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A NEW RP-UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND CANAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The aim of the investigation was to develop a new RP-UPLC method for simultaneous estimation of Metformin and Canagliflozin in Bulk and pharmaceutical dosage forms. Chromatography was carried out on UPLC (WATERS) SYMMETRY® C 18(4.6x50mm) 3.5µm with an isocratic mobile phase composed of Buffer, Acetonitrile and methanol(30:40:30) at a flow rate of 0.8mL/min.(pH adjusted to 6.0 with KH_2PO_4.) The column temperature was maintained at 25°C and the detection was carried out using a UV detector at 260nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as stated in the ICH guidelines. The retention times for Metformin and Canagliflozin were 1.74min and 2.95min respectively. The percentage recoveries of Metformin and Canagliflozin were 99.99% and 100.78% respectively. The relative standard deviation for assay of tablets found to be less than 2%.The proposed method was successfully applied to the analysis of Metformin and Canagliflozin in bulk and pharmaceutical formulation without interference from other additives. The parameters values were found within the limits and the method was found to be Satisfactory. This validated UPLC method is economic, sensitive, precise and less time consuming than other chromatographic procedures.

INTRODUCTION:

Metformin (MET) was discovered in 1922. MET is chemically named as N,N-dimethyl limido di carbonimidic diamide[(1)](Fig.1). It is a drug of biguanide group. It is used in the treatment of Type-II Diabetes Mellitus. It is of oral anti hyper glycemic agent. Metformin decreases blood glucose level by decreasing hepatic glucose production, decrease intestinal absorption of glucose and improving insulin sensitivity by increasing peripheral

glucose uptake and utilization. Activation of the E-regulating enzyme AMP activated proteinkinase (AMPK), principally in muscle and the liver, is considered as major mode of MET action.[2] . MET is used to treat over weight, Polycystic overy syndrome, cardio vascular diseases, cancer etc. **Canagliflozin(CAN)** is chemically named as (2S,3R,4R,5S,6R)- 2-{3-[5-(4-fluoro-phenyl) - thiophen-2- ylmethyl]-4-methyl-phenyl} - 6 hydroxy methyl tetrahydro -pyran-3, 4, 5- triol with molecular formula $C_{24}H_{25}FO_5S$ (Fig.2). CAN is an anti diabetic drug used to improve glycemic control in people with Type-II diabetes[3]. CAN inhibit Na^+ dependent 14C-AMG uptake in a concentration dependent fashion. It is a novel C-glucoside with Thiopene ring. Sodium glucose co-transporter 2(SGLT2), expressed in the proximal renal tubules, is responsible for the majority of reabsorption of filtered glucose from the tubular lumen[4]. In extensive clinical trials, Canagliflozin produced a consistent dose-dependent decrease in HbA1c levels when administered either as monotherapy, in combination with metformin or Pioglitazone with insulin. Sodium glucose transport inhibitor [5,6]. Literature review reveals that very few analytical methods have been reported for the determination of MET& CAN which include ultra performance liquid chromatography[7-10], solid phase extraction-non-aq capillary electrophoresis[11] and bioequalence studies[12]. The present study was aimed to develop a novel, simple, accurate, precise, economic and validated method for the simultaneous estimation of MET&CAN studies according **to ICH guidelines[13].**

Materials and Methods:

Instrumentation: Chromatography was performed with uhplc_agilent_1220 infinity LC with high speed auto sampler with open lab_ chem station software using a UV detector at 260nm.

Reagents and Chemicals:

The sample reference of MET and CAN were provided as gift samples from Reddys Laboratories, Hyderabad**.** Acetonitrile, water, methanol, were obtained from Merck, Mumbai. and phosphate buffer, obtained from RANKEM Mumbai. All solvents used in this work are HPLC grade. Commercial tablets (INVOKAMET; Dosage: MET-500 mg & CANA- 150 mg) were kindly supplied by Janssen Pharmaceuticals [14-15].

Chromatographic conditions:

The mobile phase consisted of Buffer 0.02M Potassium dihydrogen phosphate (KH_2PO_4), Acetonitrile and methanol taken in the ratio of 30:40:30 and pH adjusted to 6.0 with NaoH and at a flow rate of 0.8 ml/min. Filtered through 0.45µm nylon membrane filter under vacuum filtration and pumped at ambient temperature. ODS (4.6x50mm) 3.5µm particle size was used as the stationary phase. MET and CAN have different λ max, by considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 260 nm was selected as the detection wavelength for UV detector. Injection volume was 2.0µl. The run time was 5.0 min and the retention time of Metformin Hydrochloride and Canagliflozin was found to be 1.74min and 2.95min respectively with resolution of 8.95. The resulting UPLC chromatogram was shown in (Figure 3).

