Modified HPLC Quantification Analytical Technique for Canagliflozin and Metformin Hydrochloride in Bulk and Tablets

[1]Aditya Trivedi, [2]Noopur Dixit, [3]D.N.Jhade

[1, 2,3]Sri Satya Sai University of Technology & Medical Sciences, Opposite Sehore (M.P.) India

Correspondence Author: Aditya Trivedi, Sri Satya Sai University of Technology & Medical Sciences, Opposite Sehore (M.P.) India

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Abstract

An accurate, precise, specific modified HPLC method was developed for the simultaneous quantification of canagliflozine and metformin in bulk and dosage forms. A C18 column (250 x 4.6mm; 5 µm Phenomenex) with mobile phase containing 0.05% v/v triethylamine (pH6.5): acetonitrile (45:55% v/v) 200C was used and isocratic pump is used for elution and eluents were monitored at 215 nm. The retention times of canagliflozine and metformin were 3.4 min and 12.7 min respectively and showed a good linearity in the concentration range of 40-200 µg/mL of canagliflozine have found correlation coefficient of 0.999 and 10- 50 µg/mL of metformin with a correlation coefficient of 0.998. The average percent recoveries were found to be 98.60% and 98.90% respectively for canagliflozine and metformin.The developed method fallows all the validation parameters like accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method was validated as per ICH guidelines and successfully applied to the simultaneous estimation of canagliflozine and metformin in bulk and dosage form.

Keywords: Canagliflozine, Metformin, Simultaneous Estimation, Phenomenax C18 Column, RP-HSTLC, PDA Detection, Validation.

Introduction

Canagliflozin inhibit SGLT2 by binding more potently (250-times) than SGLT1.The 50% inhibitory concentrations (IC50) are 2.2-4.4 nmol/L and 684 - 910 nmol/L for SGLT2 and SGLT1 respectively. Chemically it is 2S,3R,4R,5S,6R)-2-{3-[5-[4-Fluoro-phenyl)-thiophen-2-ylmethyl]-4-methyl-phenyl}-6-hydroxymethyl-tetrahydro-pyran-3,4,5-triol   and Metformin is an oral antihyperglycemic agent that improves glucose tolerance in patients with NIDDM, lowering both basal and postprandial plasma glucose.[2,2] Metformin is not chemically or pharmacologically related to any other class of oral antihyperglycemic agents.chemically it is N, N-Dimethylimidodicarbonimidic diamide.they are used for management of diabetis.[3]

Literature survey reveals that few methods have been reported on analysis of canagliflozine and metformin individually in pharmaceutical dosage forms and several HPLC methods have been described for the determination of canagliflozine and metformin in spiked biological samples. However, there were no validated HPLC methods reported till date for the simultaneous estimation of canagliflozine and metformin in combination. Hence, the main objective of the present

work was to develop a method for the simultaneous analysis of canagliflozine and metformin in bulk and dosage forms by using HPLC. [4]

Material and Methods

Reagents and Chemicals

canagliflozine and metformin were gift samples from Sun Pharma, India. Acetonitrile, water and triethylamine were purchased from E. Merck, Mumbai, India. All the solvents and reagents were of HPLC grade.Invokamet contains canagliflozin 50 mg and metformin 500mg tablets manufactured by Janssan pharmaceuticals) were locally purchased.

Equipment

A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser .The HPLC system consisted of a model LC-20A(Prominence)Simadzu,Japan . Data acquisition was carried out using LC solutions software. The chromatographic analysis was performed on Phenomenex C18 column (250 × 4.6mm; 5µm).

Chromatographic Conditions

Mobile phase consisting of 0.05% v/v triethylamine of pH 6.5 (orthophosphoric acidis used to maintain the pH): acetonitrile (45:55% v/v) .The mobile phase was filtered through Millipore nylon disc filter of 0.45µm fallowed by ultrasonication for 3 min. The flow rate was maintained at 1.2 mL/min with an injection volume of 20 µL. Eluents were monitored at 215 nm and the separation was found.

Preparation of Stock and Standard Solutions

The stock solutions of canagliflozine and metformin of concentration 1mg/mL were prepared in 10mL volumetric flask using methanol as a solvent. The working standard solutions in the concentration ranging from 40-200 µg/mL of canagliflozin and 10-50 µg/mL of metformin were prepared by appropriately diluting the stock solutions with acetonitrile as diluents and capt for 20°c.

Method Validation

The method was validated according to the ICH guidelines.

