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Development and Validation of Stability-Indicating RP-Hplc Method for Simultaneous Determination of Canagliflozin and Metformin in Fixed-Dose Combination

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ABSTRACT: A new HPLC method has been developed and validated with different parameters for the estimation of Canagliflozin and Metformin in Fixed-Dose Combination. The chromatograms were developed using a mobile phase of Methanol: 0.1 % OPA in water (35:65) with a flow rate of 0.7 ml/min. C18 Column of 4.6 x 100 mm dimension was used as a stationary phase, particle size 5µm. The detection was carried out at 245 nm. The method was validated according to ICH guidelines for linearity, precision and Repeatability, Robustness, LOD, and LOQ. The response was found to be linear in a concentration range of 100-500 µg/ml for Metformin and 10-50 µg/mL for Canagliflozin. The stability studies of Metformin and Canagliflozin were also done through the exposure of analyte solution to different stress conditions. The developed HPLC method of Metformin and Canagliflozin was simple, precise, accurate, reproducible, and therefore suitable for routine analysis of drugs in a dosage form.

KEYWORDS: HPLC; Method Development; Method Validation; Forced degradation; Canagliflozin; Metformin.

1. INTRODUCTION

Different analytical methods have been reported for single-drug formulations but due to complexity in the multi-component formulation, method development is a challenge for the analytical chemist. The different instrumental techniques employed for the analysis of drugs are spectra-photometry, gas-liquid chromatography (GLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances [1-6]. In the RP-HPLC method, the mobile phase is polar and the stationary phase is non-polar. Chromatographic separation in HPLC is a result of the specific interaction of drugs with a mobile and stationary phase [7-10]. In the current study, Canagliflozin and Metformin were studied. Canagliflozin {chemically is a (2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl) thiophen-2-yl] methyl]-4-methylphenyl]-6-(hydroxymethyl)oxane-3,4,5-triol} is a Sodium-glucose transporter 2 (SGLT2) with antihyperglycemic activity. The sodium-glucose co-transporter2 (SGLT2), is found in the proximal tubules of the kidney and reabsorbs filtered glucose from the renal tubular lumen. Metformin {chemically is a 3-(di-amino methylidene)-1,1-dimethylguanidine}is anti-diabetic drug [11-15]. The main mechanism of action of metformin is a reduction of hepatic glucose output, largely by inhibiting hepatic gluconeogenesis. Metformin also shows intestinal absorption of sugars and improves peripheral glucose uptake and utilization [16-18].

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Aim and Objectives:

- ✓ To develop Stability-Indicating RP-HPLC Method for Simultaneous Determination of Canagliflozin and Metformin in Fixed-Dose Combination.
- ✓ To validate developed RP-HPLC Method for Simultaneous Determination of Canagliflozin and Metformin in Fixed-Dose Combination.
- ✓ To perform a forced degradation study.

3. RESULTS AND DISCUSSION:

3.1 Optimization of chromatographic condition (Method Development):

The mixtures of drugs were taken in different combinations of mobile phase for chromatographic study. The various mobile phase was tried, finally, methanol and water (0.1% with OPA) in the ratio of 35:65 were kept constant throughout the study. It was shown in Table 1 and figure 1.

Table 1. Optimized Chromatographic conditions

Sr. No.	Instrument/Equipment	Optimized condition
1	HPLC	Agilent (S.K)Gradient System UV Detector
2	Software	Chemstation
3	Column	(Agilent C18 Column (4.6mm x 100mm)
4	Particle size packing	5 µm
5	Stationary phase	C-18 (Agilent)
6	Mobile Phase	MEOH : Water (0.1% with OPA) 35 : 65
7	Detection Wavelength	245 nm
8	Flow rate	0.7 ml/min
9	Temperature	25°C
10	Sample size	20 µl
11	pH	3.0
12	Run Time	10 min
13	Filter paper	0.45 µm