Preparation of Phosphate buffer:

A 0.02M Phosphate buffer was prepared by dissolving 2.7218gm potassium di hydrogen Phosphate in 1000ml of HPLC grade water and pH was adjusted to 6.0 with Sodium hydroxide. The buffer was filtered through 0.45ìm nylon membrane filter to remove all fine particles and gases.

Preparation of mobile phase:

The above prepared Phosphate buffer, Acetonitrile and Methanol HPLC grade were mixed in the proportion of 30:40:30 v/v and was filtered through 0.45µm nylon membrane filter and

degassed by sonication and is used as diluent.

Standard stock solution Preparation: Accurately weighed and transferred 500.26mg and 150.45mg of MET and CAN working standards in to two 50ml clean and dry volumetric flask separately, add ¾ volume of diluent, sonicated for 30 minutes and make up to the volume with diluents. From the above stock solution 5.0ml was pipetted out in to a 50ml volumetric flask and then make up to the final volume with diluent.

Working Standard Solutions Preparation: Aliquot of 2.5, 3.5, 4.5, 5.5, 6.5, 7.5mL were pipette out from stock solution into 50 ml volumetric flask separately for both MET and CAN and volume was made up to 50 ml with diluent. This gives the solutions of 500, 700, 900, 1100, 1300, 1500µg/mL for MET and 150, 200, 250, 300, 350, 450µg/mL for CAN respectively.

Sample preparation:

Twenty tablets were weighed and crushed into fine powder. The average weight of tablet was weighed and dissolved in 50 ml diluent, sonicated for 20 min and filtered through PVDF 0.45µ filter. From the filtrate, 5 ml was pipette out and transferred into a 50 ml volumetric flask and the solution was made up to the volume with diluent.

RESULTS AND DISCUSSION

Method development:

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 responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH: 6.0 both drugs eluted with better separation. Thereafter, buffer: Acetonitrile: methanol were taken in isocratic ratio: %buffer / %Acetonitrile / %methanol: 30/40/30, with flow rate of 0.8mL/min was employed ODS (4.6x50mm) 3.5µm particle size was used as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to1. To analyze both drugs detection were tried at various wavelengths from 205nm to 280nm. Both MET and CAN show maximum absorption at 260nm with UV detector. The chromatogram obtained was shown in the Fig.3.

Method Validation:

The validation [16-17] of the method was carried out as per ICH guidelines the parameter assessed were System suitability, specificity, linearity, accuracy & precision, Robustness, LOD and LOQ.

System suitability:

The UPLC system was optimized as per the chromatographic conditions. One blank followed by five replicates of a single calibration standard solution of 1000µg/mL of MET and 300µg/mL of CAN was injected to check the system suitability. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates, peak asymmetry and resolution were taken and results were presented in Table 1.

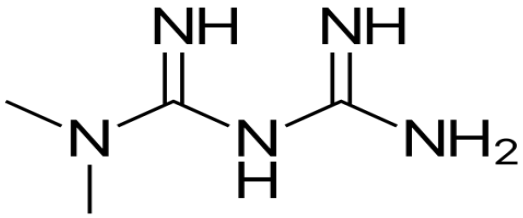
Specificity:

The effect of excipients and other additives usually present in the combined tablet dosage form of MET and CAN in the determination under optimum conditions was investigated. The specificity of the RP-UPLC method was established by injecting the blank and placebo solution into the UPLC system. The representative chromatogram of blank was shown in(Fig .4). and the readings are shown in Table 2.

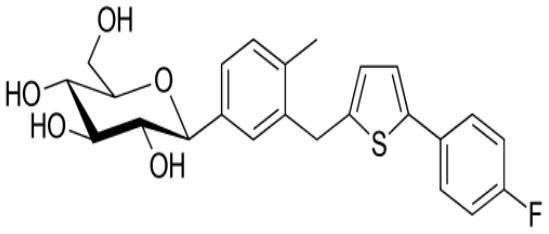
Linearity:

MET showed a linearity of response between 500-1500 µg/mL and CAN showed a linearity of response between 150-450µg/mL.

