RESEARCH ARTICLE

Development and Validation of UV-spectroscopic Technique for Simultaneous Estimation of Canagliflozin and Metformin Hydrochloride in Synthetic Mixture

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Received: 07th April, 2022; Revised: 27th August, 2022; Accepted: 06th September, 2022; Available Online: 25th September, 2022

ABSTRACT

Metformin and Canagliflozin are both anti-diabetic solid drugs. They reported harmonious action when delivered in combination. This research article established a cheap correct, rugged, and sensitive method to simultaneously estimate Canagliflozin and Metformin. The drug Canagliflozin and Metformin have absorption maxima at 290 nm and 232 nm, respectively. Canagliflozin and metformin in the developed method indicated calibration curve plot in the range of 8 to 12 µg/mL and 40 to 60 µg/mL having regression values of 0.997 and 0.988, respectively. The accuracy reading results data presented recovery of the standard drug in the range of 98–102% with a standard deviation of less than 2. Intra-day and inter-day reading effects were also obtained in the standard limits, presenting the precision of the procedure. Therefore, it could be concluded that the established technique was correct, precise, and exact with sufficient sensitivity and effectively understood for the simultaneous estimation of Canagliflozin and Metformin in their combined dosage during the routine study work.

Keywords: Simultaneous estimation, UV-spectroscopy, Vierodt's method, ICH Guidelines.

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.3.07

How to cite this article: Pathak S. Development and Validation of UV-spectroscopic Technique for Simultaneous Estimation of Canagliflozin and Metformin Hydrochloride in Synthetic Mixture. International Journal of Pharmaceutical Quality Assurance. 2022;13(3):261-266.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The biguanide family of anti-diabetics has one of the most popular drugs, metformin, which has anti-hyperglycemic activity. A relatively low level of lactic acidosis is linked with metformin. It helps lower low-density lipoprotein cholesterol and triglyceride levels is not linked to weight increase, and avoids cardiovascular diabetes problems. This drug is not digested and eliminates waste unaltered through the kidneys. It is the main agent used for managing type 2 diabetes (T2D) that can be used individually or in combination with others. Metformin has not been associated with serum enzyme rises for the duration of treatment and is an above-rare source of distinctive clinically seeming acute hepatic damage. The chemical formula is $C_4H_{11}N_5$, and the molecular weight is 129.164 g/mol. The structure is shown in Figure 1.

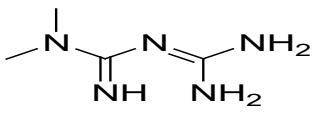

Figure 1: Metformin structure.

The IUPAC name of metformin is 3-(diaminomethylidene)-1, 1-dimethylguanidine. The Physicochemical Properties are:

- It is a white, hygroscopic crystalline powder with a bitter taste.
- It is easily soluble in water, somewhat soluble in alcohol, and almost insoluble in acetone and methylene chloride.
- The Melting point is 223–226°C.
- The drug's mechanism of action is it reduces glucose-making in the liver, reduces intestinal absorption of glucose, and increases insulin sensitivity by increasing peripheral glucose uptake and consumption. Its pharmacokinetic parameters are
- Absorption: It is given individually 1000 mg (2 tablets of 500 mg) taken after food, then Tmax (time to reach high plasma drug concentration) is attained at nearly 7–8 hours.
- Metabolism: there are no changes at the time of drug excretion in urine according to the intravenous single-dose analysis. No metabolism occurs in the liver. Renal clearance is about three times greater than creatinine clearance, showing that tubular secretion is the main method of metformin eradication. About 90% of the absorbed drug is eradicated by the renal route in the last

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one day through a plasma elimination half-life of nearly six hours. The elimination half-life is almost seventeen hours of blood.

- The side effects are faintness, diarrhea, acidity, dizziness, muscle pain, infection in the respiratory tract, low blood sugar, pain in the abdomen, deficiency of vitamin B-12, nausea, vomiting, chest uneasiness, constipation, heartburn.[1-4] Sodium-glucose co-transporter 2 inhibitor (Canagliflozin) is used in hyperglycemic patients. The FDA approved the drug on March 29, 2013. The Chemical Formula is $C_{24}H_{25}FO_5S$, and the molecular weight is 444.517 g/mol. The structure is shown in Figure 2.

