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Development and Validation RP-HPLC Method for Estimation of Antidiabetic Drugs in Pharmaceutical Dosage Form

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Abstract: *This study details the development and validation of an RP-HPLC method for estimating Canagliflozin and Metformin in pharmaceutical dosage forms. Canagliflozin, an SGLT2 inhibitor, and Metformin, an antihyperglycemic agent, were obtained from Arti Drug Pvt. Ltd. Marketed formulation Invokamet was sourced locally. Chromatographic separation was achieved on a C18 column (250 x 4.6 mm, 5 µm) using a mobile phase of Methanol: Water (80:20) with 0.1% ortho phosphoric acid at a 1.0 ml/min flow rate and detection at 254 nm. The retention times for Canagliflozin and Metformin were 8.183 min and 3.633 min, respectively. Method validation parameters included system suitability, accuracy, precision, and robustness. System suitability tests confirmed adequate resolution and repeatability. Accuracy was evaluated through recovery studies at 80%, 100%, and 120% concentration levels, yielding satisfactory results. Precision was demonstrated with intra-day and inter-day % RSD values below 2. Robustness was assessed by varying mobile phase composition and flow rate, showing consistent retention times and tailing factors. The method proved selective, accurate, precise, and robust, with a short run time, facilitating rapid quantification of samples. It is suitable for routine quality control analysis of Canagliflozin and Metformin in pharmaceutical formulations*

Keywords: RP-HPLC, Canagliflozin, Metformin, pharmaceutical dosage forms, method validation, accuracy, precision, robustness.

I. INTRODUCTION

Canagliflozin (CAN) is chemically designated as (2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl)thiophen-2-yl]methyl]-4-methylphenyl]-6-(hydroxymethyl)oxane-3,4,5-triol. It belongs to a class of medications known as sodium-glucose co-transporter 2 (SGLT2) inhibitors.[1,2] SGLT2 is a protein that plays a crucial role in renal glucose reabsorption, responsible for at least 90% of glucose reabsorption in the kidneys (with SGLT1 accounting for the remaining 10%)[3] By inhibiting SGLT2, Canagliflozin effectively reduces renal glucose reabsorption, causing up to 119 grams of glucose to be excreted in the urine daily.[4] This excretion translates to the elimination of approximately 476 kilocalories, contributing to the reduction of blood glucose levels and potentially aiding in weight loss for patients with type 2 diabetes mellitus (T2DM).[5]

Metformin, chemically known as 3-(diaminomethylidene)-1,1-dimethylguanidine, is an established antihyperglycemic agent widely used in the management of T2DM. Metformin enhances glucose tolerance in diabetic patients by lowering both basal and postprandial plasma glucose levels.[6,7] The mechanism of action of Metformin involves several pathways: it decreases hepatic glucose production (gluconeogenesis), reduces the absorption of glucose from the intestines, and improves insulin sensitivity.[8] This improvement in insulin sensitivity is achieved by increasing the peripheral uptake and utilization of glucose in muscle tissues. Additionally, Metformin has been associated with a reduction in insulin resistance, thereby promoting better glycemic control without causing significant weight gain, which is a common issue with other antidiabetic drugs.[9]

Both Canagliflozin and Metformin target different aspects of glucose metabolism and offer complementary mechanisms of action. Their combination provides a synergistic effect, enhancing overall glycemic control in patients with T2DM. This study focuses on developing and validating a robust RP-HPLC method for the simultaneous

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estimation of Canagliflozin and Metformin in pharmaceutical dosage forms, ensuring accurate and reliable quantification essential for quality control and therapeutic monitoring.[10,11]

II. MATERIALS AND METHODS

Canagliflozin and Metformin were obtained as gift sample from Arti drug Pvt. Ltd. Market formulation of this combination Invokamet was procured from the local market HPLC grade ,ortho phosphoric acid , methanol and water were obtained from Merck (India).

