Development and Validation of an RP-HPLC Method for the Simultaneous Estimation of Canagliflozin and Metformin in Tablet Dosage Form

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Abstract

The investigation's goal was to create a novel HPLC technique for measuring both metformin and canagliflozin simultaneously in pharmaceutical dose forms. Using an ODS 250mm x 4.6mm, 5 particle size, chromatography was performed using an isocratic mobile phase made up of buffer, acetonitrile, and methanol at a flow rate of 1mL/min. A PDA detector operating at 212 nm was used for detection while maintaining a column temperature of 30 °C. According to the International Conference on Harmonization's criteria, validation factors such system appropriateness, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), stability of sample and standard stock solutions, and resilience were evaluated. Metformin and canagliflozin had retention durations of 2.783 and 3.781 minutes, respectively. Metformin and canagliflozin had percentage recoveries of 100.1% and 100.2%, respectively. The analysis of tablets revealed a relative standard deviation of less than 2%. The approach may be used for regular quality control of tablets containing both medications in quality control labs and pharmaceutical enterprises since it is quick, accurate, precise, and sensitive.

Keywords: Metformin,Canagliflozin,Simultaneousestimation,ICHguidelines.
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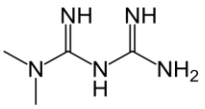
INTRODUCTION

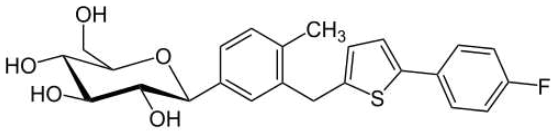
Chemically, metformin (MET) is known as N,N-dimethylimidodicarbonimidic diamide (Fig.1). The first-line oral medication for type 2 diabetes is metformin. Metformin's primary mechanism of action is thought to include activating the energy-regulating enzyme AMP-activated protein kinase (AMPK), which is mostly found in muscle and the liver [1,2].

Chemically, canagliflozin (CANA) is known as (2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl)thiophen-2- yl]methyl]-4-methylphenyl]-6-(hydroxymethyl)3,4,5-triol oxane (Fig.2). Diabetes type 2 is treated with canagliflozin. Na+-dependent 14C- AMG absorption is inhibited by canagliflozin in a concentration-dependent manner. It is a brand-new thiophene-ringed C-glucoside.

For the assessment of metformin and canagliflozin alone and in combination with other medications, several UV & HPLC test techniques are also documented in the literature [3-6]. There is no approved technique for the simultaneous measurement of Metformin and Canagliflozin by RP-HPLC in combination tablet dosage forms, according to literature review. In order to comply with the ICH standards, an effort has been undertaken to create a novel approach for the simultaneous estimate and validation of Metformin and Canagliflozin in tablet formulation.



Metformin



Canagliflozin

Figure 1. Chemical structures of drugs.

1. RESOURCES AND METHODS

1.1 Instrumentation

Chromatography was carried out using an Alliance Waters 2695 HPLC equipped with a high-speed auto sampler, column oven, degasser, and 29 96 PDA detector to create a class-leading, portable LC system.

1.2 Chemicals and reagents

The reference samples for MET and CANA were give n as freebies by Hyderabadbased Spectrum Pharma Research Solutions.
All other chemicals, including HPLC grade acetonitril e and Methanol, were purchased from Merck Chemi cal Division inBombay. During the investigation, HPL C grade water from the MilliQ water purification sys tem was utilized. The neighborhood pharmacy sold commercial pills (INVOKAMET; Dosage: MET-500 mg & CANA-50 mg).

1.3. Chromatographic state

Buffer, acetonitrile, and methanol were added to th e mobile phase at a flow rate of 1 mL/min in the pro portions of 40:40:20.
As the stationary phase, ODS 250mm x 4.6mm, 5 pa rticle size was used. 212 nm was chosen as the dete ction wavelength for PDA detector even though the MET and CANA had different max due to differences in the chromatographic parameter, sensitivity, and selectivity of the technique for both medicines.

