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RESEARCH ARTICLE

B. Krupa Karuna Vani , The Experiment,2017,Vol 40 (3),2376-2381

Extractive Visible Spectrophotometre Method development and validation for the estimation of Canagliflozin in pharmaceutical formulations

Abstract:

This work describes the development, validation and stable studies of a new, simple and reliable visible spectroscopy procedure for the analysis of Canagliflozin in pharmaceutical formulations. Studies were carried out to investigate the reaction between Canagliflozin with 5 different chromogenic dyes. All these dyes shown specific colors on reaction with Canagliflozin and prominent wavelength maxima was observed. All the developed methods were validated as per the ICH guidelines and results shows that the methods were valid. Formulation analysis shows good argument with the true values. Hence the proposed methods for the estimation of Canagliflozin are simple, rapid, accurate, economical and are useful for the estimation of Canagliflozin in pharmaceutical formulation.

Key words: Canagliflozin, colorimetry, ICH guidelines, method development, validation.

Drug Introduction:

Canagliflozin belongs to a new class of anti-diabetic drugs approved in 2013 for treating type 2 diabetes in certain patients. Canagliflozin is a sodium-glucose cotransporter 2 (SGLT2) inhibitor [1,2], which is a transport protein found in the kidney and is responsible for reabsorbing glucose that has been filtered. Canagliflozin works by decreasing the amount of sugar the body absorbs, and increasing the amount of sugar that leaves the body in the urine [3,5]. Canagliflozin is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus [6]. The drug is available as a tablet dosage form and it is also available along with other drugs like metformin as combined dosage forms. The drug is associated with side effects like increased incidence of urinary tract infections, fungal infections of the genital area, thirst, elevations in LDL cholesterol, increased urination and episodes of low blood pressure. There are concerns that it may also increase the risk of diabetic ketoacidosis [7-9]. There are very few analytical methods [10-15] have reported for analysis of Canagliflozin. Among them only there spectropotometry methods have been reported [10-12]. Hence the present work is aimed to development and validation of colorimetric method for estimation drug in pharmaceutical dosage forms.

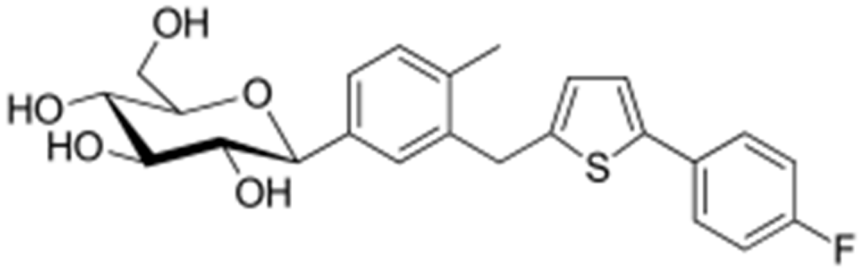

Figure.1: Chemical structure of Canagliflozin

Material and Methods:

Instrumentation:

Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Standard chromium was weighed by using Denver electronic analytical balance (SI-234).

Chemicals and reagents:

All the chemicals used were of laboratory reagent grade and were purchased from Merck chemicals private limited, Mumbai, India. The marketed formulation of Canagliflozin was purchased in local pharmacy.

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Preparation of regents:

ARS solution: weigh 200 mg of Alizarin Red S Reagent and is dissolved in 100ml of distill water.
Woll Faster Blue Black (WFBBL) solution: weigh 200 mg of WFBBL and is dissolved in 100ml of distill water.
Eryochrome Black T (EBT) solution: weigh 300 mg of EBT and is dissolved in 100ml of distill water.
HCL Solution: dissolve 8.6 ml of concentrated hydrochloric acid in 1000ml of distill water

Preparation of standard stock solution:

Standard drug solution of Canagliflozin was prepared by dissolving 10mg of Canagliflozin in 5ml methanol and was transferred to 10ml volumetric flask, it was sonicated for 5min to dissolve the drug completely in the solvent and the volume was made up to mark with methanol to obtain stock solution of 1000µg/ ml concentration. From 1000µg/ml, 250, 150 and 100µg/ml solution was prepared by selective dilution.