The analysis was carried out on a HPLC system (Youglin) equipped with UV detector. Other apparatus and instruments used were a micro analytical balance (citizen, Cyloa). Ultrasonic (Met. Lab. 1.5L50). Nylon Membrane filters (0.22 µcm, 47 mmD). All instruments and glass-wares were calibrated.[12]

Chromatographic Condition:

The chromatographic conditions for the analysis were as follows: A High-Performance Liquid Chromatography (HPLC) system equipped with a UV detector was used. The separation was achieved on a C18 column (250 x 4.6 mm, YMC) with the column oven maintained at ambient temperature. Detection was carried out at a wavelength of 254 nm. The flow rate was set at 1.0 ml/min, and the injection volume was 20 µl, with a total runtime of 10 minutes. The mobile phase consisted of a mixture of Methanol and Water in an 80:20 ratio, with 0.1% ortho phosphoric acid to enhance separation efficiency and peak resolution.[13]

Preparation of Mobile Phase:

The mixture of Methanol: Water (80:20) , 0.1% ortho phosphoric acid was prepared. Filtered and degassed the mobile phase.[13]

Preparation of Canagliflozin and Metformin Standard Solution:

Accurately weighed quantity 5mg and 50mg of Canagliflozin and Metformin was dissolved in Methanol Volume was made up to 10ml mark to get final concentration of about 500µg/ml of Canagliflozin and 5000µg/ml Metformin.[13]

Preparation of Standard Stock Solution:

Canagliflozin Standard Solution:

Accurately weighed Canagliflozin 5mg was dissolved in mobile phase and volume was make up to 10ml Methanol. The stock Solution was diluted further with mobile phase to get final concentration of about 500µg/ml of Canagliflozin. [13]

Metformin Standard Solution:

Accurately weighed Metformin 50mg was dissolved in mobile phase and volume was make up to 10ml Methanol. The stock Solution was diluted further with mobile phase to get final concentration of about 5000µg/ml of Metformin.[13,14]

III. RESULT AND DISCUSSION

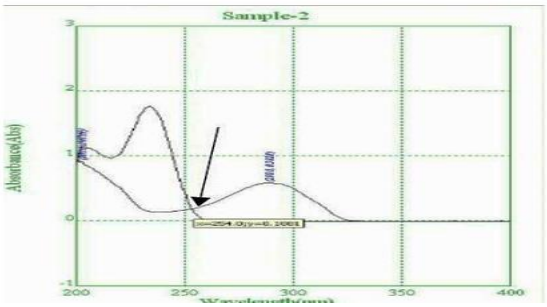

Fig 1: Overlain Spectra of Canagliflozin and Metformin

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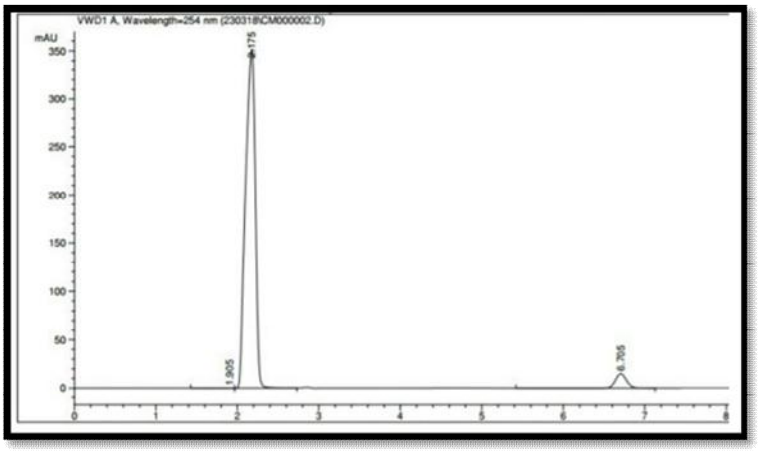

Fig 2: Selected Chromatogram of trail 1 using 80% Methanol and 20% Water (0.1% OPA) Flow rate 1.0ml at 254 nm.

