IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN AND CANAGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORM

Kanthi Silpa*, Sirisha. K, Vivek Sagar. P

Department of Pharmaceutical Analysis

Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Tarnaka, Secunderabad

CORRESPONDING AUTHOR

Dr. P. Vivek Sagar

Department of pharmaceutical Analysis

Sarojini Naidu Vanita Pharmacy Maha Vidyalaya

Abstract:

A novel, rapid, and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Metformin and Canagliflozin in their API and pharmaceutical dosage form. The detection wavelength was selected at 240 nm based on the overlay spectra of individual drugs. The chromatographic separation was achieved on a Symmetry C-18 column (4.6×150mm) 5µ column with a mobile phase consisting of methanol: pH 3 phosphate buffer (70: 30 % v/v) at a flow rate of [Flow Rate] mL/min. The retention times for Metformin and Canagliflozin were found to be 2.406 and 3.973 minutes, respectively. The method demonstrated good system suitability parameters with theoretical plates exceeding 2200 and tailing factors less than 1.3. The resolution between the peaks was 8.67, ensuring efficient separation. The developed method exhibited excellent linearity (correlation coefficient > 0.999) across a broad concentration range. The accuracy study confirmed high recovery of both drugs (around 99.5%) within the acceptable range (98-102%). Furthermore, the method demonstrated robustness by maintaining acceptable performance when subjected to slight variations in flow rate (±0.2 mL/min) and organic modifier composition (±5%) in the mobile phase. This robust, validated RP-HPLC method can be effectively employed for the routine analysis of Metformin and Canagliflozin in bulk and pharmaceutical formulations.

Key words: Metformin, Canagliflozin, RP- HPLC

1. INTRODUCTION

Metformin Hydrochloride is an orally administered biguanide derivative used to lower blood glucose concentration in patients with noninsulin dependent diabetes mellitus. Metformin Hydrochloride improves insulin sensitivity and decreases insulin resistance by inhibiting Complex1 of the mitochondrial respiratory chain and inducing AMP activatedprotein kinase-dependent signaling Metformin Hydrochloride is chemically known as 1, 1Dimethylbiguanide mono-hydrochloride. Canagliflozin is an anti diabetic drug used to improve glycemic control in patients with type 2 diabetes. Canagliflozin is an inhibitor of subtype 2 sodium glucose transport protein (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney (SGLT1 being responsible for the remaining 10%) 2. Canagliflozin is chemically known as (2S, 3R, 4R, 5S, 6R)- 2-{3- [5- [4- Fluoro- phenyl) -thiophen- 2- ylmethyl]- 4- methyl-phenyl-6-hydroxymethyl-tetra hydro-pyran-3,4,5-triol. Though several methods are reported in literature for the estimation of Metformin Hydrochloride 3 – 6 and Canagliflozin 7 - 10 individually, there are only few HPLC methods reported for the simultaneous estimation of Metformin Hydrochloride and Canagliflozin combination 11 – 14 . The objective of the present study was to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Metformin Hydrochloride and Canagliflozin and validate the method with forced degradation studies according to ICH guidelines

2. MATERIALS AND METHODS

2.1.HPLC grade acetonitrile (LichrosolR , Merck Lifesciences Pvt. Ltd., Mumbai, India), HPLC water (LichrosolvR Merck Life sciences Pvt. Ltd., Mumbai, India) Ortho phosphoric acid (Thermo Fischer Scientific Pvt Ltd., Mumbai, India), and sodium hydroxide (SD Fine - Chem. Ltd., Mumbai, India) were used in the study. The working standards of Metformin and Canagliflozin were generous gift obtained from Hetero Pharma Ltd., Hyderabad, India. Invokamet tablet containing Metformin Hydrochloride 500 mg and Canagliflozin 50mg was kindly supplied by Janssen pharmaceuticals, Inc.

2.2.Solutions:

Preparation of phosphate buffer

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluent

Mobile phase was used as the diluent.

