of canagliflozin. The results in terms of mean absorbance values, percent assay and % RSD for the intra- and inter-day precision study are demonstrated in Table 3 and Table 4 respectively.

Table 3: Intra-day precision data of UV method for canagliflozin

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.5	102.15	1.42	1.54	102.39	1.44	1.53	102.24	1.28
12	12.18	101.52	0.072	12.11	100.95	0.846	12.04	100.35	0.7368
24.5	24.69	100.78	0.260	24.78	101.15	0.680	24.81	101.26	0.7818

Table 4: Inter-day precision data of UV method for canagliflozin

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
1.5	1.54	102.11	1.38	1.53	102.16	1.44	1.53	102.04	1.19
12	12.11	100.94	0.551	12.13	101.10	0.99	12.16	101.34	0.386
24.5	24.76	101.07	0.574	24.75	101.03	0.34	24.75	101.02	0.425

% RSD values of intra-day precision study were found to be in between 0.072% and 1.44 % whereas those of inter-day precision study were in between 0.34 and 1.44. Overall, % RSD values of less than 2 showed that proposed UV method is precise.

Robustness:

During analysis, slight variation in analytical method parameters like solvent composition, buffer strength, pH, and change in wavelength etc. may occur and hamper its performance. Considering such events, it is expected that an analytical method should resist such changes during its application. Capacity of a method to remain unaffected by the variations in the method parameters is called robustness. In order to prove the ability of a proposed method to resist internal changes, robustness studies were performed. Robustness of

proposed UV method was established by scanning the sample solution at 288nm (±1nm wavelength from 288 nm) and change in the ratio of solvent composition (50:50) by ±1ml for canagliflozin. Change in the wavelength by ±1nm and solvent composition by ±1 ml did not affect/alter the performance of developed method. The% RSD values of wavelength were found to be in between 0.096 and 1.98 and % RSD values of ratio of solvent composition were found to be in between 0.63 and 1.07. Proposed method was found to be robust as the % RSD values were below 2.

Table 5: Robustness data of UV method for canagliflozin at different wavelength

Concentration (µg/ml)	**Wavelength**	**Absorbance**	**% RSD**
12(µg/ml)	287	0.5163	1.9804
12(µg/ml)	288	0.5192	0.0969
12(µg/ml)	289	0.5178	0.2238

Table 6: Robustness data of UV method for canagliflozin at different solvent composition

Concentration (µg/ml)	**Ratio (MeOH: H2O)**	**Absorbance**	**% RSD**
12(µg/ml)	**49:51**	**0.5196**	**1.07**
12(µg/ml)	**50:50**	**0.5203**	**0.63**
12(µg/ml)	**51:49**	**0.5201**	**0.99**

Ruggedness:

Ruggedness of analytical method is the ability of a method to resist the change in its performance in spite of influential environmental factors like instrumentation, analyst, etc. Rugged analytical methods are preferred as these methods are free from impact of environmental/external factors. In order establish the ruggedness of proposed UV method, canagliflozin solution was analyzed using two different UV-Visible spectrophotometers of two different labs including two different analysts. Sample analysis and data processing resulted into % RSD values between 0.0842 and 0.1007 Results revealed that proposed UV method was rugged as it showed % RSD values less than 2 as shown in Table 7,8.

Table 7: Ruggedness data of UV method for canagliflozin

Concentration (µg/ml)	**Instruments**	**Absorbance**	**% RSD**
12	Jasco	0.5194	0.0882
12	Bioage	0.5176	0.0972

Table 8: Ruggedness data of UV method for canagliflozin

Concentration (µg/ml)	Analyst	Absorbance	%RSD
12	Analyst I	0.5209	0.0842
12	Analyst II	0.5198	0.01007

Limit of Quantitation (LOQ) and Limit of Detection (LOD):

LOQ represents the lowermost concentration that can be analyzed with acceptable accuracy and precision. LOD and LOQ of proposed UV method was found to be 0.4680 and 1.4183 µg/ml respectively as shown in Table 9.

Table 9: LOD & LOQ data for UV method for canagliflozin

LOD	0.3010 µg/ml
LOQ	0.9123µg/ml

Lower LOQ value indicated that proposed method would be suitable for analyzing the samples containing even small quantities of canagliflozin.

Estimation of canagliflozin content in marketed formulation

The developed UV method was successfully applied for estimation of canagliflozin content in marketed formulation-I and II. The canagliflozin content in the marketed formulation-I and II was found to be 99.8 % and 101.4% and respectively.

In vitro dissolution studies:

The result of the in vitro dissolution studies of the formulation I and formulation II presented in (fig 4). The total percent release of Formulation I is 100.8% in 30 min and formulation II is 99.36% in 30 min.

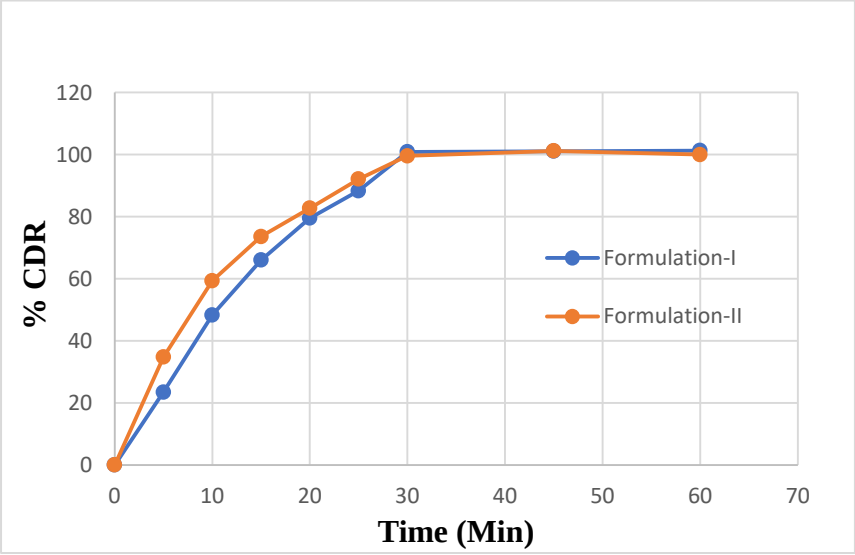

Fig 4: In-Vitro Drug Release of Marketed Formulation

IV. CONCLUSSION

A simple, yet precise and accurate UV-Visible spectrophotometric method for the estimation of Canagliflozin was developed and validated. Utility of the proposed method was successfully demonstrated for canagliflozin estimation in bulk as well as formulation and its probable industrial use during routine analysis of canagliflozin.

V.ACKNOWLEDGEMENT

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