Specificity

Canagliflozin and Metformin in pure form were evaluated by comparing the standard and sample solutions with blank. Specificity is a measure of the degree of interference in the analysis of the sample mixtures into which known impurities were added. Specificity of the method was carried out by comparing chromatogram analysis was done to check interference peaks.

Linearity

An aliquot of five different consentration was evaluated across the range of the analytical procedure. A series of standard dilutions of canagliflozine and metformin were prepared over a concentration range of 40- 200µg/mL (40, 80, 120, 160 & 200µg/mL) and 10-50µg/mL (10, 20, 30, 40 & 50µg/mL) respectively from stock solutions .Peak area was determined with correspondence to

the analyte concentration, and the test results were evaluated by appropriate statistical methods where by slope, intercept, and regression (R2) & correlation coefficients (R) were calculated.

Precision

Precision was measured in the same analytical condition in the terms of repeatability of procedure, application and measurement. Repeatability of standard application was carried out using six replicates of the standard concentration of canagliflozine (80µg/mL) and metformin (20µg/mL).Peak area is indicated less than 2% RSD which indicates precision of method.

Accuracy

Accuracy of this method was found in the whole analytical procedure. Accuracy (recovery) of the method was tested by spiking 80, 100 and 120% of canagliflozine (80µg/mL) and metformin (20µg/mL) standard concentrations. These solutions were analyzed by developed method in triplicate. The calculation of %RSD and % recovery was done throughout the addition level.

Robustness

In this method by changing the flow rate and wave length the result withstand during rigorous testing. The HPLC parameters like capacity factor, tailing factor, theoretical plate number and % assay were observed. The flow rate of the mobile phase was maintained at 1.2mL/min. To prove robustness of this method, the flow rate was changed by ±20% and wavelength by ±5nm.

Limit of Detection and Quantification

Calibration curve method were used to determined LOD and LOQ. Standard solutions of canagliflozine and metformin were prepared in the range of 40-200µg/mL and 10-50µg/mL injected (20µL) in triplicate. Average peak area of two drugs was plotted against concentration. LOD and LOQ were calculated by using following equations: $LOD = (3.3 \times \sigma)/m$; $LOQ = (10.0 \times \sigma)/m$

System suitability

System suitability was carried out by injecting a standard concentration (40µg/mL of canagliflozin and 10µg/mL of metformin) at different injection volumes in the range of 10-50µL and % RSD was calculated.

Assay

As combined dosage forms were presently available in the market, of canagliflozin (containing 50 mg) and Metformin (containing 500 mg) were used in these studies. Powder blend (from 10 tablets of each brand) equivalent to 10 mg of canagliflozin and Metformin were separately weighed and transferred to a 10 mL volumetric flask. 5 mL of methanol was added fallowed by s sonication for 5 min and volume was made up to the mark with methanol. The solutions were centrifuged and the supernatant were filtered using syringe filter (13 mm, 0.45 µm). Aliquots of the drugs of concentration 80µg/mL and 20µg/mL of canagliflozine and metformin were prepared and analyzed in triplicate. The amount present in the each tablet was calculated by comparing the areas of standard canagliflozine and metformin with that of the sample.

Results and Discussion

Validation of the Chromatographic Conditions

The present work was carried out with a view for development of HPLC method for the simultaneous estimation of canagliflozine and metformin in bulk and dosage forms. Initial trials were carried out with Phenomenex C18 column (250 x 4.6 mm; 5µm) using mobile phase 0.05% v/v formic acid and methanol (60:40% v/v) at a flow rate of 1.2mL/min and