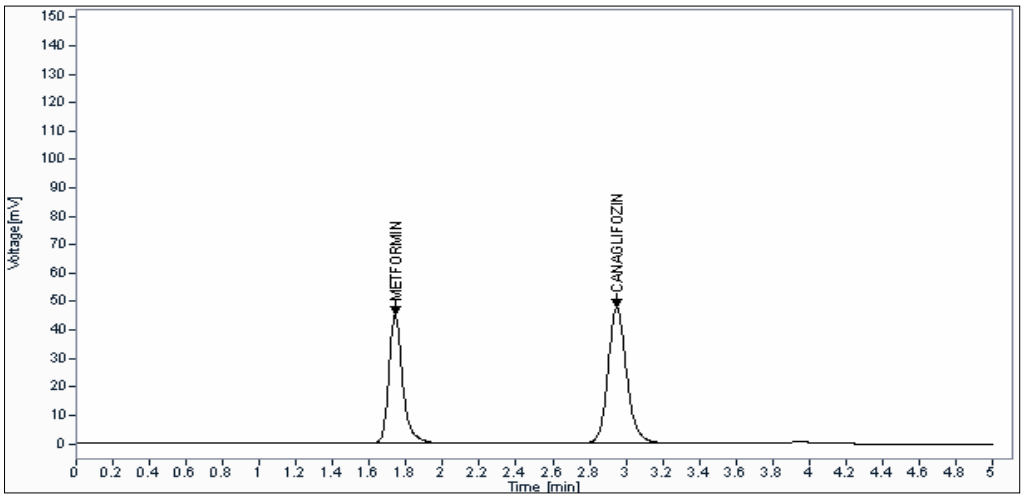
(Fig.1) Chemical Structure of Metformin



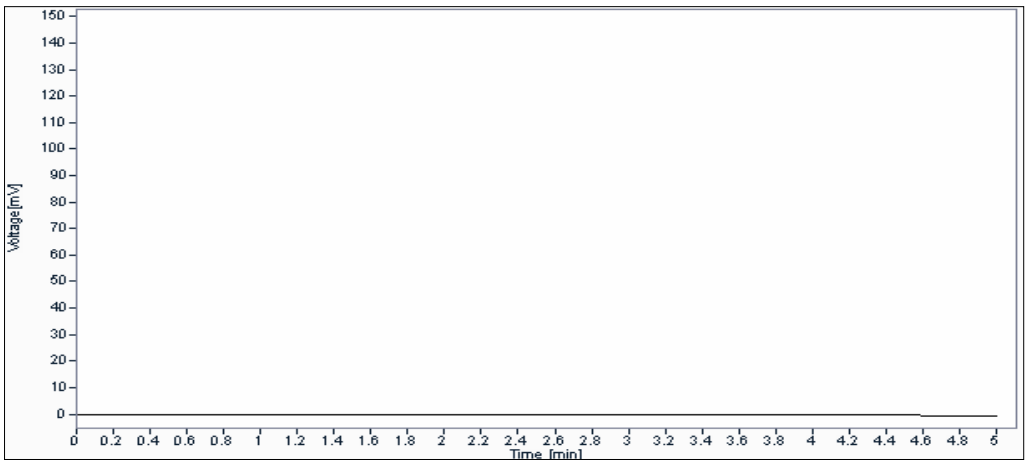
(Fig.2) Chemical Structure of Canagliflozin



