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Development and validation of uv spectroscopic method for the estimation of canagliflozin in bulk and tablets

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Abstract

The present work was aimed to develop and validate an improved UV Spectroscopic method for the determination of Canagliflozin in bulk and tablet dosage forms. The diluent used was Methanol: Distilled water (1:1) and the drug was estimated 224 nm. The developed UV Spectroscopic method for Canagliflozin used Methanol: Distilled water (1:1) as the diluent and estimated at 224 nm. Percent recovery for the drug at 80, 100 and 120 % levels was 99.82-100.09. % RSD for intra-day and inter-day precision were 0.75 and 0.84 respectively. Linearity was observed from 10-60µg/ml with a correlation coefficient of 0.999. LOD and LOQ were 0.33 and 1.00µg/ml respectively. The percentage purity of the drug was found to be 99.79 from the assay results. The method developed was simple, sensitive and cost-effective and could be used for the analysis of the drug in educational institutions.

Keywords: Canagliflozin, UV Spectroscopic method, Methanol.

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Introduction:

Globally, about 463 million people are suffering from type-2 Diabetes mellitus [1] which is manifested by polydipsia, polyuria, polyphagia and requires lifetime treatment with anti-diabetic drugs [2]. The treatment goals involve the achievement of glycemic control and reducing the diabetes associated cardio-vascular risk. Patients suffering from recent onset of diabetes are treated with Metformin, an insulin sensitizer. The risk of hypoglycemia is insignificant with Metformin and drug interactions are less making it a highly safe and acceptable first-line of drug for the treatment of early type-2 Diabetes mellitus [3]. The pathogenesis of type-2 Diabetes mellitus is multiplex; involving several organs and treatment using combination of drugs with different mechanism of actions effectively controls the plasma glucose levels. Sodium glucose co-transporter type-2 (SGLT-2) inhibitors are the new option for the treatment of type-2 Diabetes mellitus. These agents act by inhibiting SGLT-2 transporter in kidneys, thereby promoting the excretion of glucose in urine and reducing the plasma glucose levels [5]. Among SGLT-2 inhibitors, Empagliflozin, Ertugliflozin and Canagliflozin are widely used for efficient management of plasma glucose levels with decreased risk of cardio-vascular deaths and heart failure hospitalizations [6].

Canagliflozin is a SGLT-2 inhibitor anti-diabetic with a pKa of 12.57 and log P of 3.44. It is soluble in organic solvents like ethanol, methanol, acetone and tetrahydrofuran and insoluble in water [7, 8]. The structure of Canagliflozin was shown in Fig. 1. The risk of cardio-vascular deaths and

heart failure hospitalizations was reduced with Canagliflozin in addition to the management of increased plasma glucose levels [6]. The UV Spectroscopic methods reported [12-14] for the determination of Canagliflozin in bulk and tablets were expensive. In the present study, an attempt was made to develop and validate a cost effective UV Spectroscopic method for the estimation of Canagliflozin in bulk and tablets.

Materials and methods:

Materials:

Gift sample of reference standard of Canagliflozin was obtained from Laurus Labs, Hyderabad. Analytical Reagent grade methanol was procured from Merck, Mumbai and Distilled water were used in the present work. The marketed Canagliflozin tablet formulation Invokana (Janssen Pharmaceuticals) with a label claim of 100 mg was used for the analysis.

Instrumentation:

UV-Visible double beam Spectrophotometer (model no. 3000+: Lab India) with UV-win software and 10mm matched quartz cells was used. Shimadzu digital weighing balance (ATX 224), Bio-Technics ultra-Sonicator (BTI-48) were used for developing the method. Whatmann filter paper was used for filtering the prepared solutions.

Method Development [13]:

Preparation of diluent:

Canagliflozin, being insoluble in distilled water, the diluent selected was Methanol and distilled water (1:1). The diluent was prepared by mixing about 125 ml each of Methanol and distilled water in a 250 ml volumetric flask.

Preparation of Standard solutions of Canagliflozin:

About 10 mg of Canagliflozin reference standard was weighed accurately and transferred into a 10 ml volumetric flask. The drug was dissolved in 5 ml of diluent and sonicated for 10 mins in ultra-sonicator to dissolve the drug absolutely and the volume was made up with the diluent [Stock solution:1000µg/ml]. Accurately 0.4 ml of standard stock solution was diluted to 10 ml in a 10 ml volumetric flask with the diluent [Working standard: 40µg/ml].

