IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
July 2018 Vol.:12, Issue:4

Analytical Method Development and Validation of Canagliflozin and Metformin HCl by Using RP – HPLC

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Anjali Bakshi [*], Reddy NS, Shweta Bhutada, M Bhagvan Raju

Department of Pharmaceutical Analysis and Quality Assurance, Sri Venkateshwara College of Pharmacy, Madhapur, Hyderabad, India.

Submission: 23 June 2018
Accepted: 29 June 2018
Published: 30 July 2018

Keywords: Metformin, Canagliflozin, High-performance liquid chromatography

ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method was developed for Simultaneous estimation of Canagliflozin and Metformin HCl. Separation of Metformin and Canagliflozin hydrochloride was successfully achieved. The column used was WATER'S (250x 4.6mm, 5um) in an isocratic mode utilizing 0.1% OPA: Methanol (60:40) at a flow rate of 0.5mL/min and elute was monitored at 273 nm, with a retention time was 2.693 and 4.227 minutes for Metformin hydrochloride and Canagliflozin respectively. The linearity for Metformin HCl and Canagliflozin were in the range of 50-150µg/ml. The values of the correlation coefficient were found to be 0.999 for Metformin and 0.999 for Canagliflozin. Percentage recovery for Metformin and Canagliflozin was found to be 100.23 and 100.1 indicates respectively. Results show that the proposed method is highly accurate. The method was extensively validated according to ICH guidelines for Linearity, Accuracy, Precision, Specificity, and Robustness. The proposed method was validated and successfully applied for the estimation of Metformin HCl and Canagliflozin.

1. INTRODUCTION

Metformin (MET) is chemically named as N, Ndimethylimidodicarbonimidic diamide (Fig.1). Metformin is a first line oral pharmacotherapy for type 2 diabetes. Activation of the energy-regulating enzyme AMPactivated protein kinase (AMPK), principally in muscle and the liver, is considered a major mode of metformin action [1].

Canagliflozin (CANA) is chemically named as (2S, 3R, 4R, 5S, 6R)-2-[3-[[5-(4-fluorophenyl) thiophen-2-yl] methyl]-4-methylphenyl]-6-(hydroxymethyl) oxane- 3, 4, 5-triol (Fig.2). Canagliflozin is used for type 2 diabetes. Canagliflozin inhibits Na+- dependent 14CAMG uptake in a concentration-dependent way. It is a novel C-glucoside with thiophene ring [2].

Various UV & HPLC assay methods are also reported in the literature for the estimation of Metformin and Canagliflozin individually [3, 4] and in combination with other drugs [5-6]. According to the literature survey, there is no official method for the simultaneous estimation of Metformin and Canagliflozin by RP-HPLC in combined tablet dosage forms. Hence, an attempt has been made to develop a new method for simultaneous estimation and validation of Metformin and Canagliflozin in tablet formulation in accordance with the ICH guidelines [7-10].

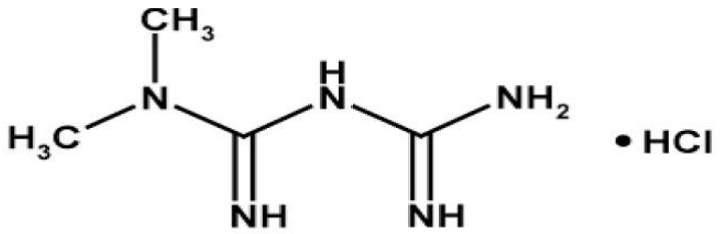

Figure 1: Chemical structure of Metformin Hydrochloride

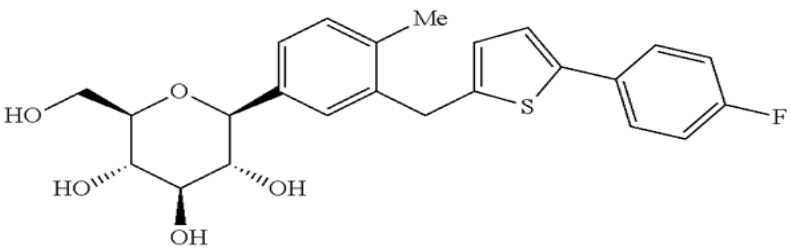

Figure 2: Chemical structure of Canagliflozin

2. MATERIALS AND METHODS:

2.1 Instrumentation

Chromatography was performed with WATERS C_{18} 4.6X250MM, 5µm HPLC with a high-speed autosampler, column oven, degasser and 2996 PDA detector with Empower-2 software.