Preparation of standard Metformin Solution

10 mg of Metformin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.5 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of standard Canagliflozin Solution

1 mg of Canagliflozin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to

Dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 3 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of test solution

10 mg of Metformin and 1 mg Canagliflozin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Preparation of standard solution

10 mg Metformin and 1mg Canagliflozin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

2.2.1. Cc standards;

Preparation of stock solution: 10 mg of Metformin and 1mg of Canagliflozin working standard were accurately weighed and were transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Preparation of Level – I (50ppm of Metformin and 5 ppm of Canagliflozin)

0.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – II (100ppm of Metformin and 10ppm of Canagliflozin)

1 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – III (150ppm of Metformin and 15ppm of Canagliflozin)

1.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – IV (200 ppm of Metformin and 20ppm of Canagliflozin)

2 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

Preparation of Level – V (250 ppm of Metformin and 25ppm of Canagliflozin)

2.5 ml of stock solution was taken in to 10ml of volumetric flask and diluted up to the mark with diluent.

2.3. Chromatographic conditions:

A new method was established for simultaneous estimation of Metformin and Canagliflozin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Metformin and Canagliflozin by using SYMMETRY C18 column (4.6×150mm) 5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer (KH2PO4and K2HPO4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2.

2.4.System suitability:

Standard solutions were prepared according to the test method and injected into the chromatographic system. System suitability parameters were determined according to the ICH guidelines. The parameters include plate count, tailing factor, and resolution. The plate count should be more than 2000, the tailing factor should be less than 2, and the resolution must be more than 2.

2.5. Method validation

The method validation was performed in accordance with ICH guidelines

2.5.1. Linearity

The linearity of an analytical procedure is its ability (within given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. For linearity prepared series of solutions of different concentrations in given range (50-250µg/ ml for metformin and 5-25µg/ ml for Canagliflozin) were injected and chromatogram was recorded in duplicate. A calibration curve was constructed by taking concentration on X- axis and average peak area on Y- axis.

2.5.2. Accuracy

Accuracy was determined by the recovery studies of the analyte. The recovery studies were performed by the standard addition method. In this method, a test solution of known quantity is spiked with standard solutions at three levels, namely 50%, 100%, and 150%. The spiked solutions are then analyzed by HPLC. The mean percentage recoveries at all the levels were calculated. The recovery studies were performed to ensure that the method was accurate. The accuracy of the method was expressed as the percentage recovery of the analyte. The percentage recovery should be within acceptable limits.

2.5.3. Precision

The precision of the method was established at two levels: system precision (Intermediate) and method precision (repeatability). System precision was assessed by taking six injections from a homogenous working standard solution. Method precision (repeatability) was demonstrated by preparing sample stock solution and six working sample solutions of the same concentrations. An injection was given from each working sample solution. Reproducibility was determined by injecting a single injection from each of six working sample solutions that were prepared. The average area, standard deviation, and % RSD were calculated for the two

drugs. The limit for precision was less than 2%. The precision of the method was found to be within acceptable limits. This means that the results of the analysis are reproducible and reliable.

2.5.4. Robustness

The robustness of the method was studied by making deliberate changes in the flow rate and change in organic phase ratio in the mobile phase. After making each change, chromatograms were recorded by injecting the standard solutions in six replicates. System suitability parameters were checked at each level.

2.5.5. Specificity:

The specificity of the method was established by examining a blank chromatogram for any interfering peaks. The specificity of the method was also evaluated with regard to interference due to the presence of any other excipient. The specificity of the method was found to be within acceptable limits. This means that the method is able to distinguish between the analyte of interest and other substances that may be present in the sample.

2.5.6. Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value based on the Standard Deviation of the Response and the slope

LOD = (3.3 * σ) / S

σ = Standard deviation

S = Slope of the calibration curve of the analyte

2.5.7. Limit of Quantification (LOQ)

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. From the linearity data calculate the limit of detection and quantification, using the following formula,

LOQ = (10 * σ) / S

σ = Standard deviation

S = Slope of the calibration curve of the analyte

3. RESULTS AND DISCUSSION

3.1 Assay of formulation:

The assay of the formulation was performed according to the given procedure. This was done in triplicate. The amount of drug present in the formulation was calculated from the standard graph. The % assay of Metformin and Canagliflozin in pharmaceutical dosage form was found to be 98.24 and 100.27% respectively..