1.4 Making the usual stock solution

To make standard stock solutions, 50 mg of metfor min and 10 mg of canagliflozin were dissolved into c lean, dry volumetric flasks measuring 25 mL and 50 mL, respectively. To these flasks, 15 mL of diluent w as then added, and they were sonicated for five min utes to produce stock solutions.

Creating Working Standard Solutions, 1.5.Pipette ou t aliquots of 0.25, 0.5, 0.75, 1, 1.25, and 1.5 mL from stock solution into separate 10 mL volumetric flasks for MET and CANA, and then add diluent to get the volume to 10 mL.
This results in solutions for Metformin of 50, 100, 15 0, 500, 250, and 300 g/mL and for Canagliflozin of 5, 10, 15, 20, 25 and 30 g/mL, respectively.

1.5 Example setup

After being weighed, twenty pills were crushed into a fine powder.
Five pills' worth of powder was weighed, diluted in 250 mL of diluent, sonicated for 20 minutes, and the n passed through a PVDF 0.45 filter.
1 mL of the filtrate was pipetted into a 10 mL volum etric flask and the solution was diluted to the requir ed volume.

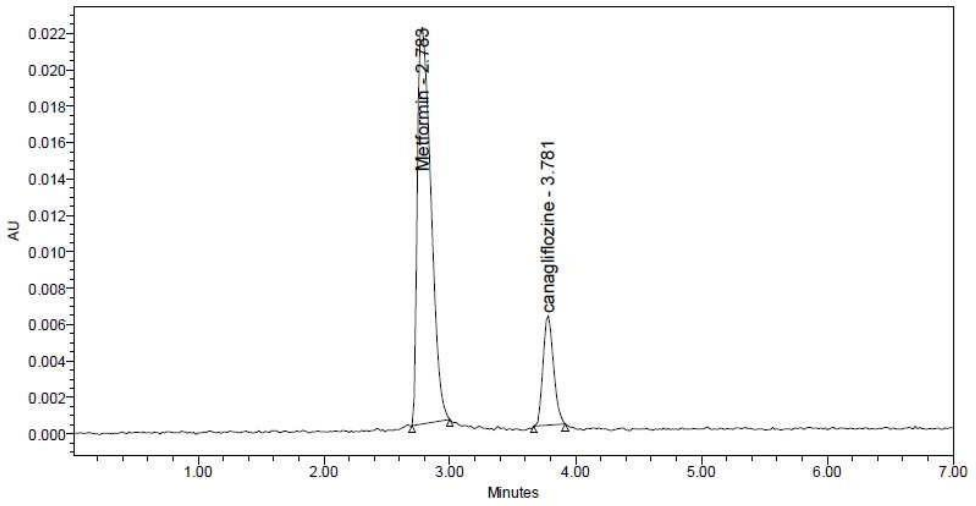


Figure2: AtypicalChromatogramofMetforminandCanagliflozin.

2. Results and discussion,

2.1 Development of a method

Originally, different ratios of acetonitrile and water and methanol and water were used as the mobile phases in an effort to establish reverse phase liquid chromatography separation. Nevertheless, neither medication behaved as expected, and the resolution was also subpar. To maximize the separation of both medicines, the organic content of mobile phase was also examined. The mobile phase's pH is a key element in enhancing the tailing effect. Both medicines separated more clearly at a pH of 4.5. After that, buffer, acetonitrile, and methanol were added in the following isocratic ratios: 10/50/40, 15/50/35, 20/40/40, 35/40/25, and 40/40/20, at a flow rate of 1mL/min. To increase resolution, ODS 250mm x 4.6mm, 5. particle size was chosen as the stationary phase, and the tailing of both peaks was significantly decreased and brought near to 1. The detection of both medicines was tested at different wavelengths between 205 and 280 nm. The wavelength of 212 nm was chosen as the PDA detector's detecting wavelength since it was the wavelength at which both MET and CANA displayed their highest absorption. For MET and CANA, the retention periods were found to be around 2.783 min and 3.781 min, respectively. The chromatogram that was produced was shown in Figure 2.