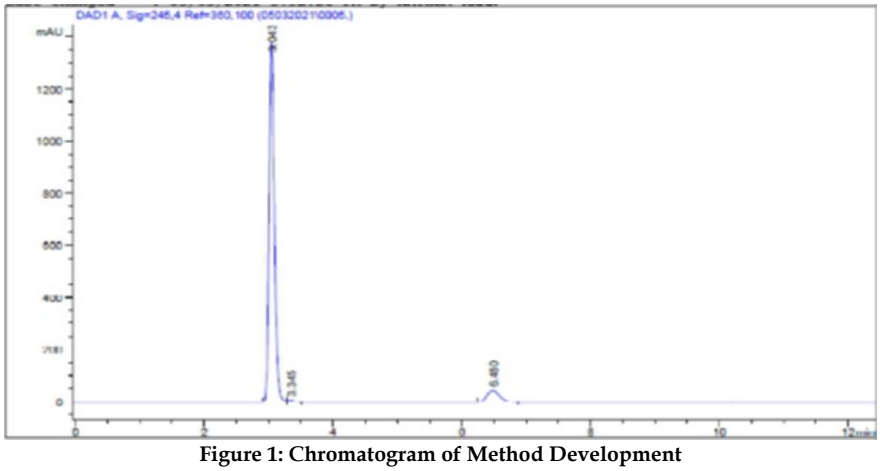

Figure 1: Chromatogram of Method Development

3.2 HPLC Method Validation:

3.2.1 Calibration Experiment (Linearity study)

While studying linear regression analysis, it shows a linear relationship between peak areas and concentrations in the range 100-500 µg/ml for Metformin and 10-50 µg/mL for Canagliflozin. Table No. 2 and 3 depict the calibration data of Metformin and Canagliflozin respectively. The respective linear equation for Metformin was y = 25.216 X + 2031.9 and Canagliflozin equation y= 22.948 X + 25.341 where x is the concentration and y is area of peak. The correlation coefficient was 0.999 and 0.999. The calibration curve of Metformin and Canagliflozin is depicted in Figure 2 and Figure 3.

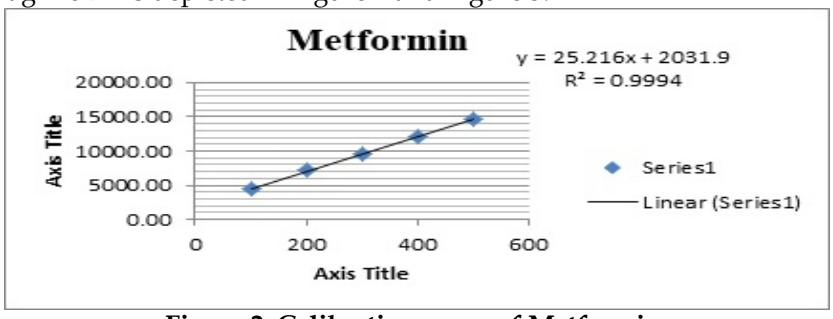

Figure 2: Calibration curve of Metformin.

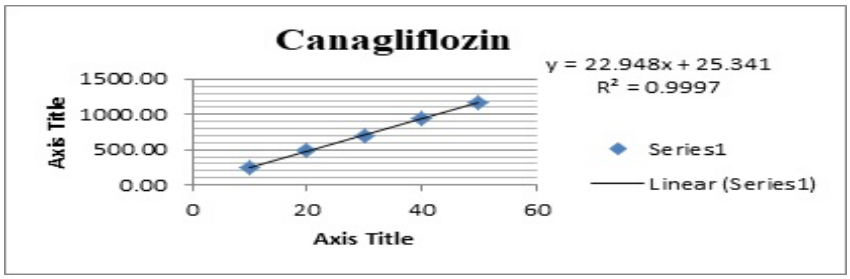

Figure 3: Calibration curve of Canagliflozin.

3.2.2. Accuracy

Recovery studies were performed to validate the accuracy of the developed method. To pre-analyze tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed**.** Statistical validation of recovery studies is shown in Table 2.

Table 2. Result of Recovery Data for Metformin and Canagliflozin

Method	**RP-HPLC**					
Drug	**Metformin**			**Canagliflozin**		
Level (%)	80 %	100 %	120 %	80 %	100 %	120 %
Amount added (ug/ml)	80	100	120	8	10	12
Absorbance Mean *±S.D.	180.25±0.27	201.99±0.16	217.66±0.83	18.00±0.12	20.13±0.094	22.03±0.64
Amount recovered Mean *	80.25±0.27	101.99±0.16	117.66±0.83	8.00±0.12	10.00±0.094	12.03±0.64
% Recovery Mean *	100.31±0.3	101.99±0.16	98.05±0.70	100.00±1.6	101.29±0.94	100.21±0.5

*mean of each 3 reading for RP-HPLC method

Accuracy of RP-HPLC method Spectrophotometric method is ascertained by recovery studies performed at different levels of concentrations (80%, 100%, and 120%). The % recovery was found to be within 99-101%.

3.2.3. System suitability parameters :(Ruggedness)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Metformin and Canagliflozin system suitability parameters were studied. The result is shown in Table 3.

Repeatability studies on the RP-HPLC method for Metformin and Canagliflozin were found to be, the %RSD was less than 2%, which shows a high percentage amount found in between 99.91 % to 100.25 % indicates the analytical method that concluded. (Table 3)

Table 3. Repeatability studies on RP-HPLC for Metformin and Canagliflozin.

