



Original Research Paper

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF METFORMIN AND CANAGLIFLOZOIN

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin(MFM) and Canagliflozin (CGF) in pharmaceutical dosage form. Chromatogram was run through Kromosil 250 x 4.6 mm, 5 μ column. Mobile phase containing Buffer and Acetonitrile in the ratio of 65:35 with gradient method was pumped through column at a flow rate of 1ml/min. Buffer used in this method was OPA. Temperature was maintained at 30°C. Optimized wavelength was 220nm. Retention time of MFM and CGF were found to be 2.424 min. and 3.730 min, %RSD of method precision for MFM and CGF were and found to be 0.47, and 0.38% respectively. % recovery was obtained as 101.60% and 100.19% for MFM and CGF respectively. LOD, LOQ values are obtained from regression equations of MFM and CGF were 0.005, 0.049 and 0.049, 0.150 respectively. Regression equation of MFM was $y = 21120x + 631.6$ ($R^2=0.999$) and CGF was $y = 9125x + 322.7$ ($R^2=0.999$) founded respectively. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for routine estimation in pharmaceutical dosage form.

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1. INTRODUCTION :

MFM is an oral antidiabetic drug from the biguanide class. Chemical name of MFM is N, N-dimethyl imidodicarbonimidic diamide. It is a drug of choice for the treatment of type 2 diabetes in overweight and obese people.^[1] The mechanism of action of MFM is due decrease in hepatic glucose production, reduces intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization of AMP-activated protein kinase (AMPK) and inhibition of glucagon-induced elevation of cAMP.^[2]

CGF is a drug of choice for the treatment of type 2 diabetes.^[3] Chemical name of CGF is 2-(3-[[5-(4-fluorophenyl)thiophen-2-yl]methyl]-4-methylphenyl)-6-hydroxy methyl) oxane-3,4,5-triol. CGF is an inhibitor of subtype 2 sodium-glucose transport protein (SGLT2). This SGLT2 is responsible for 90% of the renal glucose reabsorption from urine. By blocking SGLT2 the blood glucose level decreases in the body. ^[4] Literature ^[5-10] reveals different methods for its analysis in formulations. Hence our present plan is to develop a new, simple, precise & accurate method for its analysis in

formulation, after a detailed study, a new RP-HPLC method was decided to be developed and validated as per ICH norms. For the estimation of this method we used a combination of potassium di hydrogen ortho phosphate buffer and Acetonitrile in the quantitative relation of 65:35 %v/v was used as a mobile section at a pH 3.5 adjusted with Ortho phosphoric acid.

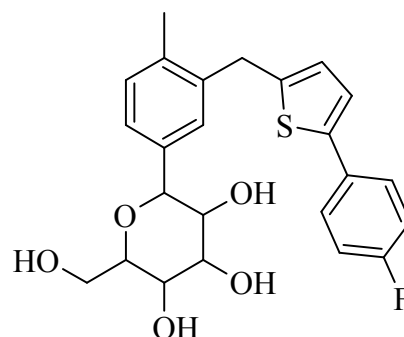
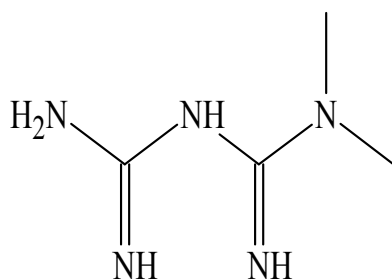


Figure: 1 Structures for MFM and CGF

2. MATERIALS AND METHODS

Apparatus and chromatographic parameters:

A Waters HPLC 2695 series with quaternary pumps, Photo Diode array detector and auto sampler integrated with empower software, Kromasil column, Denver Electronics Balance, BVK enterprises, India, pH meter, BVK enterprises, Ultrasonicator and Labindia UV double beam spectrophotometer with UVwin5 were used in the study.

Drug samples:

The drug used for estimation for this study was procured from tablet. The label claim was 500mg of MFM, and 50mg of CGF.

Reagents and solutions:

Water -HPLC grade, Acetonitrile - HPLC grade, Triethylamine- AR grade, Potassium dihydrogen ortho phosphate - AR grade, Orthophosphoric acid - AR grade, all the chemicals and solvents are purchased from Ranchem pvt. Ltd., A combination of potassium di hydrogen ortho phosphate buffer and Acetonitrile in the quantitative relation of 65:35 %v/v was used as a mobile section at a pH 3.5 adjusted with Ortho phosphoric acid and also used as a diluent for getting ready the working solution of drug. The mobile section was degassed in ultrasonic water bath for 5 minutes and filtered under vacuum filtration.

Analytical Methodology:

Standard Solution Preparation:

Accurately Weighed and transferred 50mg & 5mg of MFM and CGF working Standards into 25ml and 25ml clean dry volumetric flasks separately, add 3/4th volume of diluents, sonicated for 30 minutes and make up to the final volume with diluents. From this stock solution, 1 ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Solution Preparation:

20 tablets were weighed and calculate the average weight of each tablet then the one tablet powder weight equivalent to 500mg of MFM and 50mg of CGF was transferred into a 250ml volumetric flask, 20ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent.