ARS Method [M1]:

In a series of 125 ml separating funnels containing aliquots of standard drug [0.5-3.0ml; 150µg/ml] solution was taken. To this 6ml of HCl solution and 2ml of dye solutions were added successively. The total volume of the aqueous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 443nm against a similar reagent blank.

WFBBL Method [M2]:

In a series of 125 ml separating funnels containing aliquots of standard drug solution (0.5-3ml; 100µg/ml) was taken. To this 6ml of HCl solution and 2ml of WFBBL solutions were added successively. The total volume of the aqous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 584nm against a similar reagent blank.

EBT Method [M3]:

In a series of 125 ml separating funnels containing aliquots of standard drug solution (5-30ml; 250µg/ml) was taken. To this 6ml of HCl solution and 2ml of EBT solutions were added successively. The total volume of the aqueous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 410nm against a similar reagent blank.

All the developed methods have been validation for linearity, precision, ruggedness, sensitivity etc parameters under ICH guidelines [16, 17].

Result and Discussion:

Method Development:

The drug Canagliflozin is in basic nature and forms an ion association complex with acid dyes ARS, WFBBL and EBT. The formed complex is extractable in to Chloroform from the aqueous phase. The protonated nitrogen positive charge of the drug molecule in acid medium is expected to attack the positive charge of the dye. Hence form a colored complex which is extracted with Chloroform. The obtained color chromogen shows absorbance at 443nm for ARS Method, 584nm for WFBBL method and 410nm in EBT method.

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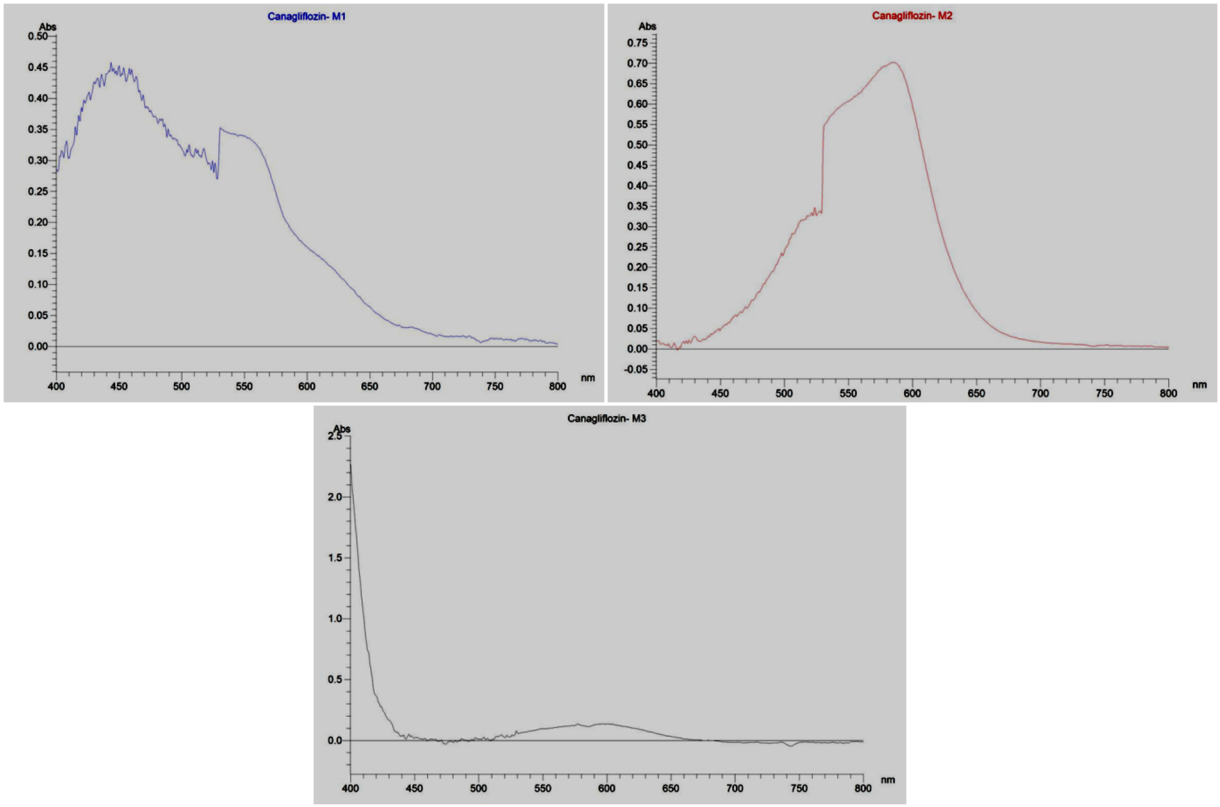