acetonitrile as the diluent. The quantification was carried out at 215nm. Under these conditions canaglifrlozin was eluted at 3.16 min and metformin at 4.88 min. The canagliflozin was almost eluted with the solvent front. In the other modification trial, methanol was replaced with acetonitrile and mobile phase combination of 80:20% v/v and kept at 20°c and a flow rate of 1.2mL/min the eluetion time for canagliflozin was 3.61 min and metformin was 6.70 min. However, the resolution between the solvent front and the canagliflozin peak not prominent. Then, 0.05% v/v triethylamine was taken (adjusted to pH 6.5 with orthophosphoric acid) with acetonitrile in the ratio of 50:50% v/v and the flow rate is 1.2mL/min. Under these conditions the cangliflozin was eluted at 5.14 min and metformin at a longer retention of 20.23 min. Finally, the ratio of the mobile phase was 45:55% v/v of 0.05% v/v triethylamine and acetonitrile .Flow rate of 1.2mL/min is maintained in order to achieve proper resolution of the both canagliflozine and metformin peaks respectively. Under these conditions the canagliflozine and metformin peaks were eluted at 3.47 min and 12.34 min respectively. Symmetrical peaks were found and tailing factor was within the limits. For quantitative analytical purpose wavelength was set at 215 nm, which provided better reproducibility without interference. The peak purity was found to be greater than 0.9999 of both the drugs canagliflozine and metformin used in the analysis. A sample chromatogram of canagliflozine and metformin were given in Figure 1 along with UV spectra.
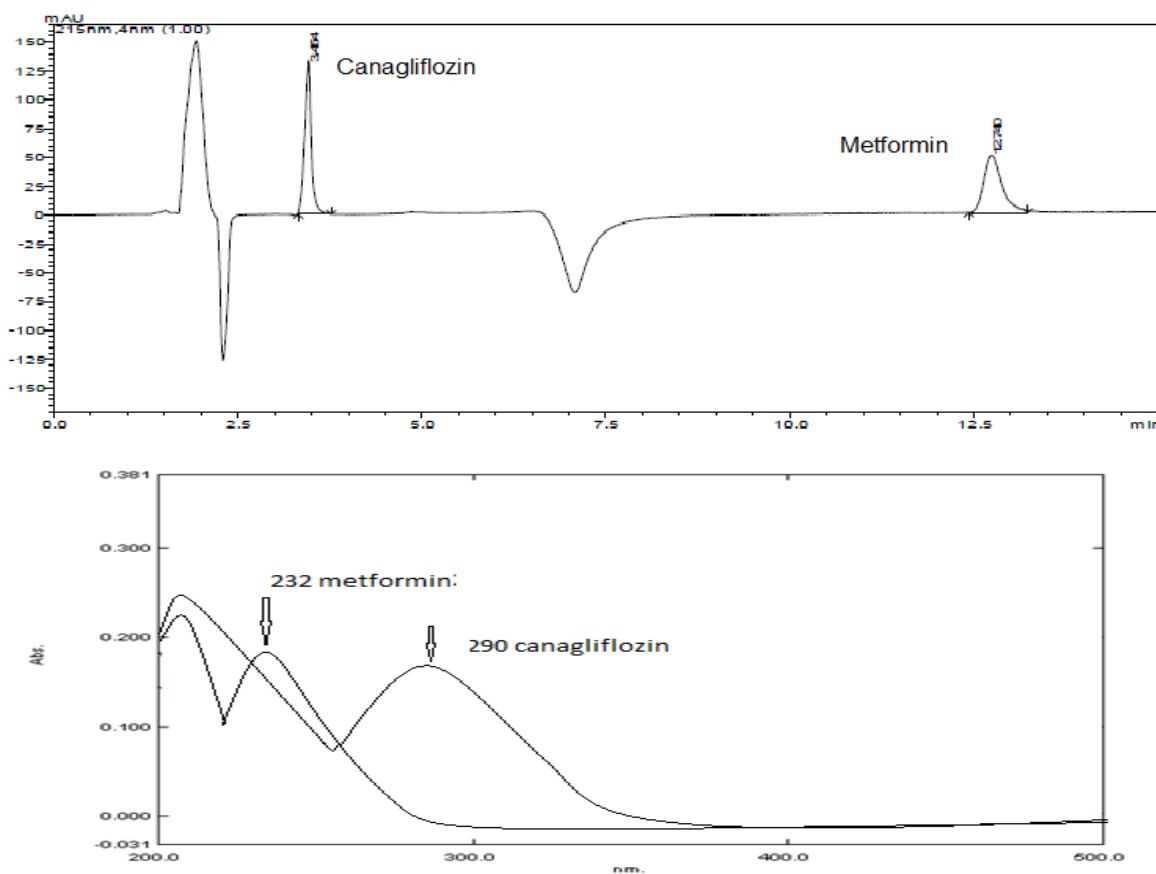

Figure 1: Standard Chromatogram of canagliflozin (40µg/mL) and Metformin (20µg/mL) mixture (A) and (B) overlay UV spectra of canagliflozin and metformin