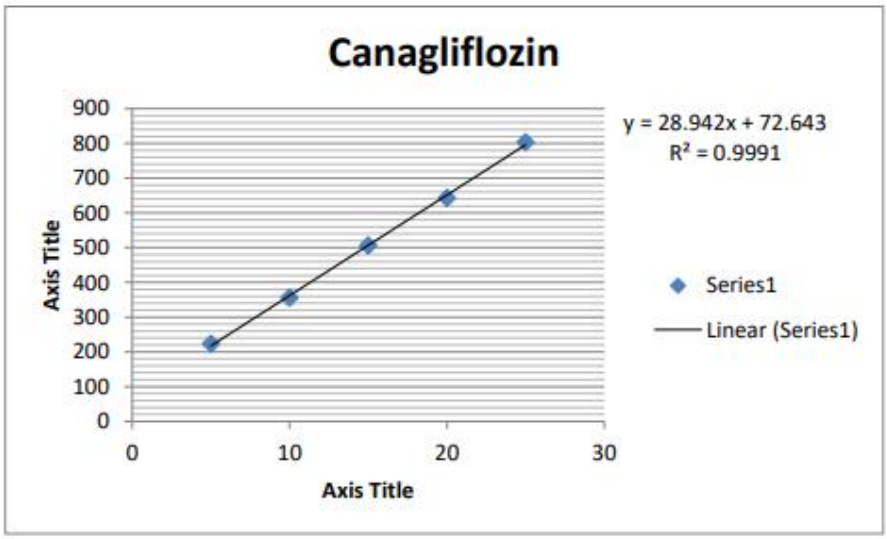

Fig 3: Observations of Standard Calibration Curve of Canagliflozin.

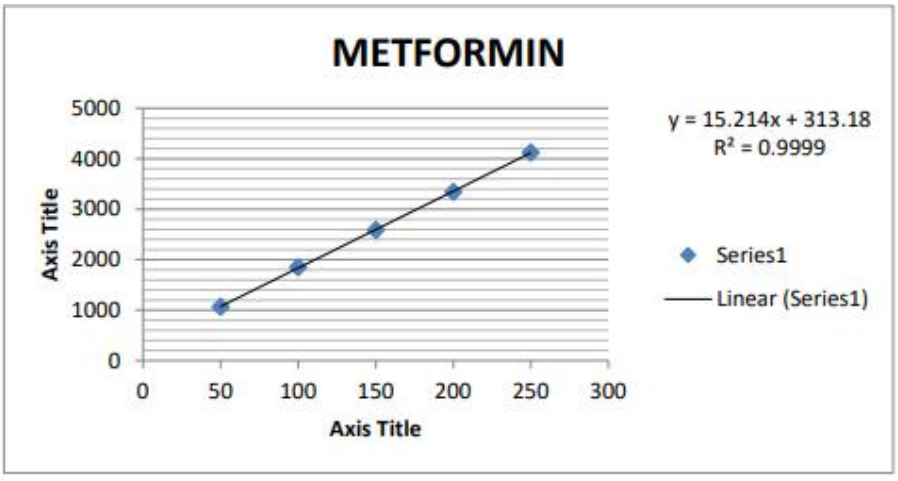

Fig 4: Concentration and Average Area of Metformin.

RP-HPLC Method was developed and validated for Canagliflozin and metformin in combine dosage form.

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The separation was achieved by C18 (YMC) column of (4.6×250 mm) with particle size packing 5 µm and methanol :water ,0.1% OPA (80:20) as mobile phase at a flow rate of 1.0 ml/min. The detection was carried out at 254 nm. The retention time of was found to be canagliflozin and metformin 8.183 min and 3.633 min respectively.

Table No.1 System suitability parameters for Canagliflozin and Metformin.

Parameters	Canagliflozin	Metformin
Retention time	8.18	3.63
Theoretical plate	5148.1	4574.95

Table No.2 Recovery studies of Canagliflozin and Metformin.

Level of Recovery (%)	80		100		120	
	CAN	MET	CAN	MET	CAN	MET
Amount present (mg)	8.94 9.02	90.49 89.92	10.00 9.88	101.24 89.92	10.96 10.83	111.84 110.56
Amount of Std. Added (mg)	4 4	40 40	5 5	50 50	6 6	60 60
Amount recovered (mg)	3.94 4.02	40.49 40.92	5.00 4.88	51.24 50.44	5.96 5.83	61.18 60.56
% Recovery	98.02 100.5	101.03 102.0	100.00 97.60	102.48 100.88	99.37 97.17	101.91 100.93

Table No. 3. Statistical Validation of Recovery Studies.

Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
80	CANAGLIFLOZIN	99.53	1.37	1.38
	METFORMIN	101.76	0.76	0.75
100	CANAGLIFLOZIN	98.80	1.70	1.72
	METFORMIN	101.68	1.13	1.11
120	CANAGLIFLOZIN	98.27	1.56	1.59
	METFORMIN	101.45	0.73	0.72

Table No. 4 Precision data of Canagliflozin and Metformin.

Compound (n=6)	Intraday Precision		Interday precision	
	% Amt. found	% RSD	% Amt. found	% RSD
Canagliflozin	98.11	0.95	98.12	0.55
Metformin	98.08	0.52	99.79	0.52

Table No.5: Result of Robustness Study of Canagliflozin.

Parameters	Conc.	Area (Mean ±SD)	%RSD
Mobile phase composition-(79+21)	20	634.76 ± 5.96	0.94
Mobile phase composition-(81+19)	20	677.17± 0.41	0.06
Wavelength change253nm	20	613.93 ± 2.96	0.48
Wavelength Change 256nm	20	629.04 ± 1.01	0.16
Flow rate change(0.9ml)	20	738.98 ± 1.41	0.19
Flow rate change(1.0ml)	20	508.87 ± 1.24	0.24

Table No.6: Result of Robustness Study of metformin.

Parameters	Conc.	Area (mean ±SD)	%RSD
Mobile phase composition-(79+21)	200	3326.29 ± 5.20	0.16
Mobile phase composition-(81+19)	200	3593.33 ± 3.66	0.10
Wavelength change253nm	200	3555.90 ± 2.43	0.07
Wavelength Change 256nm	200	3475.12 ± 2.88	0.08

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Flow rate change(0.9ml)	200	3590.15 ± 0.81	0.02
Flow rate change(1.0ml)	200	3587.92 ± 16.78	0.47

IV. METHOD OF VALIDATION

System suitability:

To determine the adequate resolution and repeatability of the proposed method, system suitability test were carried out. The parameters like retention time, no. of theoretical plates, asymmetry factors we studied by injecting standard solutions of the drug five times. The values given in table 1 were obtained within the limits.

Accuracy:

The accuracy of the method was evaluated in triplicates by recovery studies at three different concentration levels of 80%, 100% and 120% known amounts of standard drug concentration were added to the sample. The accuracy data and the corresponding results are as shown in table 2 and 3.

Precision:

The precision of this method is determined by intra-day and inter-day precision. The % RSD was found less than 2, this indicate that the method is precise. The results of precision study are shown in table 4.

Robustness:

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in ± 1 ml proportion and the flow rate was varied by ± 0.1 ml min-1, of optimized chromatographic condition. The results of robustness studies are shown in Table No.5 & 6. System suitability parameters were also found satisfactory hence the analytical method would be concluded.

V. CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of Canagliflozin and Metformin in pharmaceutical dosage forms is both efficient and reliable. The method demonstrated excellent separation using a C18 column with a mobile phase of Methanol: Water (80:20) containing 0.1% ortho phosphoric acid, achieving retention times of 8.183 min for Canagliflozin and 3.633 min for Metformin. Validation parameters, including system suitability, accuracy, precision, and robustness, were thoroughly assessed and found to be within acceptable limits, confirming the method's reliability. The method's high precision was evident from the low % RSD values in intra-day and inter-day precision studies. Accuracy was substantiated through recovery studies at various concentration levels, showing consistent and satisfactory results. Robustness testing indicated that minor variations in chromatographic conditions did not significantly affect the outcome, highlighting the method's robustness. Overall, this RP-HPLC method is precise, accurate, and robust, with a short run time, making it suitable for the routine analysis of Canagliflozin and Metformin in pharmaceutical formulations. Its ability to provide rapid quantification without interference makes it a valuable tool for quality control in pharmaceutical laboratories.

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