Figure 2: Colorometric spectra of Canagliflozin for ARS Method [M1], WFBBL Method [M2] and EBT Method [M3]

Method Validation:

Linearity test was evaluated by measuring the absorbance values of standard solutions. For Canagliflozin linearity was found to be in the range of 3-18 µg/ml for ARS Method[M1], 2-12 µg/ml for WFBBL Method [M2] and 5-30 µg/ml for EBT Method [M3] respectively measured at specific wavelength of the method. Linearity graph was constructed by taking concentration versus absorbance in each method. It was found that all the developed methods show good linear relation with in the concentrations under the study. Summary results of the Linearity were shown in table 1. Wavelength scanning spectra was shown in figure 2. Accuracy of the developed methods was confirmed by performing recovery studies 50%, 100% and 150% to the standard level. Each level was performed in triplet. Average recovery in each level by the method was compared with the standard values. Average recovery was found to be 99.73- 100.61 % for ARS Method [M1], 99.79-100.82% for WFBBL Method [M2] and 100.18-100.72% for EBT Method [M3]. All the results were within the acceptance limit this indicates that the proposed methods are accurate. Summary results of accuracy were shown in table 2.

In order to assess the intra and inter-day precision of the assay, standard concentration of Canagliflozin in each method was prepared as described above. Absorbance of the each solution at 9µg/ml for M1 and 6µg/ml for M2 and M3 were measured at corresponding wavelength; % RSD of the six repeated values in each method was calculated and was found to be within the acceptance limit. This indicates that the proposed methods were precise. Results of the precision were shown in table2. The limit of detection was calculated by LOD = 3.3σ/S, and found to 0.07µg/ml for M1, 0.05µg/ml for M2 and 0.10µg/ml for M3 respectively, which shows the sensitivity of the methods. The limit of quantification was calculated by LOQ = 10σ/S and found to 0.25µg/ml for M1, 0.20µg/ml for M2 and 0.40µg/ml for M3 respectively. Percentage of recovery was found to be more than 98% in all the methods when proposed methods were applied to its marketed formulation (sulisent – 100mg). This indicates

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that the proposed method can successfully applied for thee routine estimation of Canagliflozin in pharmaceutical dosage forms. Results of the formulation analysis were shown in table 2.

Conclusion:

These Visible spectrophotometric methods proposed for estimation of Canagliflozin on their pharmaceutical dosage forms were accurate, precise, reproducible and sensitive. All the methods have good linearity range to analysis and high degree of accuracy. All methods were found sensitive and successfully used for quantification of Canagliflozin in its pharmaceutical dosage forms. The validation procedure confirms that this is a workable methods for their quantification in the formulations.

Table 1: Results of Linearity test for Canagliflozin:

S No	M1		M2		M3	
	Concentration in µg/ml	Absorbance	Concentration in µg/ml	Absorbance	Concentration in µg/ml	Absorbance
1	3	0.156	2	0.142	5	0.174
2	6	0.264	4	0.256	10	0.286
3	9	0.379	6	0.364	15	0.412
4	12	0.485	8	0.478	20	0.546
5	15	0.596	10	0.586	25	0.665
6	18	0.702	12	0.698	30	0.795