Method	**RP-HPLC**	
Drug	Metformin	Canagliflozin
Conc. (mg/ml)	300	30
Peak area	9712.20 9704.41	707.54 700.24
Mean	9708.31	703.90
Amount found (mg)	304.53	29.58
% Amount found	101.51	98.60
% RSD	0.072	0.76

3.2.4. Precision

The method was established by analyzing various replicates standards of Metformin and Canagliflozin. All the solution was analyzed thrice to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday and interday precision is shown in Table 4.

Table 4: Result of Intraday and Inter day Precision studies of Metformin and Canagliflozin

Method	**Drug**	**Conc. (µg/ml)**	**Intraday Precision**		**Interday Precision**	
			Mean± SD	**% Amount Found**	**Mean± SD**	**% Amount Found**
RP-HPLC Method	MET	200	7146.13±0.36	101.45	980.91±0.46	97.05
		300	9670.10±0.39	101.01	1507.03±0.11	98.48
		400	12352.35±0.88	102.35	1940.00±0.91	98.74
	CAN	20	470.62±1.67	97.05	480.62±1.67	99.23
		30	703.10±2.13	98.48	723.10±2.13	101.39
		40	930.40±1.87	98.74	930.40±1.87	98.36

*Mean of each 3 reading for RP-HPLC method

3.2.5. 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, wavelength on retention time, and tailing factor of drug peak was studied.

The mobile phase composition was changed in 34:66 and 36:64 proportion and the flow rate was varied by (± 0.2ml/min**)**, and wavelength change (± 2 nm**)** of optimized chromatographic condition. The results of robustness studies are shown in Table 5. Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

The robustness study was carried out by changes in flow rate (0.6 and 0.8 ml/ min), PH of mobile phase composition (34 + 66 ml and 36+64 ml), and Wavelength (244 nm and 246 nm). %RSD for peak area was calculated which should be less than 2%. The result is shown in Table 5.

Table 5. Result of Robustness Study of Metformin and Canagliflozin.

Parameters	Conc. (µg/ml)	Metformin		Canagliflozin	
		Amount detected (mean ±SD)	% RSD	Amount detected (mean ±SD)	% RSD
Flow rate 0.6 ml/min	40+400	10816.20±0.46	0.004	1168.52±0.80	0.07
Flow rate 0.8 ml/min	40+400	8976.59±0.18	0.002	955.46±0.98	0.10
Mobile Phase 34 + 66 ml	40+400	10871.60±0.67	0.01	1185.3±0.23	0.02
Mobile Phase 36 + 64 ml	40+400	11036.01±0.62	0.01	1178.56±0.52	0.04
Wavelength 244 nm	40+400	12585.5±0.24	0.002	1183.8±0.22	0.02
Wavelength 246 nm	40+400	9709.42±0.93	0.001	1208.27±0.52	0.04

3.2.6. Limit of Detection

The LOD is the lowest limit of drug that can be detected. Based on the S.D. deviation of the response and the slope the limit of detection (LOD) may be expressed as:

LOD = 3.3 X **(SD)/S**

Where, SD = Standard deviation of Y intercept

S = Slope

Limit of detection (MET) = 3.3 X34.17/ 25.21= 4.47(ug/mL)

Limit of detection (CAN) = 3.3 X 3.60/22.94 = 0.51 (ug/mL)

The LOD of Metformin and Canagliflozin was found to be 4.47(ug/mL) and 0.51 (ug/mL).

3.2.7. Limit of Quantitation.

The LOQ is the lowest concentration that can be quantitatively measured. Based on the Standard deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 X (SD)/ S

Where SD = Standard deviation Y-intercept

S = Slope

Limit of Quantitation (MET) = 10 X 34.17/25.21 = 13.55(ug/mL)

Limit of Quantitation (CAN) = 10 X 3.60/22.94= 1.5693 (µg/mL)

The LOQ of Canagliflozin was found to be 13.55 (ug/mL) and 1.56 (ug/mL).

3.2.8. Analysis of tablet formulation

Procedure:

Weigh 20 Metformin and Canagliflozin combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 276mg Metformin and Canagliflozin into a 10 ml volumetric flask. Add about 10ml MEOH of diluents and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through a 0.45 µm filter. Further pipette 0.4ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (40+ 264µg/ml). The amounts of Metformin and Canagliflozin per tablet were calculated by extrapolating the value of area from the calibration curve. The analysis procedure was repeated five times with tablet formulation. The result of %Label claim and %RSD of Metformin and Canagliflozin is shown in Table 6 and the chromatogram is shown in Figure 4 and Figure 5.