Buffer: (0.01N Ammonium acetate buffer)

Weigh 0.77gm of ammonium acetate and taken in a conical flask, dilute with 1000 ml of water, the PH 3.5 was adjusted with dil. acetic acid.

Mobile phase:

650ml (65%) of above buffer and 350 ml of Acetonitrile HPLC (35%) were mixed well and degassed in ultrasonic

water bath for 15 minutes. The solution was filtered through 0.45µm filter under vacuum filtration.

Linearity Sample Preparation:

Linearity solutions were prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml and 1.5ml from the Stock solutions of MFM and CGF were taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, 300ppm of MFM and 5ppm, 10ppm, 15ppm 20ppm, 25ppm, 30ppm of CGF.

3 METHODOLOGY

METHOD DEVELOPMENT

Initially the mobile phase was pumped for about 30 minutes to saturate the column thereby to set the baseline corrected. Then 10 µl of the standard and sample solutions were injected separately. A quantitative determination of the active ingredients was made by comparison of the peak area of the sample injection with the corresponding peak area of the standard injection. The amount of MFM and CGF present in the sample was calculated through the standard calibration curve.

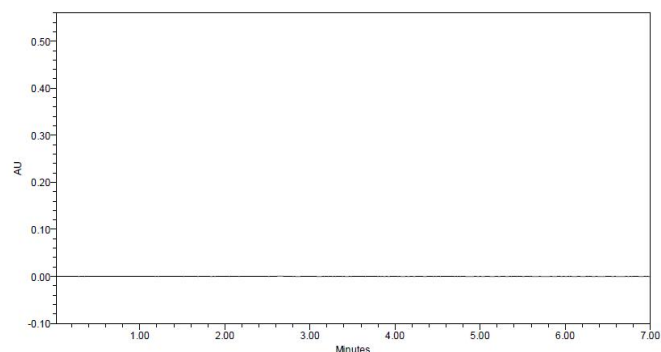


Figure: 2 Chromatogram showing no interference of diluents for MFM and CGF

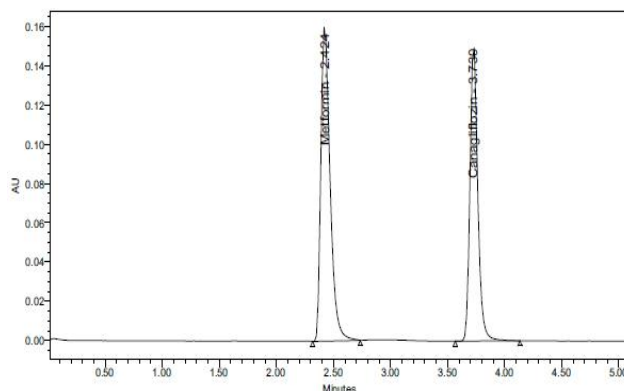


Figure: 3 HPLC Chromatogram showing Control Sample profile of MFM and CGF

Optimized Chromatographic conditions:

Trials were performed for the method development and The best peak with least fronting factor was found to be with RT= 2.424min for MFM and 3.730min for CGF, and shown in figure 3.

Table: 1 Optimized Chromatographic conditions for MFM and CGF

Parameter	Content
Column	Kromosil 250mm x 4.6 mm, 5µ. Column
Mobile Phase	Ammonium acetate Buffer and Acetonitrile taken in the ratio 65:35v/v with a pH 3.5.
Flow Rate	1 ml/min
Run time	20 minutes
Temperature	Ambient
Injection Volume	10 µL
Detection & Wavelength	UV Detector & 220nm
Retention times	2.424 min. for MFM and 3.730 min. for CGF

Performance Calculations:

To ascertain the system suitability for the method, a number of parameters such as retention time, theoretical plates, and HETP, tailing factor, % Peak area, and LOD, LOQ, resolution and peak asymmetry have been calculated with the observed readings and the results are recorded in Table: 2.

METHOD VALIDATION:

The following parameters were used to validate the method for the proposed assay procedure of MFM and CGF in pharmaceutical dosage forms. The developed HPLC method for the determination of MFM and CGF was validated as per the ICH guidelines.

Precision: Precision of the developed method was assessed by measuring the response on the same day (intraday precision) and next two consecutive days (inter day precision). The precision of the method was assessed by six replicate injections of 100% test concentration.

Acceptance Criteria: The % RSD should not be more than 2%

Table: 2: Validation Summary / System Suitability

Parameter	MFM	CGF
Theoretical Plates (N)	4968 ± 113.715	8823.2±168.576
Tailing factor	1.152 ± 0.013	1.066± 0.009
Retention time (min)	2.991± 0.008 min	4.211±0.0009 min
Resolution	---	---
% Peak Area	101.60%	100.19%
LOD (µg/mL)	0.005	0.049
LOQ (µg/mL)	0.015	0.150

