Method Development and validation of simultaneous estimation of Metformin & Canagliflozin by using RP HPLC

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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 method involves separation on Kromasil C18 column (250mm x 4.6mm x5µm particle size). The optimized mobile phase consists of Acetonitrile:Buffer:Methanol (52:38:10 v/v) with a flow rate of 1ml/min and UV detection at 254nm. Retention time was 2.216min for Metformin Hydrochloride, 3.223min min for Canagliflozin. RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Canagliflozin in their combine dosage form was developed and validated as per the ICH guidelines. Linearity was observed in the range of 50-300 µg/ml for Metformin Hydrochloride and 5-30 µg/ml for Canagliflozin with correlation coefficients (r^2=0.999). The percentage recoveries of Metformin Hydrochloride and Canagliflozin were in the range of 98.2-101.4% which was with in the acceptance criteria. The percentage RSD was NMT 2% which proved the precision of the developed method. When applied for tablet assay, drug content was within 98.55-101.4% of labeled content. When the validation parameters of the method developed are compared with those of the earlier reported methods the developed method was found superior in certain respects such as RT, LOD and the method was more economical when compared to others. Accuracy and precision, ruggedness and robustness were similar to earlier reported methods.

KEYWORDS: Metformin Hydrochloride, Canagliflozin, RP-HPLC Method, Simultaneous estimation Validation as per ICH guidelines.

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1. INTRODUCTION

Metformin 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.

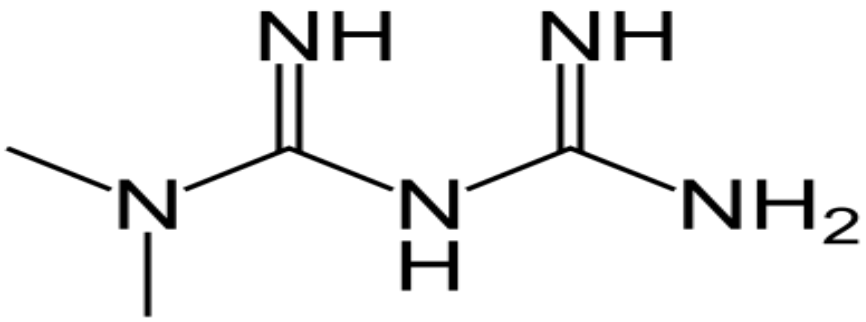

Fig 1: Structure of metformin

Canagliflozin 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.

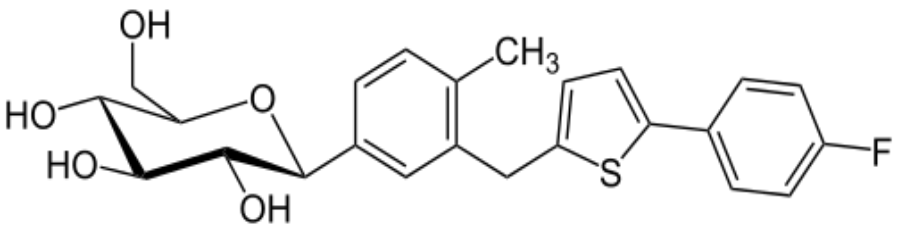

Fig 2 : Structure of Canagliflozin

Various UV & HPLC assay methods are also reported in the literature for the estimation of Metformin and Canagliflozin individually and in combination with other drugs . 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.

2. METHODS & MATERIALS:

2.1 Instrumentation

The chromatographic system consisted of WATERS, Chromatography system consisted of WATERS HPLC fitted with Prominence LC 20 AD Series pump and PDA detector using Empower, 2695 Solutions software as data handling system. Kromasil C18 column (250mm x 4.6mm x5µm particle size) was used for this method. Ultrasonic bath (Toshcon by Toshniwal), digital Ph meter (Systronics model 802) were used in the study.

2.2 Reagents and chemicals

The reference samples of Metformin and Canagliflozin 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: Metformin -500 mg & Canagliflozin - 100 mg) were purchased from the local pharmacy.

2.3 Chromatographic condition

The mobile phase consisted of buffer,Acetonitrile and Methanol were mixed in the proportion of 58:32:10v/v at a flow rate of 1 mL/min. Although the Metformin and Canagliflozin have different λ max, considering the chromatographic parameter, sensitivity, and selectivity of the method for both drugs, 254 nm was selected as the detection wavelength for PDA detector

2.4 Preparation of buffer:

Accurately weighed 1.36gm of Potassium di-hydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of HPLC Grade water added and degas to sonicate and finally make up the volume with water and pH adjusted to 4.2.

2.5 Preparation of mobile phase:

The above prepared buffer,Acetonitrile and Methanol were mixed in the proportion of 58:32:10v/v , was filtered and degassed by sonication.