2.2 Reagents and chemicals

The reference samples of MET and CANA were provided as gift samples from Rainbow labs, Hyderabad. HPLC grade Methanol and all other chemicals were obtained from Hetero Labs, Hyderabad. HPLC grade water obtained from a Milli-Q water purification system was used throughout the study. Commercial tablets (Dosage: MET-500 mg & CANA- 100 mg) were purchased from the local pharmacy.

2.3 Chromatographic condition

The mobile phase consisted of Buffer, and methanol is taken in the ratio of 60:40 at a flow rate of 0.5 mL/min. Although the MET and CANA have different λ max, considering the chromatographic parameter, sensitivity, and selectivity of the method for both drugs, 273 nm was selected as the detection wavelength for PDA detector.

2.4 Preparation of standard stock solution

Accurately weigh and transfer 500 mg of Metformin and 100 mg canagliflozin into 100 ml of volumetric flask and add 10 ml of Methanol and sonicate 10 min (or) shake 5min and makeup with HPLC grade water. Transfer 1ml above solution into 10 ml volumetric flask dilute to volume with HPLC grade water.

2.5 Preparation of Working Standard Solutions

Aliquot of 0.25, 0.5, 0.75, 1, 1.25 & 1.5 mL were pipette out from stock solution into 10 mL volumetric flask separately for both MET and CANA and volume was made up to 10 mL with diluent. This gives the solutions of 50, 100, 150, 500, 250and 300µg/mL for Metformin and 5, 10, 15, 20, 25 and 30 µg/mL for Canagliflozin respectively.

2.6 Sample preparation

Commercially available 20 tablets were weighed and powdered the powdered equivalent to the 789 mg of Metformin and Canagliflozin of active ingredients were transferred into a 100ml of volumetric flask and add 10 ml of Methanol and sonicate 20 min (or) shake 10 min and makeup with water.

Transfers above solution 1ml into 10ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through a 0.45µm filter before injecting into the HPLC system.

3. RESULTS AND DISCUSSION:

3.1 Method development

Initially, the reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases, in which both the drugs did not respond properly, and the resolution was also poor. The organic content of the mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of the mobile phase becomes an important factor. At pH: 4.5 both drugs eluted with better separation. Thereafter, buffer: methanol were taken in the isocratic ratio: %buffer / %methanol: 10/40, 15/35, 20/40, 35/25, and 60/40 and with a flow rate of 0.5mL/min was employed. ODS 250mm x 4.6 mm, 5 particle size was selected as the stationary phase to improve resolution and the tailing of both peaks was reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 205 nm to 280 nm. Both MET and CANA showed maximum absorption at 273 nm of wavelength and selected as the detection wavelength for PDA detector. The retention times were found to about 2.693 min and 4.227 min for MET and CANA, respectively. The chromatogram obtained was shown in the Fig. 3.

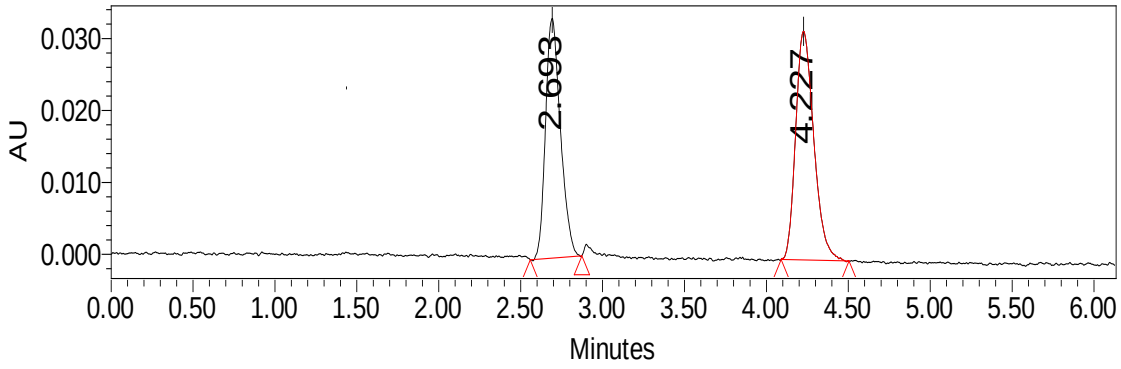

Figure 3: A typical Chromatogram of Metformin and Canagliflozin.

3.2 Method Validation:

3.2.1 System suitability and Specificity

System suitability parameters such as the number of theoretical plates, peak tailing, and retention time and resolution factor were determined. The total run time required for the method is only 6 minutes for eluting both MET and CANA. The results obtained were shown in Table 1.