Table:3 Method Precision (Inter and Intraday) studies for MFM and CGF

S.No.	MFM		CGF	
	Peak area	Day_day Precision	Peak area	Day_day Precision
1	1473362	Peak area	1516248	Peak area
2	1468555	1467908	1515201	1506421
3	1457230	1454539	1526856	1507288
4	1462516	1471612	1516243	1511942
5	1457910	1461502	1513521	1510545
6	1456693	1458311	1525590	1500741
AVG	1462711	1462774	1518943	1507387
SD	6883.9	6961.0	5740.2	4354
%RSD	0.47	0.50	0.38	0.3

Accuracy:

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. In the current study recovery at three spike levels 50%, 100% and 150% were carried out.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table: 4 Recovery studies for MFM and CGF by proposed method

%Concentration	MFM			CGF		
	50%	100%	150%	50%	100%	150%
Trail-I	100.58	100.20	99.45	100.42	100.37	102.0
Trail-II	99.44	102.86	100.40	102.12	1020.32	101.8
Trail-III	102.31	101.78	101.26	99.18	99.87	99.2
AVG (%Recovery)	100.8	101.60	100.38	100.57	100.19	101.0
SD	1.4	1.33	0.91	1.48	0.28	1.6
%RSD	1.4	1.31	0.90	1.47	0.28	1.6

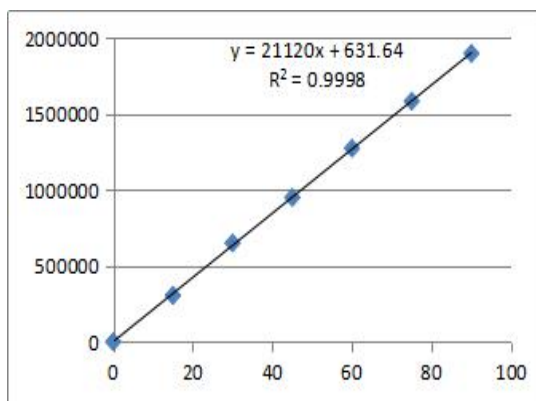
Linearity and Calibration curve:

Operating dilutions of MFM and CGF within the vary of 75-450 ppm and 0.125-0.75 ppm were ready by taking appropriate aliquots of operating commonplace solutions of drug in several 10ml volumetrical flask and diluting up to the mark with mobile section. 10µl amount of every dilution was injected in to the column at a rate of 1ml/min. the drug within the rinse was monitored at 246 nm. The regression of the plot was computed by least sq. regression methodology. The slope and intercept value for calibration curve for MFM was $y = 21120x + 631.6$ ($R^2=0.999$) and CGF was $y = 9125x + 322.7$ ($R^2=0.999$) founded respectively.

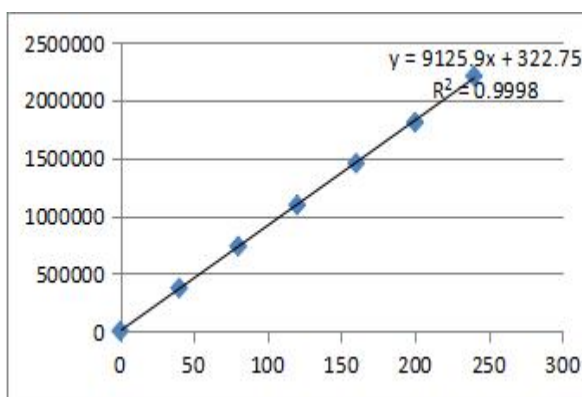
Table 5 Linearity studies for MFM and CGF by proposed method

S. No	Conc. µg/mL	MFM Peak area	Conc. µg/mL	CGF Peak area	% Level
1	75	1553899	0.125	63355	25
2	150	3017263	0.25	136283	50
3	225	4501568	0.375	202737	75
4	300	6092288	0.5	269922	100
5	375	7582239	0.625	326236	125
6	450	9109932	0.75	401217	150
Slope		21120		9125	
Intercept		631.6		322.7	
CC(r)		0.9998		0.9998	
RSQ(r ²)		0.999		0.999	

Figure 4: Calibration curves



Metformin



Canagliflozin

Robustness:

Robustness is set by creating deliberate amendments within the natural action conditions like change in rate, mobile section composition and temperature and evaluated for the impact on the strategy. A deviation of ± 0.2 mL/min in the flow rate, $\pm 5\%$ change in the organic phase were tried individually. It had been discovered from the chromatograms that the results were at intervals the boundaries. This means that the strategy developed is powerful.

Table-6 Robustness studies for MFM and CGF

Parameter		%RSD	
		Metformin	Canagliflozin
Flow rate (± 0.2 mL/min)	0.8 mL/min	0.1	0.2
	1.2 mL/min	0.3	0.02
Mobile phase (± 5)	70:30	2.9	2.7
	60:40	0.1	0.6
Temperature ($\pm 2^\circ\text{C}$)	28 $^\circ\text{C}$	2.9	2.7
	32 $^\circ\text{C}$	0.4	0.03

3. CONCLUSION

The projected study describes new and easy RP-HPLC method for the estimation of MFM and CGF. The above proposed method obviates the need for any preliminary treatment and is simple, sensitive and reliable. Thus, the present procedures constitute the first ever reported RP-

HPLC method with good precision, accuracy and sensitivity for the estimation of MFM and CGF in pure stage and also in marketed products.

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