2.6 Preparation of diluents:

Mobile phase was used as diluent.

2.7 Preparation of standard stock solutions of Metformin Hydrochloride and Canagliflozin:

Standard stock solutions of Metformin Hydrochloride and Canagliflozin were prepared by dissolving 500mg of Metformin Hydrochloride and 50mg of Canagliflozin in100ml of diluent into a 100ml clean dry volumetric flask and the standard solutions was filtered and degassed by sonicator to get the concentration of 5000µg/ml of Metformin Hydrochloride and 500µg/ml of Canagliflozin.

2.8 Preparation of standard solutions of Metformin Hydrochloride and Canagliflozin for assay:

From the above standard stock solution of 5000µg/ml of Metformin Hydrochloride and 500µg/ml of Canagliflozin further pipette 0.4ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 200µg/ml of Metformin Hydrochloride and 20µg/ml of Canagliflozin.

2.9 Preparation of sample solutions of Metformin Hydrochloride and Canagliflozin:

Twenty tablets were accurately weighed and powdered and tablet powder equivalent to 500mg of Metformin Hydrochloride and 50mg of Canagliflozin were taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent. Further pipette out 0.4ml from the above Metformin Hydrochloride and Canagliflozin sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 200µg/ml of Metformin Hydrochloride and 20µg/ml of Canagliflozin.

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: 4.2 both drugs eluted with better separation. Thereafter, Acetonitrile:Buffer:Methanol (52:38:10 v/v) and with a flow rate of 1mL/min was employed. PDA detector with Kromasil C18 column (250mm x 4.6mm x5µm particle size) as 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 Metformin and Canagliflozin showed maximum absorption at 254 nm of wavelength and selected as the detection wavelength for PDA detector. The retention times were found to about 2.216 min and 3.223 min for Metformin and Canagliflozin, respectively. The chromatogram obtained was shown in the Fig. 3.

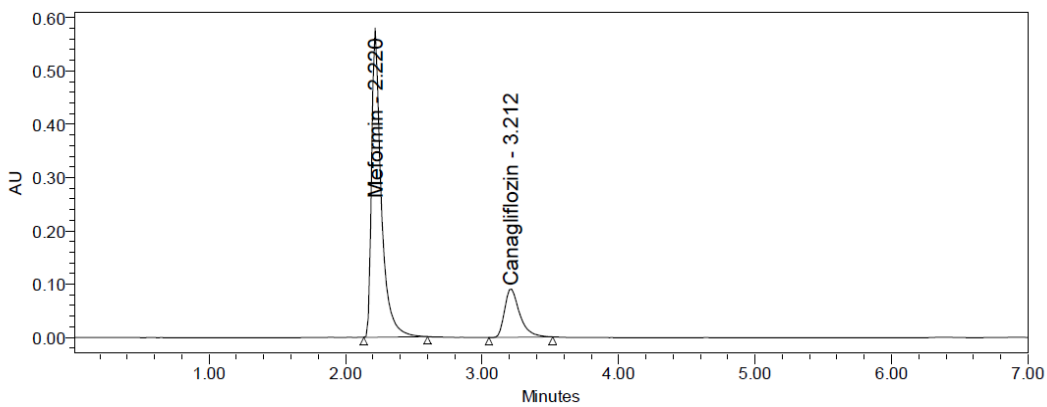

Figure 3: A typical Chromatogram of Metformin and Canagliflozin.

Table 1: System suitability studies of Metformin and Canagliflozin.

Property	**Metformin**	**Canagliflozin**
Retention time (t_R)	**2.220**	**3.312**
Theoretical plates(N)	**2563**	**3047**
Tailing factor (T)	**1.04**	**1.99**

3.2 Linearity:

Six Linear concentrations 50(µg/ml) - 300(µg/ml) of Metformin and 5(µg/ml)-30(µg/ml) of Canagliflozin are prepare and Injected .Regression equation of the Metformin and Canagliflozin are found to be,y = 7002.x + 400.3 and y = 6344.x + 898.8. and regression co-efficient was 0.999.