Method Validation

Specificity

The specificity of this modified method was established by using solutions of diluent, placebo, standard and test sample (Tablets). The inference from the 3D plots of placebo and test samples shown in Figure 2, that there were no co-eluting peaks at the retention time of canagliflozin and metformin.These results show that peak of drug candidate was pure and the excipients in the formulation did not interfere with the analysis. The purity of peak for sample and standard were found to be greater than 0.999 hence the method confirms specificity.
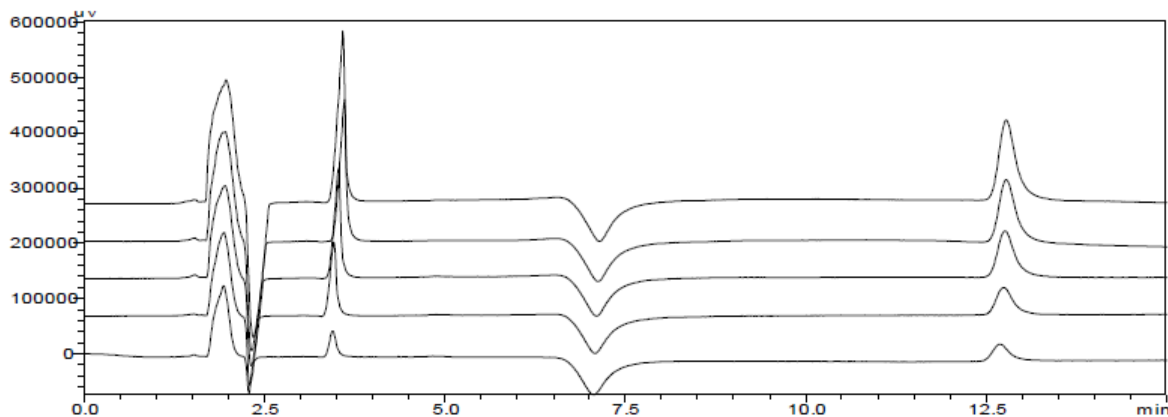

Figure 2: Overlaid canagliflozin (40-200µg/mL) and metformin (10-50µg/mL) standard chromatograms

Linearity

A linear relationship evaluated across a concentration range 40-200µg/mL of cangliflozin and 10-50µg/mL of metformin in triplicate (n=3). The concentration range was selected based on 80, 100 and 120% of the test concentration for assay. Peak area and concentration data was subjected to least square regression analysis. The correlation coefficients (R) were found to be 0.999 and 0.998 respectively for canagliflozin and metformin and indicate a good linearity within the concentration range selected. The data of the calibration curve was given in Table 1 and chromatograms were shown in Fig 2.

Precision

Precision studies were carried out in terms of repeatability. Repeatability of standard application was assessed by using six replicates of concentration at 80µg/mL of canagliflozin and 20µg/mL of metformin and the data was given in Table-2. The % RSD was found to be less than 2 for peak areas; this shows the closeness of the data values to each other, indicating the method was precised.

Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies using the standard addition method by spiking the known quantities of standards at 80, 100, and 120% to the test solution of 80µg/mL of canagliflozin and 20µg/mL of metformin. The analyte peak is evaluated by 3D plots of the chromatogram in order to confirm the existence of components at 3.4 min and 12.7 min elution time of canagliflozin and metformin respectively and shown in Figure 3. The recoveries were found to be 99.15-101.73%, 99.04-100.04%, and 98.24-99.50% at 80, 100 and 120% respectively for
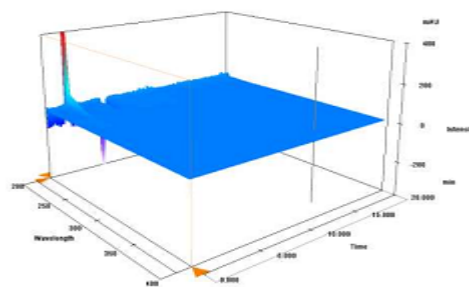
canagliflozin and metformin. These results indicate a good accuracy of the method to that of the labeled claim. The obtained recovery results were given in Table 1.
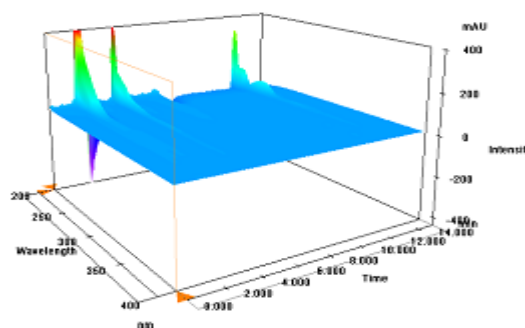
3d chromatogram of sample
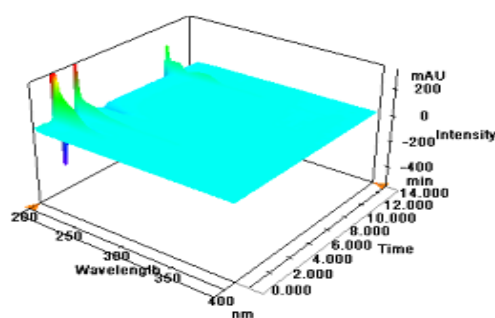
3D chromatogram of standerd

3d chromatogram of diluent solution

Figure 3: 3D plots of Sample Standard and Diluent

Robustness

As part of the robustness, a deliberate change in the flow rate and wavelength was made to evaluate the impact on the method. Significant change of Retention times was found with flow rate and showing no change with wavelength. Moreover % assay values were within limits and these results indicated minor changes in the flow rate and wavelength didn't affected the assay results. The results were given in Table 2.

