



**ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE  
SIMULTANEOUS ESTIMATION OF CANAGLIFLOZIN AND METFORMIN IN DRUG  
PRODUCT BY RP-HPLC**

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**ABSTRACT**

The purpose of the research is to develop a simple, precise, economical, accurate, reproducible, and sensitive method for the estimation of Metformin and Canagliflozin drug product by rp-hplc method. The chromatographic separation was achieved on C18 column (Inertsil ODS 3V C18 250\*4.6mm) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v TFA in water: Acetonitrile (20:80). The flow rate was 1.0ml/minute and ultra violet detector at 254nm. The average retention time for Metformin and Sacubital found to be 2.32 min and 4.20 min. the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 200.0 – 600.0µg/ml for Metformin and 20.0 – 60.0µg/ml of Canagliflozin.

**KEYWORDS:** Metformin and Canagliflozin, Isocratic, HPLC, Inertsil ODS, TFA, Acetonitrile, Methanol and validation.

**METFORMIN**

**Metformin**, marketed under the trade name **Glucophage** among others, is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome. Limited evidence suggests metformin may prevent the cardiovascular disease and cancer complications of diabetes. It is not associated with weight gain. It is taken by mouth.

**Metformin** is chemically designated as N,N-Dimethylimidodicarbonimidic diamide. Its molecular formula is C<sub>4</sub>H<sub>11</sub>N<sub>5</sub>, and its molecular weight is 129.16364 g/mol.

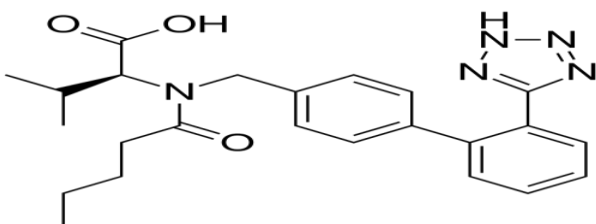


Fig. 1: Structure of Metformin.

**CANAGLIFLOZIN**

**Canagliflozin** is a medication used for the treatment of type 2 diabetes. It is of the gliflozin class or subtype 2 sodium-glucose transports (SGLT-2) inhibitors class. This mechanism is associated with a low risk of hypoglycemia (too low blood glucose) compared to sulfonylurea derivatives and insulin. In 2017, the FDA concluded that canagliflozin causes an increased risk of leg and foot amputations. The FDA began requiring a Boxed Warning to be added to the canagliflozin drug labels to describe this risk.

**Canagliflozin** is chemically designated as (2*S*,3*R*,4*R*,5*S*,6*R*)-2-({3-[5-[4-Fluoro-phenyl]-thiophen-2-ylmethyl]-4-methyl-phenyl}-6-hydroxymethyl-tetrahydro-pyran-3,4,5-triol). Its molecular formula is C<sub>24</sub>H<sub>25</sub>FO<sub>5</sub>S, and its molecular weight is 444.52 g/mol.

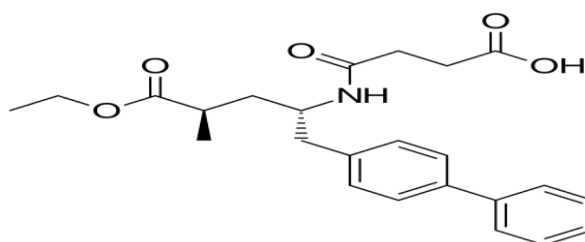


Fig. 2: Structure of Canagliflozin.

## EXPERIMENTAL

**Equipments:** The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (Inertsil ODS 3V 250\*4.6,5 $\mu$ ) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vaccum micro filtration unit with 0.45 $\mu$  membrane filter was used in the study.

**Materials:** Pharmaceutically pure sample of Metformin/Canagliflozin were obtained as gift samples from Fortune pharma training institute, sri sai nagar colony, KPHB, Hyderabad, India.

HPLC-grade Methanol was from qualigens reagents pvt ltd. Trifluoro acetic acid (AR grade) was from sd fine chem.