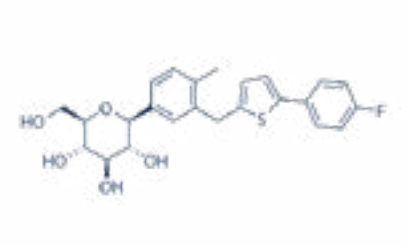

Figure 2: Canagliflozin structure.

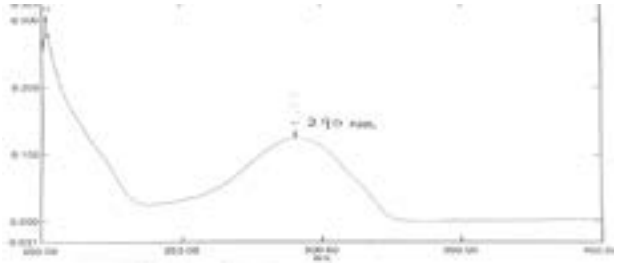

Figure 3: Absorption spectra of canagliflozi.

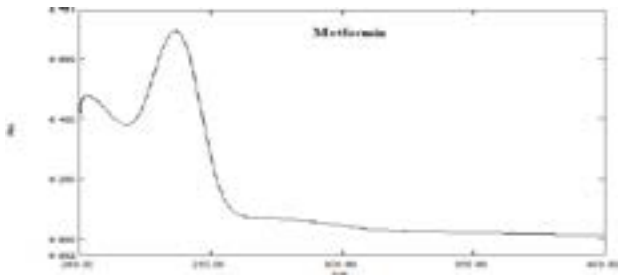

Figure 4: Absorption spectra of metformin hydrochloride.

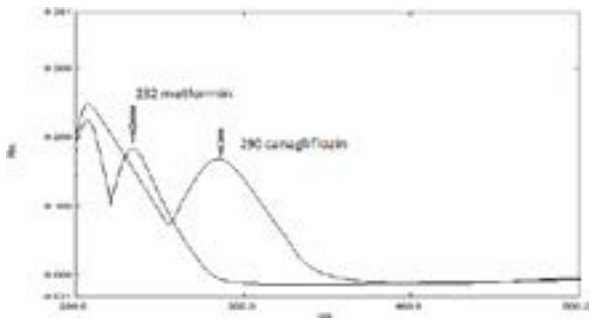

Figure 5: Absorption spectra of overlay of canagliflozin and metformin (100 µg/mL).

The IUPAC name is (2S,3R,4R,5S,6R)-2-(3-{[5-(4-fluorophenyl)thiophen-2-yl]methyl}-4-methylphenyl)-6-(hydroxymethyl)oxane-3,4,5-triol.

The physicochemical properties are:

- Canagliflozin drug is a white to off-white powder in color.
- It is soluble in different organic solvents like ethanol, methanol, tetrahydrofuran, and acetone but insoluble in water.
- Melting point: 68–72°C
- The drug's mode of action originates in the kidney's proximal tubules, and it reabsorbs filtered glucose from the renal tubular lumen. Canagliflozin inhibits SGLT2 co-transporter, leading to poor reabsorption of filtered glucose into the body and failing the renal threshold for glucose, most important to improved glucose excretion in the urine. Its pharmacokinetics parameters are:
- Absorption: The complete oral bioavailability of the drug is nearly 65% labeled. Steady-state concentrations are attained later, 4 to 5 days of daily dose management between 100 to 300 mg series.
- Protein binding: The primary component of canagliflozin is albumin. This medication contains 99% plasma protein binding.
- Metabolism: O-glucuronidation metabolism is used for this drug. Two inactive metabolites of O-glucuronide are predominantly glucuronidated by the enzymes UGT1A9 and UGT2B4.
- The side effects are urinating a lot, including at night, increased thirst, constipation, and dry mouth[5-7].

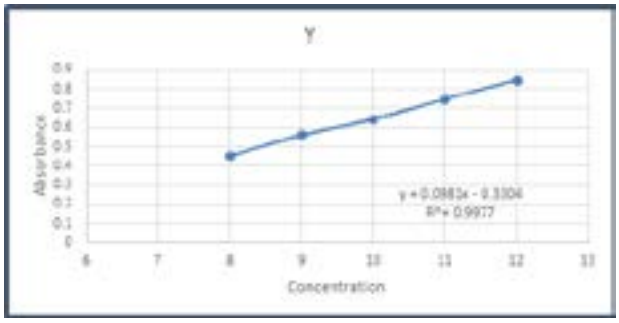

Figure 6: Calibration plot of canagliflozin at 290 nm.