Table:1 Linearity, Accuracy and Precision data.

Validation parameters	Parameters	Canagliflozin	Metformin
Linearity (n=3)	Range	40-200 µg/mL	10-50µg/mL
	Regression equation	Y=11632x-1152	Y=53152x-7026
	Regression coefficient(R^2)	0.999	0.998
	Correlation coefficient(R)	0.998	0.997
Accuracy(n=3)	% Level of addition	Mean%recovery(RSD)	Mean%recovery(RSD)
	80	99.59(1.95)	100.03(1.08)
	100	99.12(0.89)	101.07(1.02)
	120	99.82(0.64)	100.09(0.86)
Precision (n=6)			
System precision	Average peak area of the standred sample (RSD)	839193(1.94)	9404(1.13)
Method precision	Average peak area of the Assay sample (RSD)	855010(0.81)	8891(0.16)

Table 2: Robustness data for Wavelength

Drug	Parameter range	Retention Time in Min.	Theoretical plates(N)	Tailing factor	Capacity Factor(k)	% Assay
Canagliflozin	210	3.5	5274.5	1.0	1.1	98.5
	215	3.54	6079.5	1.0	0.8	100.2
	220	3.5	5317.1	1.3	0.8	100.3
Metformin	210	12.7	14152.6	1.4	5.6	101.2
	215	12.7	14886.4	1.3	5.6	100.3
	220	12.7	14086.0	1.3	5.6	99.15

Table 2: Robustness data for Flow rate

Drug	Parameter range	Retention Time in Min.	Theoretical plates(N)	Tailing factor	Capacity Factor(k)	% Assay
Canagliflozin	1.0	4.5	5282.3	1.42	0.99	98.6
	1.2	3.7	4895.4	1.37	0.94	101.2
	1.4	3.4	4906.2	1.39	1.1	99.8

Metformin	1.0	13.8	13827.6	1.47	5.54	101.8
	1.2	12.7	13040.7	1.39	5.62	100.2
	1.4	11	13633.8	1.38	5.79	98.2

Detection and Quantification Limits

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve. LOD for Canagliflozin and Metformin was found to be 0.326 and 0.436 mg/mL respectively. LOQ for Canagliflozin and Metformin was found to be 0.990 and 1.321 mg/mL respectively.

System suitability:

System suitability studies were carried out by injecting a 40mg/mL standard of Canagliflozin and 10μg/mL of Metformin respectively at injection volumes ranging from 10-50μL. With increment of injection volumes, the % RSD for tailing factor and theoretical plate number were calculated and were found to be within limits.

Assay

Assay of Canagliflozin and Metformin in tablets was performed by the proposed method and the % assay was calculated as an average of 3 determinations. These results indicate that the present HPLC method can be successfully used for the simultaneous assay of Canagliflozin and Metformin respectively in bulk and dosage forms. The assay values were found to be within the limits and the data was given in Table 3.

Formulation	Drug	Labled claim in mg	Amount Found mean±SD	%Assay	%RSD
Invokamet	Canagliflozin	50mg	47.69±0.11	98.2	1.19
	Metformin	500mg	498.51±0.34	99.8	1.2

Stability of the Stock Solution

The stability of the stock solution was determined by analyzing the samples under refrigeration (8±1°C) at different time intervals up to 48hrs. The % variation in assay values at different time intervals were found 0.825 for Canagliflozin and 0.546 for Metformin from the initial zero time interval solution, thus indicating that the solutions were stable for a period of 48hrs when stored at 8±1°C.

Conclusion

The proposed HPLC method was validated as per International Conference on Harmonisation (ICH) Guidelines, and can be used quality control analysis for the simultaneous estimation of Canagliflozin and Metformin using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective and simultaneous quantification of Canagliflozin and Metformin without interferences from diluent and placebo. Overall, the proposed method is highly sensitive, reproducible, reliable, rapid and specific and can be employed in quality control for simultaneous estimation of Canagliflozin and Metformin in bulk andin dosage forms that may available in near future.

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