Table 1: System suitability of MET and CANA

Sr. No.	METFORMIN				CANAGLIFLOZIN			
	Retention time	Area	Plate count	Tailing factor	Retention time	Area	Plate count	Tailing factor
Injection-1	2.683	1370099	4090	1.45	4.226	518631	6657	1.33
Injection-2	2.684	1370132	4308	1.43	4.221	518306	6954	1.32
Injection-3	2.690	1389202	4151	1.45	4.221	522064	6885	1.30
Injection-4	2.690	1383831	4125	1.43	4.218	520877	6866	1.32
Injection-5	2.6694	1392955	4310	1.43	4.219	522181	7049	1.28

3.2.2 Linearity

MET showed a linearity range was between 50-150 µg/mL for MET and CANA. These were represented by a linear regression equation as follows: y (MET) = 43363X (r^2=0.999), y (CANA) = 5205x (r^2=1) and a regression line were established by least squares method and

correlation coefficient (r2) for MET and CANA is found to be greater than 0.98. Hence the curves established were linear.

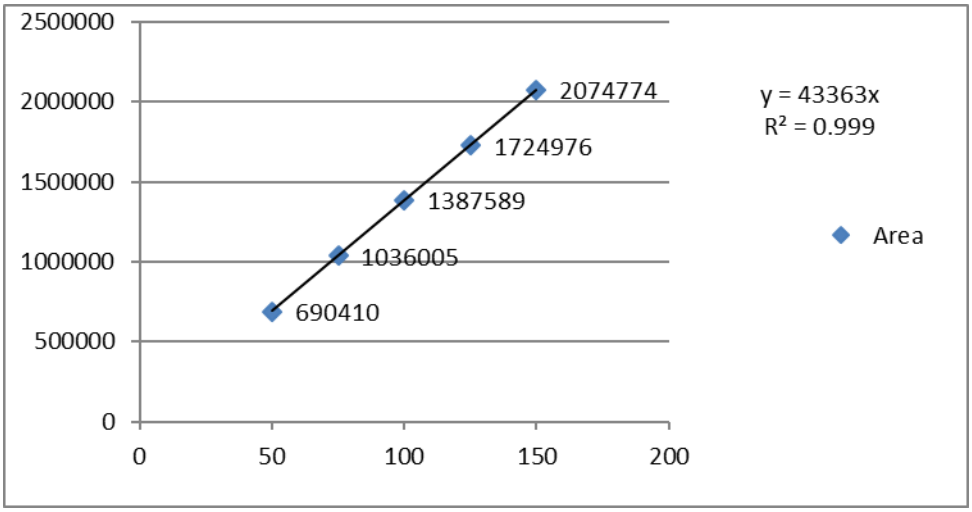

Figure 4.1: Linearity plot of Metformin

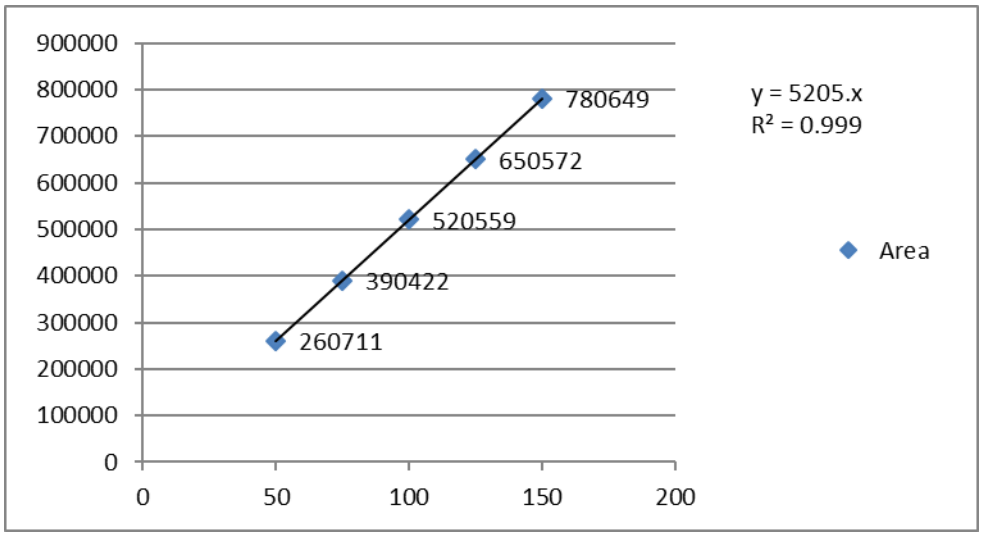

Figure 4.2: Linearity plot of Canagliflozin

3.2.3 Accuracy

To pre-analyzed sample solution, a definite concentration of standard drug (50%, 100% & 150 % level) was added and recovery was studied. The % Mean recovery for MET and CANA are 100.23 and 100.1 respectively and these results are within the acceptable limit of 98-102.