Brand Name: Invokamet 500 +50 mg.

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Table 6: Analysis of marketed formulation:

Drug	Conc.	Amt. Found	%Label Claim	SD	%RSD
MET	300	304.6887	101.56	0.75	0.76
CAN	30	29.58	99.13	0.22	0.76

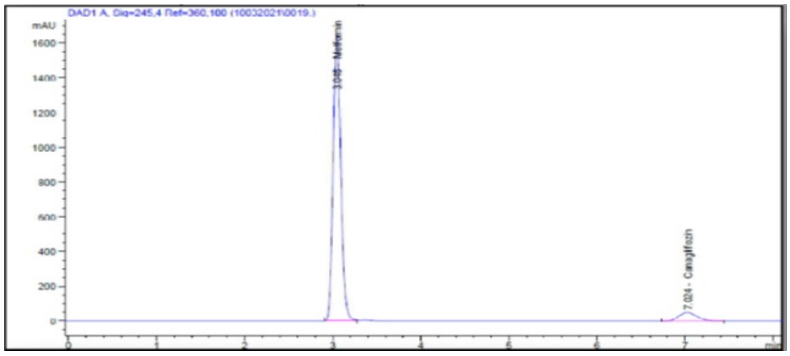

Figure 4. Chromatogram of Assay (Sample 1)

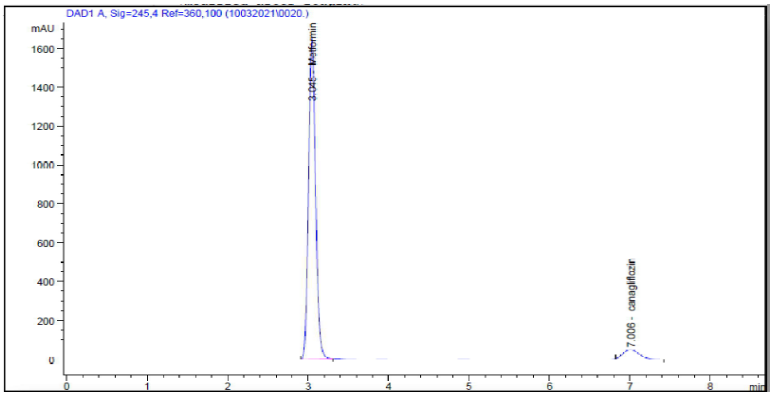

Figure 5. Chromatogram of Assay (Sample 2)

4. CONCLUSION

The proposed method is specific, rapid, reproducible, precise, and accurate. The method was completely and accurately validated showing satisfactory data for all the tested method validation parameters. The developed method was found to be robust in the separation and quantification of Metformin and Canagliflozin.

5. MATERIALS AND METHODS

5.1 Chemicals and Reagents

Canagliflozin and Metformin are obtained from Kopran Ltd. Ortho-Phosphoric acid are obtained from Avantor Performance material India Ltd. Thane, Maharashtra, and Methanol from Merck Specialities Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai, Maharashtra. Canagliflozin and Metformin marketed formulation is obtained from the local medical store (Brand name: Invokamet, contains Metformin 500 mg and Canagliflozin 50mg.)

5.2 Instrumentation:

Agilent technology HPLC having gradient system and UV detector was used for analysis purpose. Agilent C18 Column (4.6mm x100mm) having particle size 5 µm was used. A 940D pump, 20µl injection loop, UV 740D Absorbance detector, and running Chemstation software were used for analysis.

5.3 The standard Stock solution of Metformin and Canagliflozin:

Accurately weight and transfer 10 mg Canagliflozin and Metformin 100mg working standard into 10 ml volumetric flask as about diluents methanol completely and make volume up to the mark with the same solvent to get 1000 & 10000 µg/ml standard (stock solution) and sonicate to dissolve it and remove the

unwanted gas. 0.1 ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10 +100ug/ml. (5-7)

5.4 Tablet solution preparation for Assay:

To determine the content of Canagliflozin and Metformin in marketed tablets (label claim 10 mg of Canagliflozin and 100mg Metformin), 20 tablets powder weighed in 276 gms and an average weight of powder was calculated in 13.8. Tablets were triturated and powder equivalent to weighing 218mg the drug was extracted from the tablet powder with 10 mL MeOH. To ensure complete extraction it was sonicated for 15 min. 0.1mL of supernatant was then diluted up to 10 mL with the mobile phase. The resulting solution was injected in HPLC and a drug peak area was noted**.** (8-10)

Approval of Ethical Committee

There is no need of approval of Ethical committee, because no animal activity has been carried out.

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