Representative chromatograms for the standard and test were shown in Figures 3 and 4. The results were summarized in Table 1.

3.2 System suitability

The retention time of Metformin and Canagliflozin was found to be 2.406mins and 3.973mins respectively. The system suitability parameters for Metformin and Canagliflozin such as theoretical plates and tailing factor were found to be 2294, 1.27and 4891, 1.03. Resolution was 8.67.The results showing system suitability parameters were given in Table 2.

3.3 Validation

3.3.1. Linearity

The linearity of the method was determined in the range of 50-250µg/ ml for metformin and 5-25µg/ ml for Canagliflozin and the correlation coefficient was found to be NLT 0.999. The peak areas were plotted against concentration and the calibration curve was constructed. The calibration curve was illustrated in Figure 3. The correlation coefficient (r^2) was greater than 0.99 within the concentration range for both drugs. The results for linearity were given in Table 3. The standard graphs are shown in Figures 5 and 6.

3.3.2. Accuracy

The accuracy of the method was established at three levels of concentrations using the standard addition method. Triplicate injections were given at each level of accuracy and percentage recoveries were calculated. The mean percentage recoveries were obtained as 99.56% and 99.47% for metformin and Canagliflozin respectively. The results for accuracy were given in Table 4.

3.3.3. Precision:

The precision of the method was studied by considering system precision, method precision. The Method precision study was performed and %RSD of Metformin and Canagliflozin was found to be 0.3 and 0.3 (NMT 2). The intermediate precision was performed for %RSD of Metformin and Canagliflozin was found to be 1.3 and 0.4 respectively (NMT 2). The results for system precision, method precision, and interday precision were given in Tables 5 and 6, respectively..

3.3.4. Robustness:

The robustness of the method was studied by making deliberate changes in the flow rate and change in organic phase ratio in the mobile phase. After making each change, chromatograms were recorded by injecting the standard solutions in six replicates. System suitability parameters were checked at each level. The system suitability parameters were not much affected and all the parameters were passed. The variation in flow rate is does not affect the method significantly, Organic composition in the mobile phase also does not affect the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.. The results were given in Table 7.

3.3.5. Specificity:

The specificity of the method was evaluated by examining the blank chromatogram for any interfering peaks. The blank chromatogram showed no extra peaks, which indicates that the method is specific. The blank chromatogram showed no extra peaks, which means that there were no interfering peaks present in the

solution. This indicates that the method is specific and can distinguish between the analyte of interest and other substances that may be present in the sample.

3.3.6. Limit of detection

The limit of detection (LOD) for ivacaftor and lumacaftor was found to be 0.79 µg/mL and 2.95 µg/mL, respectively.

3.3.7. Limit of Quantification

LOQ of for Ivacaftor and Lumacaftor was found to be 0.98 µg/ml and 3.97 µg/ml respectively.

4. CONCLUSION

The developed HPLC method was found to be simple, precise, accurate, and sensitive for the simultaneous estimation of lumacaftor and ivacaftor in pharmaceutical dosage form. The results are in accordance with ICH guidelines. Hence, this method can be easily and conveniently adopted for routine analysis of lumacaftor and ivacaftor in pure and pharmaceutical dosage form. From the results, it was concluded that this newly developed method for the simultaneous estimation of lumacaftor and ivacaftor was found to be simple, precise, and accurate. The high resolution and shorter retention time make this method more acceptable and cost-effective, and it can be effectively applied for routine analysis in research institutions, quality control departments in industries, and approved testing laboratories in the near future.

5. REFERENCES:

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Table 1. Assay Results

S. No	Name of compound	Amount taken(mg)	%purity
1	Metformin	628.5	98.24
2	Canagliflozin	628.5	100.27

Table 2: System suitability parameters for Metformin and Canagliflozin

Peak Name	RT	Area	Height	USP Plate Count	USP Resolution	USP Tailing
metformin	2.464	2270870	433508	5299.2	1.2	1.1
canagliflozin	3.746	968090	144381	7292.6	8	1.1