**Chromatographic conditions** The sample separation was achieved on a (5  $\mu$ , 250 cm X 4.6 mm i.d.) Inertsil ODS 3V C18 column, aided by mobile phase mixture of 0.1%v/v TFA in water : Acetonitrile (20:80). The flow rate was 0.8 ml/ minute and ultra violet detector at 254nm, that was filtered and degassed prior to use, Injection volume is 10  $\mu$ l and ambient temperatures.

### Preparation of mobile phase

**Buffer Preparation:** Taken accurately 1ml of Trifluoro acetic acid in 1000mL of water.

**Mobile phase:** Then added 20 volumes of buffer and 80 volumes of Acetonitrile mixed well and sonicated for 5 min.

**Diluents:** Water: Methanol 50:50 v\v.

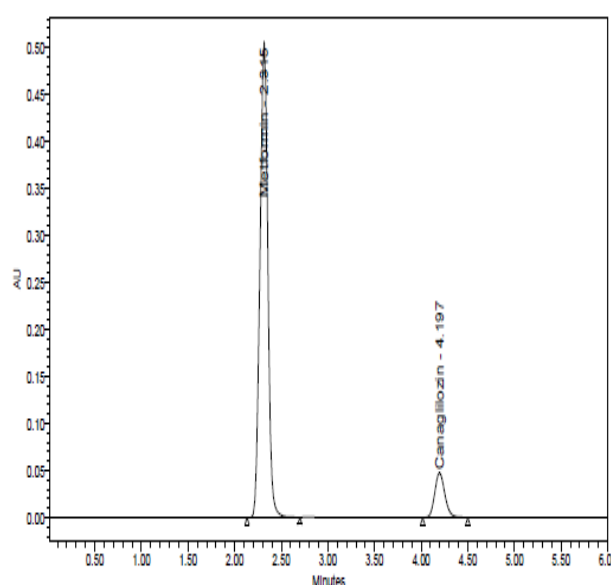
**Preparation of standard stock solution:** A 500 mg of pure Metformin and 50 mg of Canagliflozin were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution 0.4ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with water to give a solution containing 400 $\mu$ g/ml of Metformin and 40  $\mu$ g/ml Canagliflozin.

**Preparation of sample solution:** Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 500mg of Metformin and 50mg of Canagliflozin sample were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the filtered solution 0.4 ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 400  $\mu$ g/ml of Metformin and 40  $\mu$ g/ml Canagliflozin.

## RESULTS AND DISCUSSIONS

**Determination Of Working Wavelength ( $\lambda$  max):** 10 mg of the Metformin and Canagliflozin standard drug is taken in a 10 ml volumetric flask and dissolved in Diluent and volume made up to the mark, from this solution 0.1ml is pipette into 10 ml volumetric flask and made upto the mark with the Water to give a concentration of 10  $\mu$ g/ml. The above prepared solution is scanned in uv between 200-400 nm using diluent as blank. The  $\lambda$ max was found to be 254nm.

After several initial trails with mixtures of methanol, water, Aectonitrile and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v TFA in water: Methanol (20:80). The flow rate was 0.8 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1.



**Fig. 3: Chromatogram of Metformin/Canagliflozin.**

### METHOD VALIDATION

**System suitability:** The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5 and theoretical plates >3000. The retention time, peak area, theoretical plates and tailing factor were evaluated for system.

**Table. 1: System suitability data of Metformin and Canagliflozin.**

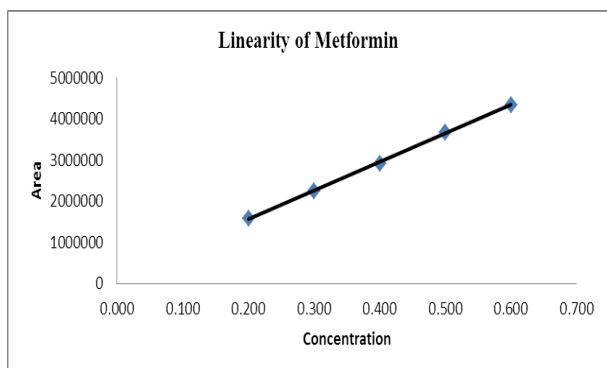
Parameter	Metformin	Canagliflozin	Acceptance criteria
Retention time	2.315	4.198	+ -10
Theoretical plates	3458	7148	>3000
Tailing factor	1.01	1.15	<1.50
% RSD	0.19	0.45	<2.00

