Simultaneous estimation of canagliflozin and metformin hydrochloride in tablet dosage form by UV spectrophotometry

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Abstract

The combination of Canagliflozin and Metformin was available as fixed dose tablets for the treatment of type 2 diabetes. The present method aims to develop a simple, precise and accurate spectrophotometric method for simultaneous determination of Canagliflozin and Metformin in commercial formulation. The method utilizes Vierordt's equation based on the measurement at two wavelengths 290nm (λmax of Canagliflozin) and 236nm (λmax of Metformin). The method exhibited linear range of 2.5 to 15µg/ml and 5 to 17.5µg/ml for Canagliflozin and Metformin, respectively, with a correlation coefficient of 0.999. The LOD and LOQ for Canagliflozin were found to be 0.43 and 1.31 respectively. For Metformin the LOD and LOQ were found to be 0.49 and 1.49 respectively. The recovery of Canagliflozin and Metformin were found to be 99.43 and 98.82 respectively. The results were validated statistically as per ICH guidelines and were found to be satisfactory. To conclude, the developed UV spectrophotometric method is more economical for analysis of Canagliflozin and Metformin in both bulk and pharmaceutical dosage form for routine analysis.

Keywords: Canagliflozin, Metformin, Vierordt's equation, UV-Spectrophotometry, ICH guidelines.

Introduction

The combination of Canagliflozin and Metformin is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type-2 diabetes. The Canagliflozin and Metformin formulation is available in four dose strengths (50/500 mg, 50/1000 mg, 150/500 mg, 150/1000 mg) and should be taken twice daily with food.[1] Canagliflozin (Fig. 1a) is chemically (1S)-1, 5-anhydro-1-[3-[[5-(4-fluorophenyl)-2-thienyl]methyl]-4-methylphenyl]-D-glucitol and belongs to the class of SGLT2 inhibitors. It is used in the treatment of type-2 diabetes.[2] Canagliflozin inhibits the reabsorption of glucose from kidneys and lowers the renal glucose threshold by inhibiting sodium-glucose transport protein (SGLT2).[3-4] By blocking SGLT2, Canagliflozin decreases reabsorption of filtered glucose and reduces the renal threshold for glucose (RT_G), thereby elevating the urinary glucose excretion (UGE) and reducing raised plasma glucose in patients with type-2 diabetes.[5] Canagliflozin can be used as monotherapy or multi therapy in the treatment of type-2 diabetes.[6-9]

Metformin (Fig. 1b) a biguanide antihyperglycemic agent used for treating type-2 diabetes. It acts by decreasing hepatic glucose production and glucose absorption, and it enhances insulin mediated glucose uptake. Metformin is recommended as first line therapy for patients with type-2 diabetes. Patients, from whom Metformin monotherapy is not sufficient to achieve glycemic goals, it is referred to use in combination with other class of antidiabetic drugs.[10]

The literature survey revealed that few analytical methods were reported for estimation of the drugs individually and in combination using, UV,[11-13] HPLC,[14-16] HPTLC[17] and LC-MS.[18] In the present study an attempt was made for simultaneous estimation of Canagliflozin and Metformin in pharmaceutical dosage form by UV spectrophomerty. The method can be applied for routine quality control analysis.

Materials and Method

Reagents and Chemicals: The pure sample of Canagliflozin and Metformin was procured from Selleckchem LLC supplied by Pro lab marketing, India. The commercial formulations (Invokamet tablets containing 150mg of Canagliflozin and 500mg of Metformin) were procured from the local market. Methanol (AR grade) was purchased Merck Chemical Division, Mumbai, India and was used as diluent. Fresh purified distilled water was used throughout the experiment.

Instrumentation: Shimadzu UV1800 Double Beam UV-Visible Spectrophotometer, using software UV Probe (version 2.42) was used for spectral studies. Shimadzu BL220H Digital Weighing Balance having sensitivity of 0.001g was used for weighing the materials.

Method Development

Standard solution preparation: About 100mg of Canagliflozin and 100mg of Metformin was accurately weighed and transferred into a 100mL clean dry volumetric flask containing 70mL of methanol. The solution was sonicated for 5min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the methanol to get a stock concentration of 1mg/mL Canagliflozin and Metformin. From the above prepared stock solution

1mL was transferred into a 10mL volumetric flask and the volume was made up to the mark with the methanol to obtain 100μg/mL. The resulting solution was further diluted to obtain the concentration of 10μg/mL for both drugs separately and scanned from 400-200nm. The spectra of Canagliflozin with 290nm as λmax and of Metformin with 236nm as λmax were obtained.