Preparation of Sample solutions of Canagliflozin:

The average weight of each tablet was calculated by weighing twenty tablets. The tablets were powdered and the tablet powder equivalent to 10 mg of Canagliflozin was weighed accurately and transferred into a 10 ml volumetric flask. About 5 ml of the diluent was used for dissolving the drug by sonicating for 10 mins in Ultra-sonicator. The resulting solution was filtered using Whatmann filter paper and final volume was made up with the diluent [Stock solution: 1000µg/ml]. Working sample solution was prepared by diluting 0.4 ml of sample stock solution to 10 ml with the diluent in a 10 ml volumetric flask [Working sample: 40µg/ml].

Determination of λ max:

λ max of the drug was determined by scanning 10 µg/ml of working standard solution from 200-400 nm against the blank (diluent) [Fig. 1].

Validation [14]:

The developed UV Spectroscopic method was validated for Accuracy, Precision [Intra-day and Inter-day Precision], Linearity, Limit of Detection [LOD] and Limit of Quantitation [LOQ].

Accuracy: Accuracy of the developed method was ascertained by recovery studies at 80, 100 and 120 % levels of the target concentration. In the present method, standard solution of Canagliflozin [32, 40 and 48 µg/ml] was spiked to fixed amount of pre-analyzed sample solution [40µg/ml]. Accurately 0.4 ml of sample stock solution was spiked with standard stock solution (0.32ml, 0.4 ml and 0.48 ml) and made up to 10 ml in three separate 10 ml volumetric flasks with the diluent. The absorbance of three samples of solution at each level was measured at 224 nm against the blank. Percent recovery of the drug at three levels was calculated from the absorbance of the drug at each level.

Precision: Intra-day and inter-day precision was performed for verifying the precision of the developed method by measuring the absorbance of six samples of working standard solution of Canagliflozin on the same day for intra-day precision, on two consecutive days for inter-day precision at 224nm and % RSD of absorbance was calculated.

Linearity: Linearity of the developed method for Canagliflozin was ascertained in the concentration range of 10-60µg/ml. Suitable aliquots [0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml] of standard stock solution were diluted to 10 ml in six separate 10 ml volumetric flasks using the diluent. The absorbance of the resulting solutions was measured at 224 nm against the blank. Calibration curve was constructed by plotting absorbance on y-axis against concentration [10-60µg/ml] on x-axis and. The correlation coefficient of the calibration curve was calculated by using the method of least squares in MS Office Excel 2007. LOD and LOQ: LOD and LOQ were calculated using the formulae based on the standard deviation of the y-intercepts of regression lines [σ] and the slope of the calibration curve [S].

$$\mathbf{LOD} = \mathbf{3.3\ X}\ \frac{\sigma}{S} \qquad \mathbf{LOQ} = \mathbf{10\ X}\ \frac{\sigma}{S}$$

Assay: The absorbance of six samples of the working sample solution [40µg/ml] was measured at 224 nm against the blank.

Results and Discussion:

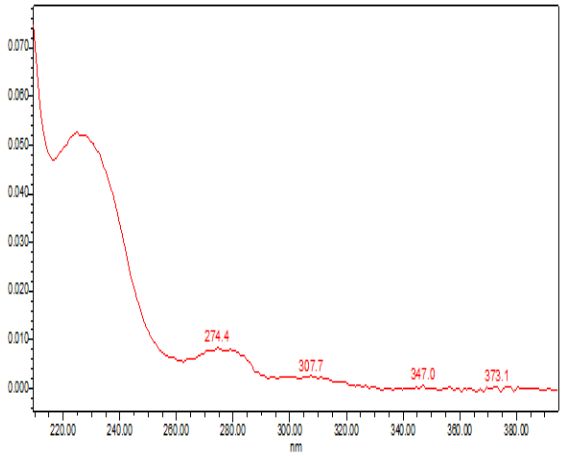

Fig. 1: UV Spectrum of Canagliflozin

Table 1: Accuracy [% Recovery] of Canagliflozin

% Spiked level	Fixed sample concentration (µg/ml)	Amount Spiked (µg/ml)	Mean Amount recovered (µg/ml)	Mean % Recovery ± SD	% RSD
80	40	32	72.07	100.09 ± 0.55	0.55
100	40	40	79.86	99.82±0.37	0.37
120	40	98	87.91	99.89±0.59	0.60