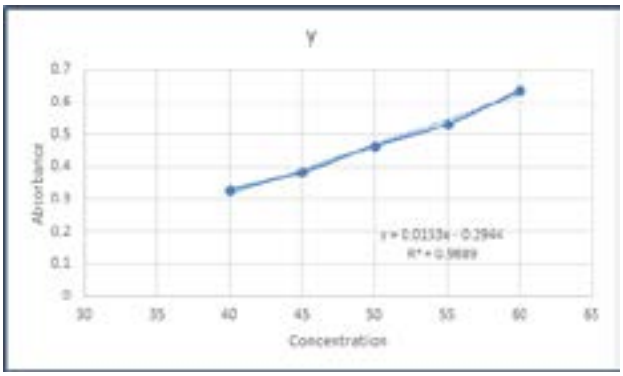

Figure 7: Calibration plot of metformin at 232 nm.

Table 1: Linearity for both drugs.

Canagliflozin		*Metformin*	
Concentration (µg/mL)	*290 nm*	*Concentration (µg/mL)*	*232 nm*
8	0.451	40	0.329
9	0.563	45	0.384
10	0.641	50	0.466
11	0.750	55	0.532
12	0.848	60	0.637

Table 2(a): Precision result (System) for both drugs.

Canagliflozin		*Metformin*	
Concentration (µg/mL)	*Absorbance*	*Concentration (µg/mL)*	*Absorbance*
10	0.644	40	0.325
10	0.642	40	0.326
10	0.640	40	0.328
10	0.641	40	0.328
10	0.644	40	0.327
10	0.641	40	0.325
Mean	0.642	Mean	0.3265
SD	0.00167	SD	0.00137
%RSD	0.15	%RSD	0.30

As per the literature survey there are many methods of UV-spectroscopy, as in individual canagliflozin[8,9] and metformin[10] drugs, but very few analytical papers are reported in a combination of both. One paper is from LC/MS analysis in human plasma for the same combination of drugs.[11] Another research is given by Attimarad *et al.* for the combination of metformin and remogliflozin.[12] Hassib *et al.* developed HPLC and UV methods for the three gliflozin drugs and Metformin with C-1 cyanoguanidine.[13,14] So, there is no simultaneous estimation of canagliflozin and metformin using eco-friendly solvent ethanol.

MATERIAL AND METHOD

Chemicals and Reagent

The canagliflozin was procured from CHEMSCENE, NJ, USA, and metformin was received as a gift sample from Aurobindo Pharmaceuticals, Hyderabad, India. The combinations of these two drugs (marketed formulation) tablets were procured from the local pharmacy. Ethanol was from Merk Specialties Pvt. Ltd., Mumbai. Analytical-grade chemicals were used throughout the studies. HPLC water from spectrochem was used to prepare all solutions.

Instrumentation

A UV-vis double beam spectrophotometer (UV-1800, Shimadzu), with 1-cm matched quartz cell, and UV probe software was used to analyze the result. All (weighing) was performed over 0.1 mg sensitivity citizen CX 220, and a sonicator (Hicon, model 1.5L (H)) was used in the study.

EXPERIMENTAL

Preparation of Stock Solution

Canagliflozin 50 mg (accurately weighed) was dissolved in 50 mL of ethanol in a volumetric flask (1000 µg/mL). 1-mL of the solution was diluted to 10 mL to give 100 µg/mL. Metformin 100 mg (accurately weighed) was separately dissolved in 100 mL of ethanol in a separations volumetric flask (1000 µg/mL). This was diluted in the same way as canagliflozin to give 100 µg/mL.

Selection of Working Wavelength

Both the solution of canagliflozin and metformin were suitable diluted to give 10 µg/mL. These were then separately scanned in a UV-spectrophotometer. Metformin hydrochloride showed absorption maxima at 232 nm (Figure 3), and canagliflozin showed absorption maxima at 290 nm (Figure 4).

One mL of each solution (100 µg/mL) of both, *i.e.,* canagliflozin and metformin, was taken in a ten mL volumetric flask in ethanol. This mixture was then scanned in the UV region. A mixture (100 µg/mL) of canagliflozin and metformin was then scanned and shown in Figure 5.

The wavelength selected was 290 and 232 nm. A set of two simultaneous equations was developed using the absorption coefficient.