Table1: SystemsuitabilityofMETandCANA

SYSTEMSUITABILITYPARAMETERS	MET	CANA
Nooftheoreticalplates	3088	9339
TailingFactor	1.62	1.19
Resolution	-	5.5
RT	2.853min	3.781min
MeanArea	5374289	866114
RSD	0.51	0.7

Table 2: Results of accuracy of MET and CANAPre analyzed sample solutionconc. (ug/ml) tandard drug conc. (ug/ml)
amount recovered(ug/ml) Recovery (%)

MET	CANA	MET	CANA	MET	CANA	MET	CANA
200	20	100	10	97.856	10.07	98.813	100.714
200	20	100	10	102.63	9.97	101.153	99.452
200	20	100	10	100.85	10.07	100.262	100.63

200	20	200	20	200.36	20.14	100.314	100.72
200	20	200	20	199.36	20.22	99.369	101.10
200	20	200	20	199.58	20.38	99.895	101.91
200	20	300	30	297.03	30.14	99.021	100.36
200	20	300	30	298.79	30.01	99.895	100.05
200	20	300	30	301.05	30.453	100.236	101.51
					MEAN	99.9	100.74
					SD	0.230384	0.451193
					%RSD	0.22	0.45

2.2 System appropriateness and specificity:

2.2.1 Method Validation

The number of theoretical plates, peak tailing, retention period, and resolution factor were all identified as system appropriateness criteria. Just 7 minutes of total run time are needed for the technique to elute both MET and CANA. The results were shown in Table 1.

2.2.2 Linearity

The linearity of response for MET was between 50 and 300 g/mL, whereas that for CANA was between 5 and 30 g/mL. They were represented by the following linear regression equation: The regression line was formed using the least squares approach, and the correlation coefficient (r2) for MET and CANA is determined to be larger than 0.98. y (MET) = 26632x + 519.3 (r2=0.999), and y (CANA) = 43142x + 222.8 (r2=0.999). Hence, the established curves were linear.

2.2.3 Accuracy

A specific concentration of the standard drug—at levels of 50%, 100%, and 150%—was added to the pre-analyzed sample solution, and recovery was examined. The % Mean recovery for MET and CANA are respectively 99.9 and 100.74, and these findings are within the acceptable range of 98-102. The percent RSD for MET and CANA is 0.23 and 0.44, respectively, and the percent RSD for ACB and MET is within the allowed range of 2. As a consequence, the suggested approach is correct, and Table 2 provides a summary of the findings.

Table3: ResultsofPrecisionforMETandCANA

	Repeatability		**Intermediate precision**	
S.No.	**Metformin**	**Canagliflozin**	**Metformin**	**Canagliflozin**
1	5376766	867867	5767564	877148
2	5389765	889878	5421475	871586
3	5387675	864657	5351865	864288
4	5377852	868459	5377869	868475
5	5364645	868565	5364859	862589
6	5476545	877845	5478453	878698
Mean	5389763	869041.3	5397129.67	870464
Std.Dev.	319087.7	6871.4	38297.53	6600.731
%RSD	0.58	0.73	0.70	0.75

SD=StandarddeviationRSD=Relativestandarddeviation

Table4: ResultsofRobustnessforMET

Columntemperature(ºc)			**Mobilephasecomposition**			
Evaluationparameters	**1.1**	**0.9**	**35**	**25**	**+5%**	**-5%**
MeanRT	2.58	2.98	2.39	2.82	2.77	2.80
Meanarea	5392954	5403684	5318465	5487862	5245689	5785459
SD	26458.3	24359.24	30245.86	31654.10	29845.49	28465.71

	3					
RSD	0.5	0.5	0.6	0.57	0.56	0.49
Tailingfactor	1.42	1.72	0.99	1.05	1.27	1.12
No.oftheoretical plates	5647	5846	6485	5896	5706	7861