**Linearity:** Linearity was studied by analyzing five standard solutions covering the range of 200.0 - 600.0µg/ml for Metformin and and 20.0 -60.0µg/ml Canagliflozin. From the primary stock solution 0.2ml,0.3ml,0.4ml,0.5ml,0.6 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the water to give a concentrations of 200.0 µg /mL, 300.0µg/mL, 400.0µg/mL, 500.0µg/mL and 600.0 µg/mL of Canagliflozin and 20.0g/mL,30.0µg/mL, 40.0µg/mL, 50.0µg/mL and 60.0 µg/mL Metformin.

Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

**Table. 2: Linearity data of Metformin.**

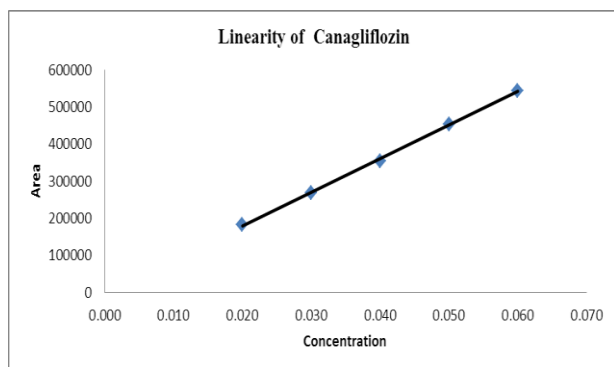
Level	Concentration (mg/mL)	Peak area
50%	0.200	1569160
75%	0.300	2250628
100%	0.400	2915279
125%	0.500	3676609
150%	0.600	4331865



**Fig. 4: Calibration curve of Metformin.**

**Table. 3: Linearity data of Canagliflozin.**

Level	Concentration (mg/mL)	Peak area
50%	0.020	182344
75%	0.030	267883
100%	0.040	353200
125%	0.050	454430
150%	0.060	543891



**Fig. 5: Calibration curve of Canagliflozin.**

**RESULT**

A linear relationship between peak areas versus concentrations was observed for Metformin and Canagliflozin in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9998 and 0.9995 for both Metformin and Canagliflozin which prove that the method is linear in the range of 50% to 150%.

**Limit of detection and limit of quantification**

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

**LOD = 3.3 σ /S ..... (1)**

**LOQ =10 σ /S ..... (2)**

Where,

σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte.

**Table. 4: LOD and LOQ values Calculated from calibration curve.**

	Metformin mg	Canagliflozin mg
<b>LOD</b>	0.013	0.002
<b>LOQ</b>	0.040	0.006

**Method precision (repeatability)**

The precision of the method was checked by repeated preparation(n=6) of 400.0µg/ml of Metformin and 40.0µg/ml Canagliflozin without changing the parameter of the proposed chromatographic method. And measure the peak areas and retention times.

**Table. 5: Summary of peak areas for method precision of Metformin.**

Sample No	Retention time	Peak area	% Assay
1	2.313	2949856	99.5
2	2.314	2957506	99.8
3	2.313	2953307	99.2
4	2.314	2957817	99.4
5	2.312	2940638	98.8
6	2.312	2951211	99.4
Mean	2.313	2951723	99.4
%RSD	<b>0.04</b>	<b>0.21</b>	<b>0.32</b>

**Table. 6: Summary of peak areas for method precision of Canagliflozin.**

Sample No	Retention time	Peak area	% Assay
1	4.198	355994	99.6
2	4.199	356973	99.9
3	4.199	356533	99.6
4	4.199	356393	99.1
5	4.198	354983	98.7
6	4.198	356975	99.9
Mean	4.199	356309	99.5
%RSD	<b>0.01</b>	<b>0.21</b>	<b>0.46</b>

**RESULT**

Results of variability were summarized in the above table. Percentage relative standard deviation (%RSD) was found to be less than 2.0% which proves that method is precise.