Table 3: Linearity data of Metformin and Canagliflozin

	Metformin		Canagliflozin	
S.No	Concentration	Peak Area	Concentration	Peak Area
1	50 ppm	800199	5 ppm	339009
2	100 ppm	1589391	10ppm	689527
3	150 ppm	2264300	15ppm	994963
4	200 ppm	3071625	20ppm	1385006
5	250 ppm	3894075	25ppm	1766425

Table 4: Accuracy table of Metformin and Canagliflozin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1184204.3	5	4.96	99.91%	99.56%
100%	2121872.4	10	9.98	99.18%	
150%	3525766.1	15	15.02	99.60%	

Table 5: Accuracy table of Canagliflozin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	52228.2	0.5	0.99	99.53%	99.47%
100%	979319	1.0	1.05	99.38%	
150%	1576651	1.5	1.495	99.52%	

Table 6: Precision data of Metformin and Canagliflozin

	System precision		Method precision	
S. No	Metformin Area	Canagiflozin Area	Metformin Area	Canagiflozin Area
1.	2270553	993413	2286415	1006874
2.	2278100	993859	2285660	1005320
3.	2282356	998213	2286224	1006642
4.	2283157	998930	2292264	1007252
5.	2285975	999663	2301304	1011606
6.	2286548	995698	2355896	1014319
Mean	2280028.2	996815.8	2304269.6	1009027.9
S.D	6001.7	2952	29539	3782.8
%RSD	0.3	0.3	1.3	0.4

Table 7: Robustness data for Metformin and Canagliflozin for change in flow rate.

S. No	**Flow rate (ml/min)**	**System suitability results For Metformin**		**System suitability results For Cangliflozin**	
		USP Plate Count	**USP Tailing**	**USP Plate Count**	**USP Tailing**
1	0.6	2590	1.39	5435	1.04
2	**0.8**	**2294**	**1.27**	**4891**	**1.03**
3	1.0	2146	1.26	4781	1.04

Table 7: Robustness data for Metformin and Canagliflozin for change in mobile phase ratio.

S. No	**Change in organic composition in the mobile phase**	**System suitability results for Metformin**		**System suitability results for Canagliflozin**	
		USP Plate Count	**USP Tailing**	**USP Plate Count**	**USP Tailing**
1	5 % less	2347	1.44	5437	0.99
2	***Actual**	**2294**	**1.27**	**4891**	**1.03**
3	5 % more	2239	1.13	4817	1.05