Table 2: Linearity, LOD and LOQ of Canagliflozin

Concentration (µg / ml)	Absorbance*
0	0
10	0.051
20	0.104
30	0.152
40	0.202
50	0.252
60	0.302
Linear Regression Equation y = mx + c	y = 0.005x + 0.001
Slope (m)	0.005
Intercept (c)	0.001
Correlation coefficient (R^2)	0.999
LOD	0.33 µg / ml
LOQ	1.00 µg / ml

*Average of three determinations

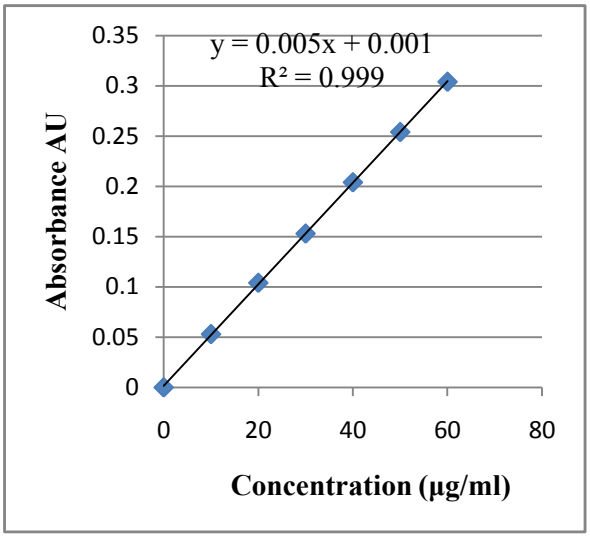

Fig. 2: Calibration Curve of Canagliflozin

Table 3: Summary of Validation Parameters of Canagliflozin

Parameter		Values
λ max		224 nm
Beer's law limits		10-60 µg/ml
Sandell's sensitivity (µg/ cm^2-0.001 absorbance units)		0.196
Regression equation		y = 0.005x + 0.001
Slope		0.005
Intercept		0.001
Correlation coefficient (R^2)		0.999
% Recovery		99.82-100.09
% RSD	Intra-day Precision	0.75
	Inter-day Precision	0.84
LOD		0.33 µg/ml
LOQ		1.00 µg/ml
Mean % Assay		99.19 ± 1.17

Canagliflozin, being insoluble in distilled water so, Methanol: distilled water (1:1) was selected as diluent. The selected λ max was 224 nm. Percent recovery is used to express accuracy and standard limits are 98-102 [10]. Percent recovery of Canagliflozin at 80, 100 and 120 % levels was 99.82-100.09 indicating that the developed method was accurate [Table 1]. % RSD is used to express precision of the developed method. Standard limits of % RSD should be less than 2.0 for intra-day precision and inter-day precision [10]. % RSD of intra-day was 0.75

and inter-day precision was 0.84, reflecting that the developed method was precise [Table 3].

Linearity should be presented in terms of correlation coefficient [R^2] and it should be NLT 0.999. The linearity observed was 10-60 µg/ml [Table 2] for Canagliflozin with a correlation coefficient of 0.999 inferring that there was linear relationship between the absorbance of the drug and concentration. The calculated LOD and LOQ for Canagliflozin were 0.33µg/ml and 1.00µg/ml respectively [Table 2] implying that the developed method was sensitive. The amount of Canagliflozin present in tablets was found to be 99.19 ± 1.17 mg [Table 3] when the developed method was applied to tablets reflecting that the developed method was suitable for the determination of Canagliflozin in tablets.

The present developed and validated method was cost-effective compared to the best method reported as Methanol: Distilled water (1:1) was used as diluent in place of phosphate buffer. LOD and LOQ values were also decreased compared to the existing methods indicating that the developed method was more sensitive.

Conclusion:

The present UV Spectrocopic method for the determination of Canagliflozin in bulk and tablets was developed using Methanol: Distilled water (1:1) as the diluent. The drug was estimated at a λmax of 245 nm. The developed method was sensitive and cost-effective as LOD and LOQ were less and Methanol: Distilled water (1:1) replaced Phosphate buffer compared to the reported best method. Hence, it could be used for the analysis of Canagliflozin in bulk and tablet dosage forms.

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Authors' contributions:

The authors have read and approved the manuscript. SB and SK designed the study. SK performed the experiment, analyzed and reviewed the data. SB supervised the experiment, reviewed the data and supported for writing the manuscript.

Competing interests:

The authors declare that they have no competing interests.

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