Where Cx and Cy are concentrations of canagliflozin and metformin, respectively.

A_1 and A_2 are absorbances at 280 and 232 nm, respectively are the absorption coefficients of canagliflozin at 290 and 232 nm, respectively.are absorption coefficients of metformin at 290 and 232 nm, respectively.

Substituting the value of, equation can be recorded as

$A_1 = 0.098C_x + 0.015C_y$ at 290 nm 1

$A_2 = 0.120C_x + 0.030C_y$ at 232 nm 2

Validation of Method

The validation of analytical procedure concerning linearity, precision, accuracy, and ruggedness and tablet analysis was done according to ICH guidelines.[15,16]

Linearity

Further, various concentrations in the series of 8–12 µg/mL and 40–60 µg/mL for canagliflozin and metformin, respectively were prepared from stock solutions, and a calibration curve plot was prepared by taking the absorbance at respective scanning λ_{max} for both the drugs (Figures 6 and 7). The linearity table is shown in (Table 1).

Precision

It was resolute by various levels of drug dilutions, prepared from stock solutions, and examined (n = 6). In system precision, six replicates recordings of absorbance at 578 nm of 10 µg/mL were taken, and %RSD was calculated. The method precision was determined by performing an assay of the sample under tests of (i) Intra-day precision and (ii) Inter-day precision. The precision of the methods was found for 10 µg/mL samples within the limit (<2%RSD) proving the precision

Table 2(b): Precision results for both drugs

Concentration 10 µg/mL	*Intra-day study (Canagliflozin)*			*Inter-day study (Canagliflozin)*		
	Morning	*Afternoon*	*Evening*	*Day 1*	*Day 2*	*Day 3*
Avg. Abs	0.642	0.646	0.641	0.644	0.644	0.643
SD	0.001	0.001	0.001	0.002	0.003	0.002
%RSD	0.15	0.15	0.15	0.31	0.46	0.31
Concentration 40 µg/mL	*Intra-day study (Metformin)*			*Inter-day study (Metformin)*		
	Morning	*Afternoon*	*Evening*	*Day 1*	*Day 2*	*Day 3*
Avg. Abs	0.323	0.323	0.322	0.325	0.326	0.329
SD	0.004	0.001	0.002	0.001	0.002	0.002
%RSD	1.2	0.30	0.62	0.30	0.61	0.60

Table 3(a): Accuracy result for canagliflozin.

Concentration (10 µg/mL)			
Level	*Abs*	*%Recovery*	*Mean %recovery*
80	0.450	99.77	
80	0.451	100	99.99
80	0.452	100.22	
100	0.644	100.41	
100	0.642	100.15	100.29
100	0.643	100.31	
120	0.845	99.64	
120	0.848	100	99.80
120	0.846	99.76	

Table 3(b): Accuracy result for metformin

Concentration (40 µg/mL)			
Level	*Abs*	*%Recovery*	*Mean %recovery*
80	0.302	99.45	
80	0.303	98.98	99.54
80	0.301	100.21	
100	0.329	99.87	
100	0.328	99.43	99.77
100	0.327	100.02	
120	0.337	99.87	
120	0.338	99.98	100.06
120	0.339	100.34	

of the methods. Various levels of drug dilution in replicates were prepared various times in a day and calculated for intra-day study. The percent relative standard deviation (%RSD) should be less than 2, and all results are mentioned in (Tables 2a and 2b).

Accuracy

It is the nearness of the measured value to the correct value for the sample. To calculate this parameter of the planned process, recovery readings were calculated by standard addition method, which incorporated the addition of various concentrations of the respective drugs to a well-known pre-considered preparation sample, and the total concentration

Table 4: Ruggedness results for both drugs

Concentration 10 µg/mL (Canagliflozin)			
Analyst	*Abs*	*SD*	*%RSD*
Analyst 1	0.642		
0.643		0.0005	0.07
0.643			
Analyst 2	0.644		
0.644		0.0011	0.17
0.642			
Concentration 40 µg/mL (Metformin)			
Analyst	*Abs*	*SD*	*% RSD*
	0.329		
Analyst 1	0.328	0.0005	0.5
	0.329		
	0.325		
Analyst 2	0.322	0.0017	0.5
	0.325		

was calculated by the planned method. The percent recovery of the added drug was measured as follows:

$$\% \text{ recovery of cana/met} = [(Ct - Cs)/Ca] \times 100$$

Ct is the total drug concentration measured after standard addition Cs drug concentration in the formulation sample Ca drug concentration was added to the formulation. So the accuracy was determined by performing recovery experiments, in which the mean %recovery of samples was carried out at three different levels (80–120%). The observed recovery was found to be between 98.81–101.8% (Tables 3a and 3b).