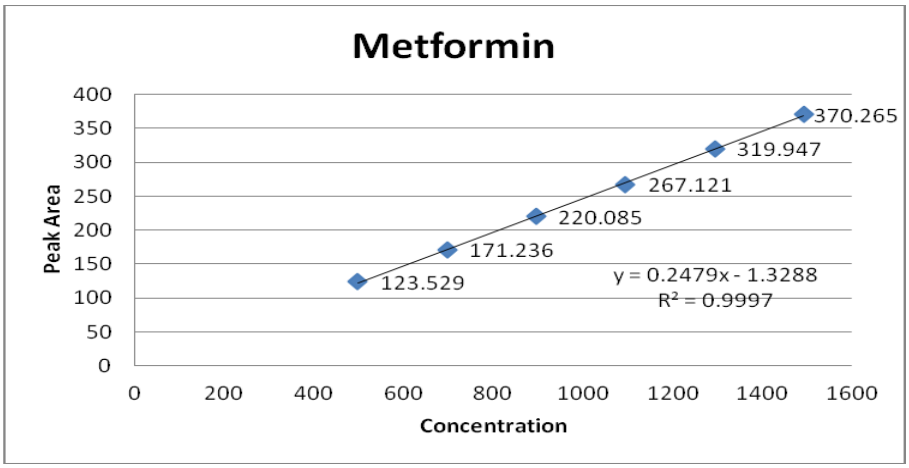
(Fig.3) Developed chromatogram



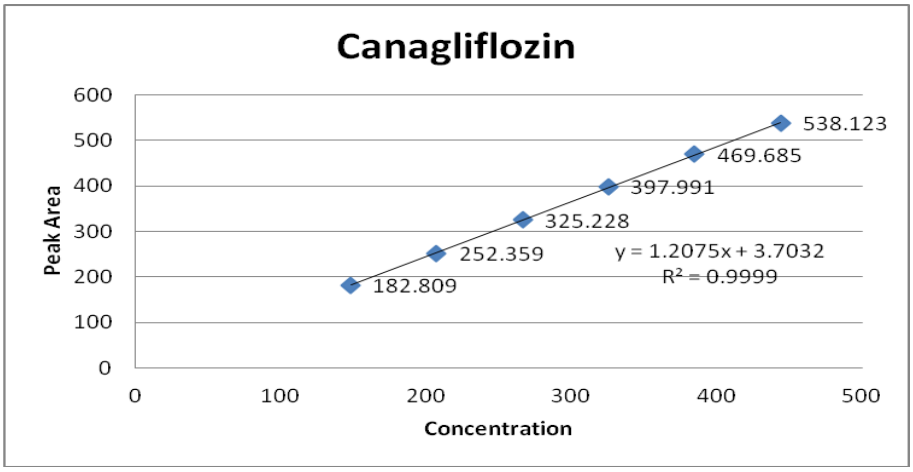
(Fig.4) Blank



(Fig. 5) Linearity of Metformin



(Fig.6) Linearity of Canagliflozin



(Table 1). System suitability

S.No	Parameter	Metformin *	Canagliflozin*
1.	Rt	1.75	2.964
2.	Theoretical plates	2852	4315.2
3.	Tailing factor	1.326	1.162
4.	Resolution	7.89	7.89
5.	Area%	41.26	58.74
6.	SD	2.74	3.49
7.	%RSD	1.136	1.017

*Mean average of five determinations

(Table 2). Specificity

S.No.	Injection	Metformin Rt	Area	Canagliflozin Rt	Area	SD	%RSD
1.	Metformin (5)	1.74	244.96587	NIL	NIL	1.41	0.58
2.	Canagliflozin(5)	NIL	NIL	2.95	345.75219	0.96	0.28
3.	Blank	About 1.74	NIL	About 2.95	NIL	NIL	NIL
4.	Placebo	About 1.74	NIL	About 2.95	NIL	NIL	NIL

(Table 3) Linearity

S.No	Linearity of Metformin		Linearity of Canagliflozin	
	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
1	497.534	123.529	147.966	182.809
2	696.547	171.236	207.153	252.359
3	895.561	220.085	266.300	325.228
4	1094.574	267.121	325.526	397.991
5	1293.588	319.947	384.713	469.685
6	1492.602	370.265	443.900	538.123
Slope	0.247		1.207	
Y-Intercept	1.328		3.703	
Correlation coefficient	0.999		0.999	

(Table 4). Recovery of Metformin

S. no.	Sample ID	Std Area	Sample Area	Calculated content of drug(mg)	Actual Assay	Recover Amount	Actual amount added	Recovery Percentage (%)
					Mg of drug/ avg. Wt			
1	Spiked with 10%	254.736	283.146	551.511	502.177	49.3337	49.7853	99.09
2	Spiked with 20%	254.736	308.813	601.339	502.177	99.1618	99.5432	99.62
3	Spiked with 30%	254.736	335.939	653.124	502.177	150.947	149.078	101.25

(Table 5). Recovery of Canagliflozin

S. no	Drug	Sample ID	Std Area	Sample area	mg/tab (Avg)	Average & Assay %	SD	%CV	%RSD
						At Individual Conc levels			
1.	Metformin	Low level	259.58	208.35	498.0853	99.62	0.30	0.31	1.12
		Middle level		263.28	503.7113	100.74	0.94	0.983	
		High level		317.1617	504.737	100.95	0.75	0.75	
2.	Canagliflozin	Low level	363.13	295.1483	150.9557	100.64	0.88	0.88	1.68
		Middle level		366.8517	150.1238	100.08	0.55	0.55	
		High level		439.0617	149.537	99.69	0.58	0.58	

(Table 6). Accuracy & precession

S. no.	Sample ID	Std Area	Sample Area	Calculated content of drug (mg)	Actual Assay	Recover Amount	Actual amount added	Recovery Percentage (%)
					Mg of drug/ avg.Wt			
1.	Spiked with 10%	367.287	405.747	165.0262	149.8280	15.1982	14.9196	101.87
2.	Spiked with 20%	367.287	442.331	179.8562	149.8280	30.0282	29.8311	100.66
3.	Spiked with 30%	367.287	478.917	194.4240	149.8280	44.5960	44.6757	99.82