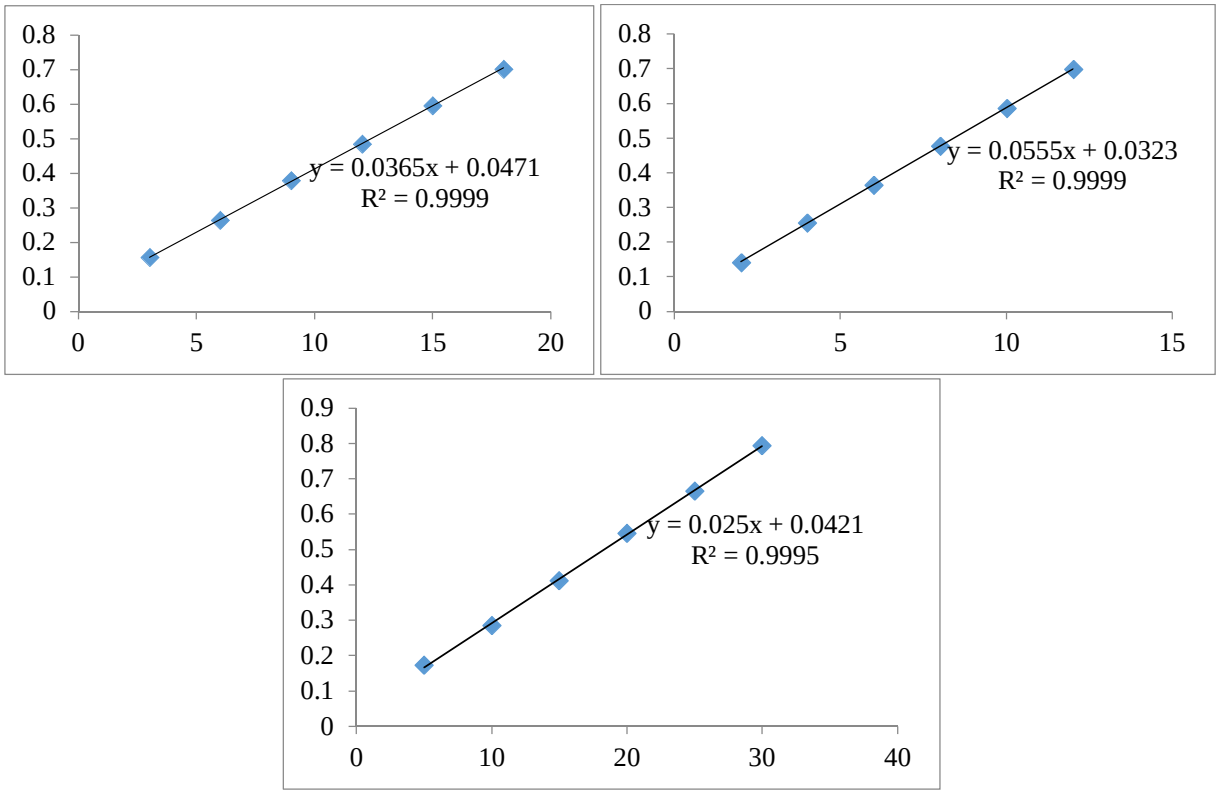

Figure 3: Linearity graph of Canagliflozin for ARS Method [M1], WFBBL Method [M2] and Canagliflozin for EBT Method [M3]

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Table 2: Summary of validation results for Canagliflozin:

S. No	Parameter	ARS Method	WFBBL Method	EBT Method
1	λ max	443nm	584nm	410nm
2	Linearity Range	3-18 µg/ml	2-12 µg/ml	5-30 µg/ml
3	r^2	0.999	0.999	0.999
4	Slope	0.036	0.055	0.025
5	Intercept	0.047	0.032	0.042
6	%RSD of Precision Intraday Interday Ruggedness	 0.496 0.694 0.786	 0.510 0.708 0.54	 0.45 0.773 0.785
7	Recovery range (50-150%)	99.73- 100.61 %	99.79-100.82%	100.18-100.72%
8	LOD	0.07µg/ml	0.05µg/ml	0.10µg/ml
	LOQ	0.25µg/ml	0.20µg/ml	0.40µg/ml
9	Stability Period	90min	80min	35min
10	Formulation assay	99.89%	99.67%	99.93%

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