**Accuracy (recovery study)**

The accuracy of the method was determined by calculating the recoveries of Metformin and Canagliflozin by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Metformin and Canagliflozin. The percentage recovery results obtained are listed in Table 7 & 8.

**Table. 7: Recovery data of Metformin.**

LEVEL	S.No	%Recovery of Metformin	Average
50	1	99.1	99.3%
	2	99.2	
	3	99.6	
100	1	99.5	99.5%
	2	99.8	
	3	99.2	
150	1	99.7	99.0%
	2	98.8	
	3	98.5	

**Table. 8: Recovery data of Canagliflozin.**

LEVEL	S. No	%Recovery of Canagliflozin	Average
50	1	99.5	99.6%
	2	99.1	
	3	100.2	
100	1	99.6	99.7%
	2	99.9	
	3	99.6	
150	1	99.5	99.3%
	2	99.3	
	3	99.2	

**RESULT**

Results of accuracy study are presented in the above table. All the results indicate that the method is highly accurate.

**Robustness:** Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied  $\pm 2$ nm and flow rate was varied  $\pm 0.2$  ml/min. The results were shown in (Table no. 9&10).

**Table. 9: Results of of Metformin.**

parameter	Rt of Metformin	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	2.631	3657	1.01
Increased flow rate (0.9ml/min)	2.061	3371	1.01
Wave Length 252nm	2.315	3474	1.01
256	2.315	3475	1.01

**Table. 10: Results of of Canagliflozin.**

parameter	Rt of Canagliflozin	Theoretical plates	Asymmetry
Decreased flow rate (0.7ml/min)	4.784	7363	1.17
Increased flow rate (0.9ml/min)	3.741	6730	1.15
Wave Length 252nm	4.197	7250	1.15
256	4.199	7165	1.15

**RESULT**

The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust.

**Ruggedness:** The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The results were shown in Table no.11&12.

**Table. 11: Results of Metformin.**

		%Assay	%RSD
Analyst-1	METFORMIN	99.5	0.21%
Analyst-2		99.8	

**Table. 12: Results of Canagliflozin.**

		%Assay	%RSD
Analyst-1	CANAGLIFLOZIN	99.6	0.21%
Analyst-2		99.9	

**RESULT**

The %RSD assay values between two analysts was calculated, this indicates the method was rugged.

**Table. 13: Summary of Metformin.**

S. NO	PARAMETER	RESULT	Acceptance Criteria
1	System suitability		
	Retention time	2.315	Not less than 2000
	Theoretical plates	3458	Not more than 2.0
	Asymmetry	1.01	
	%RSD	0.19	Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.21	Not more than 2.0%
4	Linearity Range(mcg/ml)	200.0- 600.0	
	Correlation coefficient( $r^2$ )	0.9998	Not less than 0.990
5	Accuracy (Mean % recovery)		
	50%	99.3	97 - 103%
	100%	99.5	
	150%	99.0	
6	Robustness	All the system suitability parameters are within the limits.	

\*RSD = Relative standard deviation

**Table. 14: Summary of Canagliflozin.**

S. NO	Parameter	Result	Acceptance Criteria
1	System suitability	4.198	Not less than 2000
	Retention time	7148	Not more than 2.0
	Theoretical plates	1.15	
	%RSD	0.45	Not more than 2.0
2	Specificity	Specific	Specific
3	Method precision(%RSD)	0.21	Not more than 2.0%
4	Linearity Range(mcg/ml)	20.0- 60.0	
	Correlation coefficient( $r^2$ )	0.9995	Not less than 0.990
5	Accuracy (Mean % recovery)		
	50%	99.6	97 - 103%
	100%	99.7	
	150%	99.3	
6	Robustness	All the system suitability parameters are within the limits.	

\*RSD = Relative standard deviation

**CONCLUSION**

From the above experimental results it was concluded that, this newly developed method for the simultaneous estimation of METFORMIN and CANAGLIFLOZIN was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be

effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

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