Table 2: Results of the accuracy of MET and CANA

Analyte	Concentration	Area	Amount added	Amount found	% Recovery
Metformin	**50**	690825	247.686	247.96	100.11
	100	1387759	496.000	497.96	100.39
	150	2047980	743.686	745.23	100.20
Canagliflozin	**50**	260658	49.737	49.85	100.22
	100	520060	99.600	99.62	100.02
	150	780873	149.337	149.43	100.06

3.2.4 Precision:

Repeatability Six replicates injections in same concentration were analyzed in the same day for repeatability and the % RSD for MET and CANA found to be 0.146 and 0.058 respectively and % RSD for MET and CANA found to be within the acceptable limit of ≤2 and hence the method is reproducible and the results are shown in Table 3.

Table 3: Results of Precision for MET and CANA

Injection no	Peak area of Metformin	Peak area of Canagliflozin
1	1384762	520159
2	1387359	520535
3	1383028	520242
4	1382785	520861
5	1386367	520168
6	1387168	520730
Mean	1385244.833	520449.166
SD	2031.048	303.977
%RSD	0.146	0.058

SD= Standard deviation RSD= Relative standard deviation

3.2.5 Robustness

The robustness was established by changing the flow rate, column temperature, and composition of the mobile phase within allowable limits from actual chromatographic

conditions. It was observed that there was no marked change in mean Rt and RSD is within the limit of ≤2. The tailing factor, resolution factor, and No. of theoretical plates are found to be acceptable limits for both MET and CANA. Hence the method is reliable with variations in the analytical conditions and the results of MET and CANA is shown in Table 4.

Table 4: Results of Robustness for MET and CANA

Parameters	Metformin		Canagliflozin	
	Retention time	Theoretical plate count	Retention time	Theoretical plate count
Flow rate-0.8ml/min	3.273	5036	4.20	8631
Flow rate-1.2ml/min	2.241	4274	3.427	6497
Temperature-20^{o}c	3.382	4613	5.206	1185
Temperature-30^{o}c	2.252	3976	3.493	6327

3.2.6 LOD and LOQ

LOD and LOQ for MET were 0.15 and 0.46 µg/mL respectively and for CANA were 0.19 and 0.58 µg/mL, respectively. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive.

3.2.7 Assay:

$$\%\ Assay = \frac{\text{Sample Area}}{Standard\ Area} X \frac{\text{Concentration of standard}}{concentrations\ of\ Sample} X \frac{\text{Average Weight}}{L.C} X \frac{P}{100} X100$$

Table 5: Assay Results for MET and CANA

S no	Sample name	Metformin Area	Rt	Canagliflozin Area	Rt
1	Standard	1375307	2.682	369314	4.235
2	Sample	1384762	2.697	371852	4.218

4. CONCLUSION

A new precise accurate and simple RP-HPLC method was developed and validated for simultaneous estimation of Metformin and Canagliflozin tablet dosage form. This method is

fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in QC laboratories and industries.

REFERENCES

1. Bailey CJ, Path MRC, Robert C, Turner. *New England Journal of Medicine*, 1996 :334: 574-579.
2. Edward C. Canagliflozin. *Drugs of the Future*, 2011; 36(5): 351-357.
3. Madhukar A, Prince A, Vijay K. Simple and sensitive analytical method development and validation of metformin HCl by RP-HPLC. *Int J Pharm Pharm Sci.* 2011:3(3):117-120.
4. Suchitra S. Analytical method development and Validation of Canagliflozin: Review. *Imperial Journal of Interdisciplinary research*.2017: 3(10):205-207
5. Rajesh T, Lakshmi KS, Srinivas S. Simultaneous determination of metformin and pioglitazone by Reversed-phase HPLC in pharmaceutical dosage forms. *Int J Pharm Pharm Sci.* 2009: 1(2): 112-116.
6. Dai XM, An N, Wu JM, Li HY, Zhang QM. Development and validation of HPLC-UV-MS method for the control of four anti-diabetic drugs in suspected counterfeit products. *Acta Pharm. Sinica*. 2010:45 (3): 347-352.
7. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J Chromatogr A.* 2003, 987, 57–66.
8. Method validation guidelines International Conference on Harmonization; GENEVA; 1996.
9. Sethi P, HPLC Quantitative Analysis of Pharmaceutical Formulations, 1st ed.CBS Publication and Distributor, 2001.
10. Validation of Analytical Procedures, ICH Harmonised Tripartite Guidelines, Q2B, 1997.