Preparation of sample solution: Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 150mg of Canagliflozin and 500mg Metformin of was transferred into a 100mL clean dry volumetric flask containing 70mL of methanol. The solution was sonicated for 5min and the drug was dissolved completely and to mark with methanol. The solution was subjected to filtration using Whatman filter paper No 41. The 0.5mL stock was further diluted 10mL with methanol, from which 1mL is transferred to 10mL volumetric flask and further diluted with methanol. The final drug mixture dilution was subjected for analysis.

Preparation of calibration Standards: Working standard solutions were prepared for the Canagliflozin and Metformin from the standard solution of 100μg/mL. Different aliquots were taken from standard stock solution and diluted with methanol separately to prepare 2.5 to 15μg/mL, and 5 to 17.5μg/mL for Canagliflozin and Metformin respectively. Then the construction of calibration curve was plotted by taking the above prepared solutions by taking the concentration and absorbance values.

Simultaneous equation method (Vierodt's method): The approach was possible when two analytes have dissimilar λmax, each of which exhibits absorbance at the λmax of other. Two equations are constructed based on the fact that the absorbance at a particular wavelength is sum of individual absorbance of two components. Absorptivity from all the concentration was calculated for both the drugs in methanol solution and used for the development of simultaneous equation.

$$C_X = A_2 a_{y_1} - A_1 a_{y_2} / a_{x_2} a_{y_1} - a_{x_1} a_{y_2} \ldots\ldots\ldots\ldots (1)$$
$$C_Y = A_1 a_{x_2} - A_2 a_{x_1} / a_{x_2} a_{y_1} - a_{x_1} a_{y_2} \ldots\ldots\ldots (2)$$

The concentration of Canagliflozin (C_x) and Metformin (C_y) can be obtained by solving above equation (1) and (2)

Where,
i)   504.74 (a_{x_1}) and 232.29 (a_{x_2}) are absorptivity of Canagliflozin at λ₁ (290nm) and λ₂ (236nm) respectively.
ii) 80.81(a_{y_1}) and 388.82 (a_{y_2}) are absorptivity of Metformin at λ₁ (290nm) and λ₂ (236nm) respectively.

A₁ and A₂ are absorbance of mixture at λ₁ (290nm) and λ₂ (236nm) respectively.

Method validation
The method was validated as per ICH guidelines to study various parameters like linearity, accuracy, precision etc.

Linearity: The linearity of the method was developed by plotting absorbance vs concentration (μg/mL). The linearity for Canagliflozin was established over a concentration range of 2.5-15μg/mL and for Metformin 5-17.5μg/mL respectively. The correlation coefficient was calculated by analysing regression equation.

Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analysed drug sample solution. Percent recovery was calculated by comparing the absorbance before and after the addition of the standard drug. The standard addition method was performed at three concentration levels in triplicate at 80%, 100% and 120%.

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the method was confirmed by intra-day and inter-day analysis. The concentration used for the precision studies is 10μg/mL of Canagliflozin and 10μg/mL Metformin was assumed as 100%. The precision of the developed method was expressed in terms of % relative standard deviation (RSD).

Limit of Detection and Limit of Quantification (LOD and LOQ): The LOD and LOQ were calculated based on the standard deviation of response and slope. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Results and Discussion
From the spectrum of analytes, the λmax of Canagliflozin and Metformin was found to be 290nm and 236nm respectively. The linear regression data showed a good linear relationship over a concentration range, 2.5 to 15μg/mL and 5 to 17.5μg/mL for Canagliflozin and Metformin respectively, with a correlation 0.999 for both drugs. The linearity data was submitted in Table 1 and Fig. 4 and 5. By performing assay for marketed dosage from, the percentage drug estimated was found to be 99.33 ± 0.832% for Canagliflozin and 100.80 ± 0.521% for Metformin. The results obtained were within the limit of ±2%, the accuracy data was given Table 2. The percentage recovery of pure drug for Canagliflozin ranged from 97.84 to 101.00 and for Metformin 98.40 to 99.45 respectively. The results were presented in Table 3. The method developed was proved to be precise as %RSD values for both intra-day and inter-day were within the limit of ±2%, the data was presented in Table 4 and 5. The limit of detection for Canagliflozin and Metformin was found to be 0.43μg/mL and 0.49μg/mL respectively. Limit of quantification was found to be 1.31μg/mL and 1.49μg/mL for Canagliflozin and Metformin respectively.