(Table 7). Robustness of Metformin

S. No.	Parameter	Drug Metformin	Avg Peak Area	SD	%RSD
1.	Flow rate	0.6ml/min	334.68	4.18	1.25
		1.0ml/min	202.55	1.86	0.92
2.	Mobile phase Change	10% increase in aq phase	288.77	4.48	1.55
		10% increase in Org phase	28.11	1.93	0.85
3.	Change in Wave length	258nm	251.19	2.36	0.94
		262nm	236.68	1.92	0.81

(Table 8). Robustness of Canagliflozin

S.No.	**Parameter**	**Drug Canagliflozin**	**Avg Peak Area**	**SD**	**%RSD**
1.	Flow rate	0.6ml/min	478.79	5.39	1.13
		1.0ml/min	289.04	3.22	1.11
2.	Mobile phase Change	10% increase in aq phase	411.08	4.56	1.11
		10% increase in Org phase	325.75	3.27	1.0
3.	Change in Wave length	258nm	326.38	3.02	0.92
		262nm	385.62	2.65	0.69

(Table 9). Solution Stability

S.No	**Drug**	**Conc (µg/mL)**	**Rt**	**Peak area**	**Theoretical plates**	**SD of peak area**	**%RSD of Peak area**
				Average*			
Solution Stability at 24 hours							
1	Metformin	Middle level	1.75	264.41	2925.55	3.02	1.14
2	Canagliflozin		2.87	378.14	4441.23	4.33	1.44
Solution Stability at 48hours							
1	Metformin	Middle level	1.74	280.81	3004.34	1.35	0.48
2	Canagliflozin		2.99	402.31	4521.20	2.73	0.68

*mean average of six determination

These were represented by a linear regression equation as follows: y (MET) = 0.247x-1.328 (r^2=0.999), y (CAN) = 1.207x+3.703 (r^2=0.999) and regression line was established by least squares method and correlation coefficient (r^2) for MET and CAN is found to be greater than 0.98. Hence the curves established were linear, shown in Fig.5&6) and in Table 3.

Recovery:

To pre analyzed sample solution, a definite concentration of standard drug (10%, 20% & 30 % level) was added and recovery was studied. The % Mean recovery for MET and CAN was 99.99 and 100.78 respectively and these results are within acceptable limit of 98-102. The % RSD for MET and CAN are 1.13 and 1.02 respectively and %RSD is within limit of ≤ 2. Hence the proposed method is accurate and the results were summarized in Table 4& 5.

Precision:

Six replicate injections in same concentration were analyzed in the same day for repeatability and the % RSD for MET and CAN found to be 1.12 and 1.68 respectively and % RSD for MET and CAN found to be within acceptable limit of ≤ 2 and hence method is reproducible and the results are shown in Table 6.

Robustness:

The robustness was established by changing the flow rate, composition of the mobile phase and change in wavelength within allowable limits from actual chromatographic conditions. It was observed that there was no marked change in mean Rt and RSD is within limit of ≤ 2 .The tailing factor, resolution factor and no. of theoretical plates are found to be acceptable limits for both MET and CAN. Hence the method is reliable with variations in the analytical conditions and the results of MET are shown in Table 7 and results of CAN shown in Table 8.

Stability of Solution:

The sample solutions and mobile phase solution used during the validation were stable up to 48hours at room temperature. The % RSD of the prepared MET & CAN solutions were less than 2% are shown in Table 9., which tells the stability of the solutions.

Limit of Detection and limit of Quantification

The LOD can be define as the smallest level of analytes that gives a measurable response and LOQ was determined as the lowest amount of analytes that was reproducibly quantified. These two parameters were calculated using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated using equation LOD=3.3×s/S and LOQ=10×s/S, where s= standard deviation of Y-intercept, S = average slope of calibration curve. LOD and LOQ for MET were 6.2114 & 24.845 µg/mL respectively and for CAN were 1.856 & 7.424µg/mL, respectively.

CONCLUSION

A new simple precise accurate and validated RP-UPLC method has been developed for simultaneous estimation of Metformin and Canagliflozin in bulk and pharmaceutical dosage form with good retention time, economical mobile phase, and quick run time. Hence it can be employed for routine quality control of tablets containing both drugs in QC laboratories and industries and used for the routine analysis of metformin and Canagliflozin in both bulk and pharmaceutical dosage form.

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