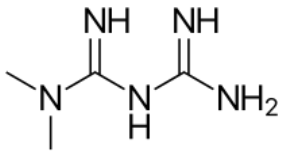

Fig 1: Structure of Metformin

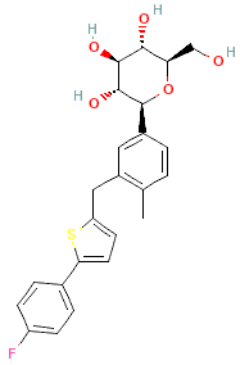

Fig.2: Structure of Canagliflozin

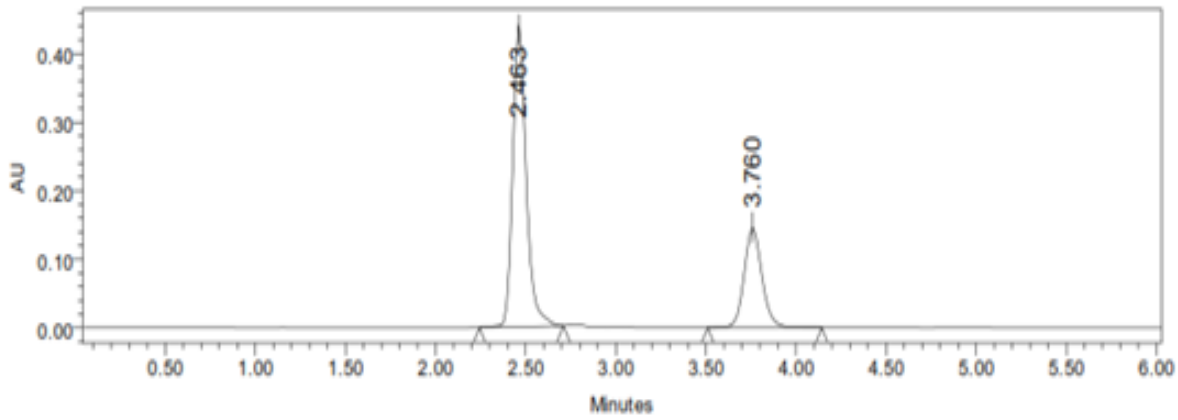

Fig 3: Representative Chromatogram of working standard solution

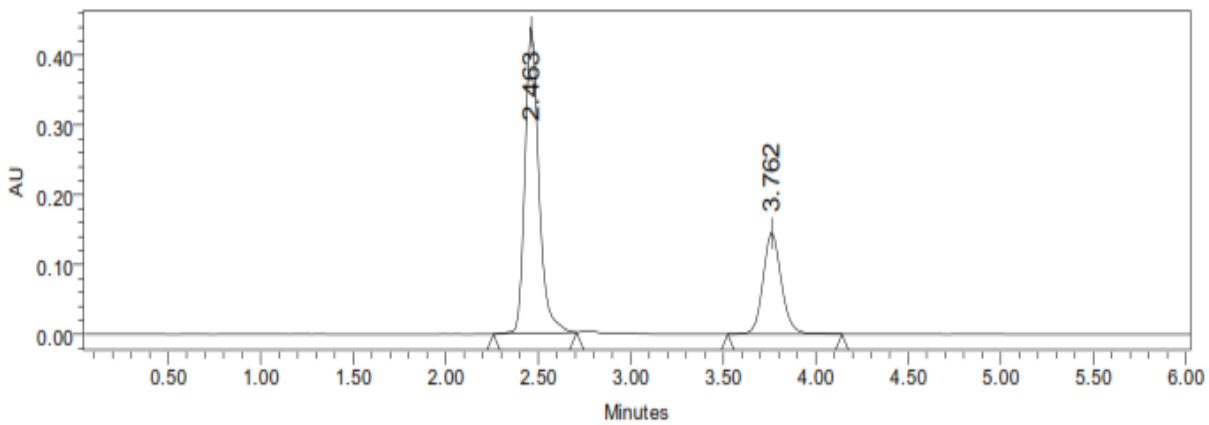

Fig 4: Representative Chromatogram of working sample solution

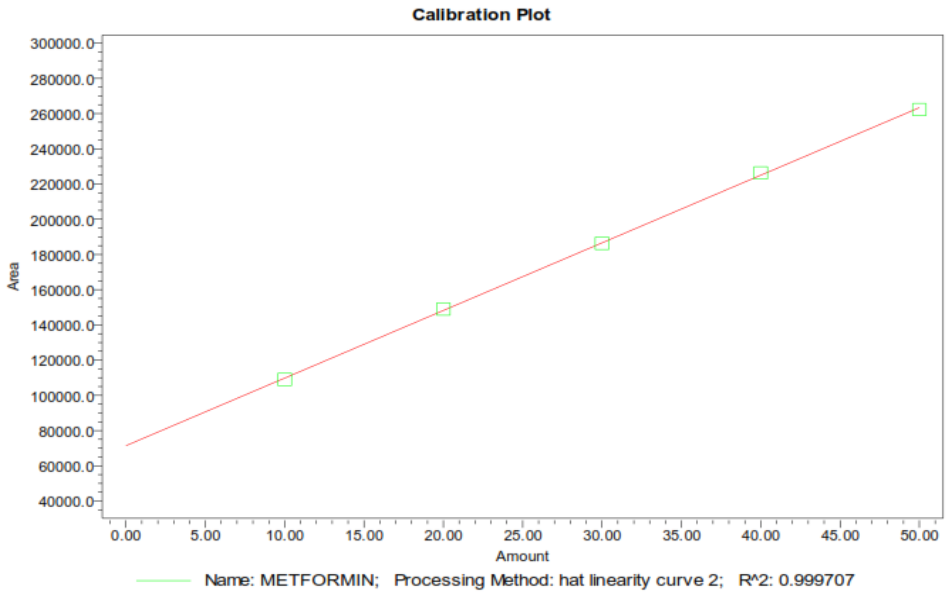

Fig 5: Calibration curve of Metformin

Fig 6: Calibration curve of Canagliflozin