Table5: ResultsofRobustnessforCANA

Columntemperature(^{O}c)			**Mobilephasecomposition**			
Evaluationparameters	**1.1**	**0.9**	**35**	**25**	**+5%**	**-5%**
MeanRT	3.83	3.70	3.76	3.64	3.96	3.45
Meanarea	842657	849365	839878	865478	790875	865479
SD	5414.5	5936.8	5684.3	6247.3	6384.54	6485.84
RSD	0.64	0.69	0.56	0.74	0.81	0.72
Tailingfactor	1.61	1.33	1.10	1.25	1.32	0.94
No.oftheoreticalplates	8465	9898	8364	8990	9364	8024

2.2.4 Precision and Repeatability

Six replicate injections of the same concentration were examined for repeatability on the same day, and the results are shown in Table 3. The% RSD for MET and CANA were found to be 0.58 and 0.73, respectively, and the% RSD for MET and CANA found to be within acceptable limit of 2, indicating that the method is reproducible.

2.2.5Intermediate Accuracy

In order to validate the variation in accuracy, six duplicate injections of the same concentration were tested on two separate days with a different analyst and column. The% RSD for MET and CANA was determined to be 0.70 and 0.75, respectively, and it is within the permissible range of 2. As a result, the approach may be used with a different analyst and column on various days. This demonstrates the accuracy of the procedure, and Table 3's data attest to this.

2.2.6 Robustness

By varying the flow rate, column temperature, and mobile phase composition while staying within safe bounds from real chromatographic circumstances, the robustness was established. It was noted that the mean Rt had not significantly changed, and the RSD was within the acceptable range of 2. For both MET and CANA, it is determined that the tailing factor, resolution factor, and number of theoretical plates are within acceptable bounds. The findings of MET are provided in Table 4 and those of CANA are shown in Table 5, indicating that the approach is dependable despite changes in the analytical circumstances[8].

Table6: ResultsofstocksolutionstabilityforMETandCANA

Drug	**%Assayat0 hr**	**%Assayat24hr**	**%Deviation**
MET	100.1	99.24	0.86
CANA	100.2	100.86	0.69

Table7: ResultsofHPLCAnalysisofTabletforMETandCANA

RSD

No.ofsampleassayed	**Label amount(mg)**		**Amountfound(mg)**		**%Assay(Mean±SD)**		RSD	
6	MET	CANA	MET	CANA	MET	CANA	MET	CANA

	500	50	200.2	20.04	100.1±1.098	100.2±1.002	0.6	0.73

2.2.7 Solution stability in the sample

The sample and standard solutions were injected at 0 hours (for the comparison sample) and after 24 hours (for the stability sample), both times at 30 degrees Celsius. The RSD for sample and standard solutions of MET at 0 hours and 24 hours is 0.58, 0.74, and 0.51, 0.96, respectively. The RSD for sample and standard CANA solutions at 0 and 24 hours is 0.73, 0.7, and 0.7, 1.21, respectively. The sample and standard stock are stable for 24 hours at room temperature thanks to RSD findings for both MET and CANA that are under the limit of 2. The results are shown in Table 7.

2.2.8 LOQ and LOD

LOD and LOQ were 0.034 and 0.102 g/mL for MET and 0.034 and 0.102 g/mL for CANA, respectively. The LOD and LOQ's lowest valuesas determined by the suggested procedure suggest that the approach is accurate.

1. Tablet Evaluation

The suggested approach was used to determine the pills' MET and CANA contents. The findings were shown in Table 8 and the RSD values for both MET and CANA were within the limit of 2.

CONCLUSION

For the simultaneous measurement of metformin and canagliflozin in tablet dose form, a novel exact accurate and straightforward HPLC approach was developed and validated. This technique may be used for regular quality control of tablets containing both medications in QC labs and industries since it is quick, accurate, precise, and sensitive[9-10].

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