Table 1: Linearity data of Canagliflozin and Metformin

S. No.	Canagliflozin at 290nm		Metformin at 236nm	
	Concentration (µg/mL)	Absorbance	Concentration (µg/mL)	Absorbance
1	2.5	0.120	5	0.199
2	5	0.234	7.5	0.298
3	7.5	0.384	10	0.384
4	10	0.511	12.5	0.475
5	12.5	0.656	15	0.576
6	15	0.799	17.5	0.668
Regression Equation	y = 0.0547x-0.0281		y = 0.0374x+0.0129	
Correlation Coefficient (r^2)	0.999		0.999	

Table 2: Assay results of Canagliflozin and Metformin in formulations

Formulation		Label claim	Amount found	%Assay
Inokamet®	Canagliflozin	150mg	148.99mg	99.33% ± 0.832
	Metformin	500mg	504mg	100.80% ± 0.521

Table 3: Recovery studies for Canagliflozin

Level	Canagliflozin		Metformin	
	% Recovery (n=3)	%RSD	% Recovery (n=3)	%RSD
80%	99.46 ± 0.420	0.422	98.40 ± 0.093	0.094
100%	101.00 ± 0.525	0.520	99.45 ± 0.493	0.496
120%	97.84 ± 0.238	0.243	98.60 ± 0.420	0.426

Table 4: Intra-day precision results of Canagliflozin and Metformin

S. No.	Time (Hours)	Canagliflozin	Metformin
1	0	0.513	0.362
2	3	0.515	0.361
3	6	0.511	0.355
4	9	0.509	0.357
5	12	0.510	0.359
6	15	0.508	0.363
Mean		0.511	0.360
SD		0.003	0.003
%RSD		0.51	0.86

Table 5: Inter-day precision results of Canagliflozin and Metformin

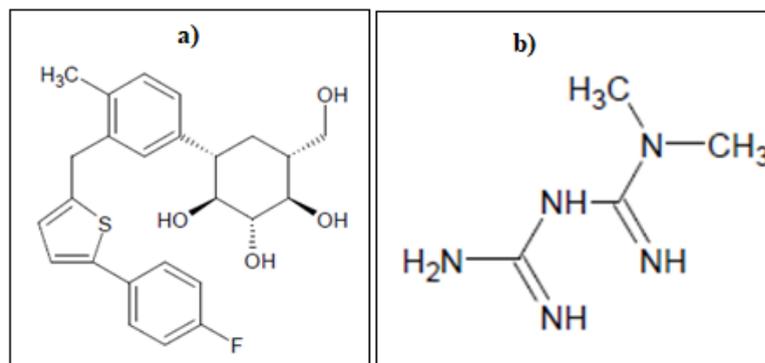| S. No.	Time (Days)	Canagliflozin	Metformin
1	1	0.515	0.362
2	2	0.509	0.359
3	3	0.510	0.360
4	4	0.514	0.365
5	5	0.507	0.365
6	6	0.501	0.359
Mean		0.509	0.361
SD		0.005	0.003
%RSD		1.00	0.78