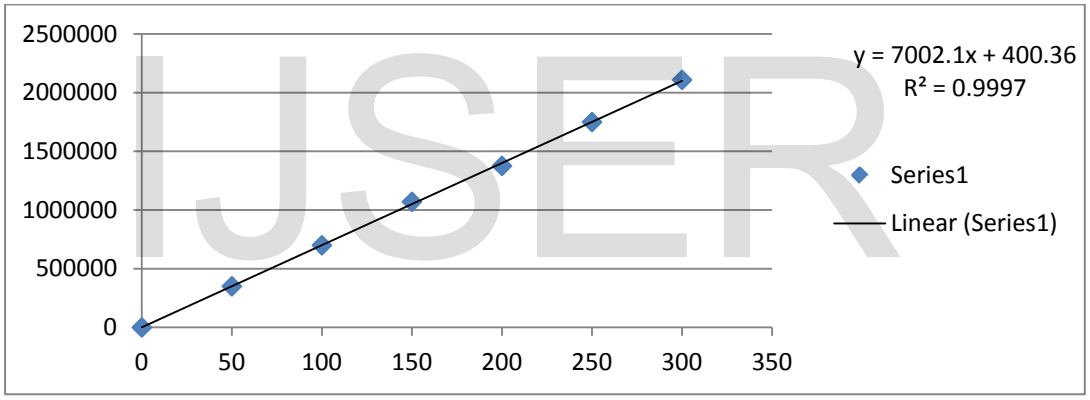

Fig 4: Calibration curve of Metformin.

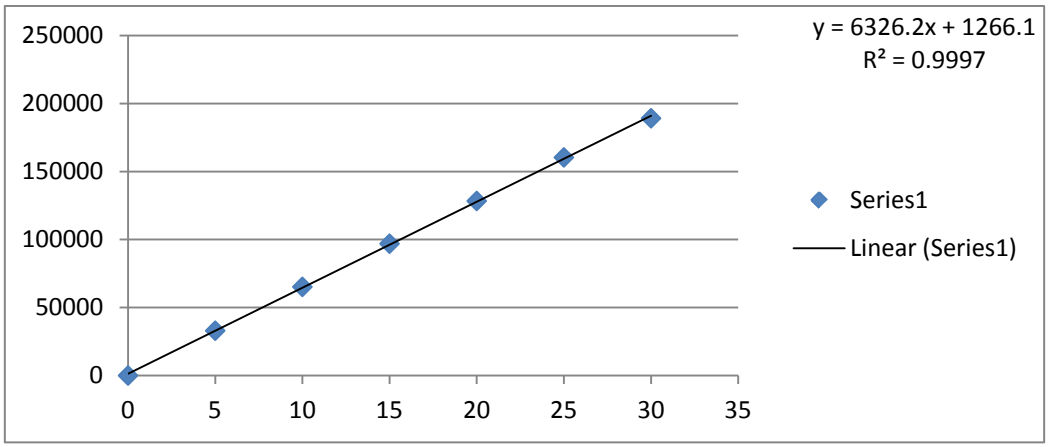

Fig 5: Calibration curve of Canagliflozin

3.3 Precision:

3.3.1Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Metformin and Canagliflozin were found to be 1.03% and 0.67% respectively.

Table 2 : Repeatability data for Metformin and Canagliflozin

S. No.	Metformin	Canagliflozin
1	1400930	130709
2	1375998	130752
3	1367248	129109
4	1368377	128941
5	171190	128849
6	1360626	129498
Mean	1374062	129643
Std. Dev.	14093.7	871.3
%RSD	1.03	0.67

3.3.2Inter day precision:

Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Metformin and Canagliflozin were 0.95 and 0.82.

Table 3: Inter day precision results for Metformin and Canagliflozin.

S. No.	Metformin	Canagliflozin
1	1403040	456940
2	1378762	462267
3	1373662	462562
4	1366739	461831
5	1370408	457291
6	1383729	469803
Mean	1379390	96054

Std. Dev.	13054.3	440.9
%RSD	0.95	0.82

3.4 Accuracy:

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 7.5.

Table 4: Accuracy data of Metformin

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	100	100.0	100.04	100.2%
	100	99.49	99.49	
	100	99.62	99.62	
100%	200	100.56	201.12	
	200	99.79	199.58	
	200	101.00	202.00	
150%	300	100.49	301.48	
	300	100.5	300.15	
	300	101.04	303.12	

Table 5 : Accuracy data of Canagliflozin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	100.46	10.05	100.83%
	10	101.34	10.13	
	10	100.83	10.08	
100%	20	100.87	20.17	
	20	101.28	20.26	
	20	101.33	20.27	
150%	30	100.8	30.24	
	30	100.7	30.21	
	30	99.9	29.96	

3.5 LOD and LOQ

LOD and LOQ for Metformin were 0.069 µg/mL and 0.210 µg/mL respectively and for CANA were 0.135 µg/mL and 0.410 µg/mL, respectively. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive.

3.6 Assay

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 99.7 and 100.1 for Metformin and Canagliflozin respectively.

Table 6 : Assay of Tablet

S. No.	Metformin %Assay	Canagliflozin %Assay
1	101.7	100.94
2	99.90	100.97
3	99.26	100.70
4	99.34	99.57
5	99.55	99.50
6	98.78	100.00
AVG	99.76	100.1
STDEV	1.02	0.6728
%RSD	1.03	0.67

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.

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