Ruggedness

Ruggedness was determined by performing the same analysis procedure carried out by two analysts for the canagliflozin and metformin drugs. The results were then compared. In this method %RSD was found to be less than two, which established the ruggedness of the procedure. The results were reported in Table 4.

Analysis of Marketed Formulation

Ten tablets were weighed at random, and the average weight of the tablet was determined. These were then extracted with

Table 5: Tablet analysis of canagliflozin and metformin.

S. no	Absorbance		Amount found per tablet (mg)		Amount claimed per tablet (mg)	
	A_1 290 nm	A_2 232 nm	Canagliflozin	Metformin	Canagliflozin	Metformin
1	1.242	2.104	50.00	501.32	50	500
2	1.243	2.106	50.00	500.00	50	500
3	1.245	2.105	50.65	506.50	50	500
4	1.241	2.105	49.60	496.00	50	500
5	1.245	2.105	50.65	506.50	50	500
6	1.246	2.107	50.65	506.50	50	500

Table 6: Summary of optical characteristics of the UV method

Parameters	Result	
	Canagliflozin	Metformin
Detection wavelength (nm)	290 nm	232 nm
Beer's Law limits(µg/mL)	8–12	40–60
Regression equation	y = 0.0981x - 0.3304	y=0.0153x-0.2944
Correlation coefficient	R^2 = 0.9977	R^2 = 0.9889
Slope (m)	0.0981	0.0153
Intercept (c)	0.3304	0.2944
Precision (% RSD)		
Intra-day	<2	<2
Inter-day	<2	<2
Accuracy (%mean recovery)		
80% level	99.99	99.54
100% level	100.29	99.77
120% level	99.80	100.06
Ruggedness		
2 Analyst (% RSD)	<2	<2

ethanol and filtered, and finally, volume was made up. Further dilutions were made with ethanol to form a working standard solution corresponding to 5 µg/mL of canagliflozin and 50 µg/mL of metformin. Aliquots of definite concentration were taken from this working standard solution in six replicates in six 10 mL volumetric flasks (in Beer's Lambert's Law limit). The volumes were made, and absorbance was noted at 290 and 232 nm. These data are incorporated in Table 5.

Optical Characteristics of the Visible Spectrophotometric method

The summary of optical characteristics and validation parameters of the visible method is shown in Table 6.

RESULT AND DISCUSSION

In the present case, one method was developed (based on a simultaneous equation). This study applied the partial simultaneous equation method (Vierodt's method).[17] The wavelength selected was 290 and 232 nm. The equation developed was

$$A_1 = 0.0643Cx + 0.00662Cy$$

$$A_2 = 0.0242Cx + 0.00924Cy$$

using this equation, the concentration of canagliflozin and metformin were estimated in commercial formulation. The analysis results showed lower values of standard deviation, standard error of the mean, coefficient of variation, and percentage range of error (within 95% confidence limit) and thus showed a precision of methods.

The linearity was found at 8–12 µg/mL and 40–60 µg/mL for canagliflozin and metformin, respectively. To test the accuracy and reproducibility, recovery experiments were performed. The percentage recovery was close to 100% for this method. The low standard deviation value indicated the method's repeatability, accuracy, and reproducibility. The system and method precision was found within limits according to ICH guidelines.[18-21] Ruggedness was found to be less than 2% relative standard deviation. The developed procedure was applied to the marketed formulation of the composition of managliflozin 50 mg and metformin 500 mg. The analysis obtained was in good uniformity in the claimed amount in the marketed sample. Thus it can be concluded that the methods developed were simple, accurate, sensitive, and precise. Hence the above method can be applied successfully in the simultaneous estimation of canagliflozin and metformin in marketed formulation.

CONCLUSION

The established process was appropriate for simultaneous estimating canagliflozin and metformin in tablet formulation. The established process is cost-effective, precise, and accurate. Recovery analysis indicated that there is no interference of excipients. In the future, this method can be used in quality control and routine analysis of the finished formulation.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENT

The author thanks the institute of pharmaceutical research, GLA University, Mathura, India, for providing the facilities for the research work.

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