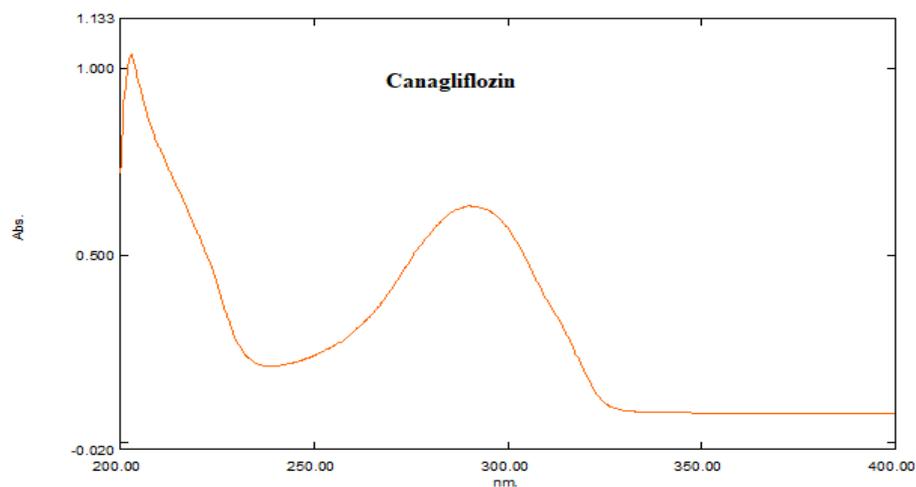**Fig. 1: Structure of Canagliflozin (a) and Metformin (b)**
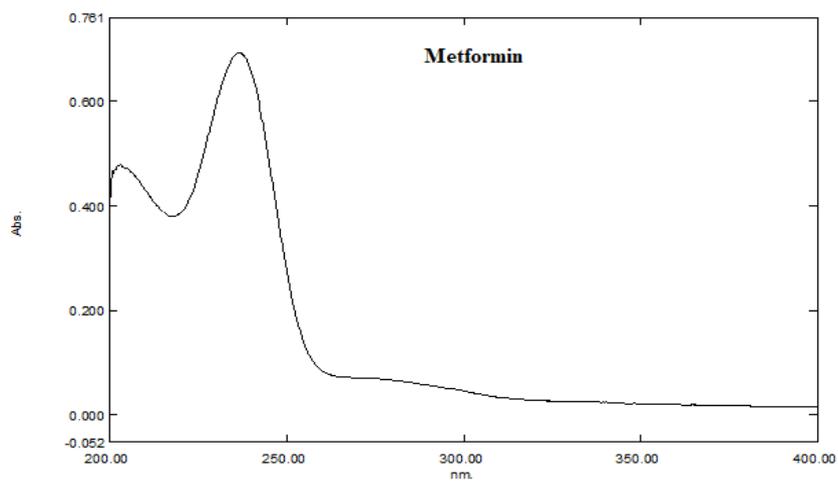

Fig. 2: Spectrum of Canagliflozin

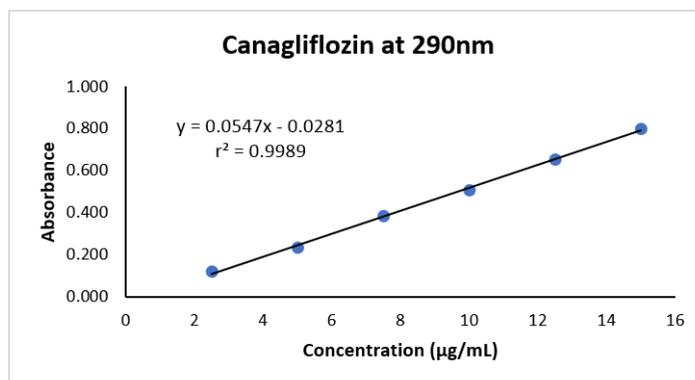**Fig. 3: Spectrum of Metformin**

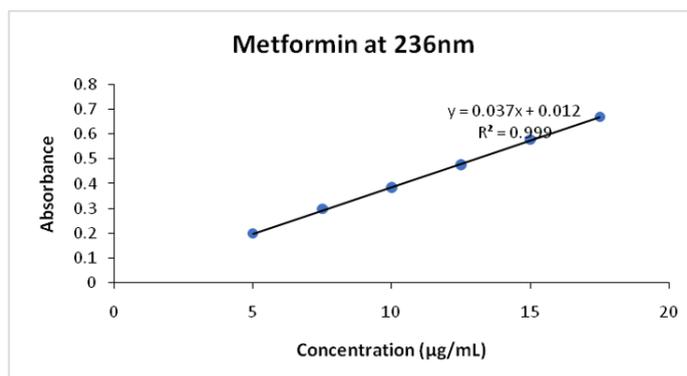**Fig. 4: Calibration curve of Canagliflozin at 290nm**

Fig. 5: Calibration curve of Metformin at 236nm

Conclusion

The proposed spectrophometric method is simple, accurate, precise and economical for the simultaneous estimation of Canagliflozin and Metformin in bulk and dosage form. The results obtained indicate method compliances with ICH limits. Percentage assay is very near to label claim which shows accuracy of the method. On the basis of validation and assay data it was concluded that the developed method can be utilized for routine analysis of selected combination.

Acknowledgement

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