IJPSR (2017), Volume 8, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 January, 2017; received in revised form, 17 May, 2017; accepted, 27 May, 2017; published 01 August, 2017

DEVELOPMENT OF A NEW STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN AND ITS VALIDATION AS PER ICH GUIDELINES

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Keywords:

Metformin Hydrochloride, Canagliflozin, RP-HPLC Method, Simultaneous estimation, Validation as per ICH guidelines, Forced degradation studies

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ABSTRACT: A new stability indicating RP HPLC method has been developed and validated for simultaneous estimation of Metformin Hydrochloride and Canagliflozin in bulk and dosage forms. The method involves separation on Kromasil C18 column (250mm x 4.6mm x5µm particle size). The optimized mobile phase consists of 0.1% OPA (pH 2.8) and Acetonitrile (45:55v/v) with a flow rate of 1ml/min and UV detection at 254mn. Retention time was 2.112 min (Metformin Hydrochloride), 2.671 min (Canagliflozin). Linearity range was 25-150ug/ml (Metformin Hydrochloride), 2.5-15ug/ml (Canagliflozin). Accuracy was in the range of 98.22-101.54% for both drugs. Precision was 0.63% and 0.65% for Metformin Hydrochloride and Canagliflozin. LOD and LOQ are 0.17ug/ml and 2.20ug/ml for Metformin Hydrochloride, 0.01ug/ml and 0.50ug/ml for Canagliflozin. The method developed is more sensitive, accurate and precise than the methods reported earlier. Retention time and run time were also less and hence the method is economical. When applied for tablet assay, drug content was within 98.55-101.4% of labelled content. Forced degradation studies indicated the suitability of the method for stability studies.

INTRODUCTION: Metformin Hydrochloride is an orally administered biguanide derivative used to lower blood glucose concentration in patients with noninsulin dependent diabetes mellitus [1]. Metformin Hydrochloride improves insulin sensitivity and decreases insulin resistance by inhibiting Complex1 of the mitochondrial respiratory chain and inducing AMP activated protein kinase-dependent signalling.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(8).3427-35

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (8).3427-35

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 [15].

Experimental:

MATERIALS AND REAGENTS: HPLC grade acetonitrile (Lichrosol^R, Merck Lifesciences Pvt. Ltd., Mumbai, India), HPLC water (Lichrosolv^R 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.

Instrumentation: Chromatography was performed on a Waters 2695 HPLC column (waters corporation, Mildord, USA) with an auto-sampler and equipped with a 2996 series of PDA detector with a spectral bandpass of 1.2nm. Components were detected using UV and that processing was achieved by Empower 2 software. A hot air oven was used for thermal degradation of the samples and a UV cross inker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 and 300nm was selected for photolytic degradation. Ultrasonic bath (Toshcon by Toshniwal), digital pH meter (Systronics model 802) were used in the study.

Chromatography Conditions: The chromatographic condition was performed on Kromosil C_{18} column (250 X 4.6mm, 5µm particle size) at an ambient column temperature. The samples were eluted using 0.1% Ortho phosphoric acid (pH adjusted to 2.8 with NaoH): Acetonitrile (45:55v/v) as the mobile phase at a flow rate of 1 ml/min the mobile phase and samples were degassed by ultra sonication for 20 min and filtered through 0.45µm Nylon (N66) 47mm membrane filter. The measurements were carried out with an injection volume of 10µL, flow rate was set to 1.0 mL/min, and UV detection was carried out at 254 nm. All determinations were done at ambient column temperature (27 °C). The chromatograms of the prepared standard stock solutions of Metformin and Canagliflozin were recorded under optimized chromatographic conditions (**Fig. 1**).

Diluent: Water and Acetonitrile in 50:50 v/v ratio.

Preparation of Standard Solutions:

Stock Solution of Metformin: Standard stock solution of Metformin Hydrochloride was prepared by dissolving 25mg of Metformin Hydrochloride in 25 ml of diluent (Water: Acetonitrile, 50:50 v/v) in a 25 ml clean dry volumetric flask and the standard solutions was filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 1000 µg/ml of Metformin Hydrochloride. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Metformin Hydrochloride.

Stock Solution of Canagliflozin: Standard stock solution of Canagliflozin was prepared by dissolving 2.5mg of Canagliflozin in 25ml of diluent (Water: Acetonitrile, 50:50 v/v) in a 25ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 100µg/ml of Canagliflozin. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Canagliflozin.

Working Standard Solution: Working standard solution of Metformin Hydrochloride and Canagliflozin was prepared by taking 1ml of stock solutions of Metformin and Canagliflozin in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 100µg/ml of Metformin and 10µg/ml of Canagliflozin.

Preparation of Sample Solutions of Metformin and Canagliflozin: Twenty tablets were accurately weighed and powdered and tablet powder equivalent to 500mg of Metformin Hydrochloride and 50mg of Canagliflozin was taken into 25ml clean dry volumetric flask, diluents was added and sonicated to dissolve completely and volume was made up to volume with the diluent. The above

sample solution suitably diluted to get a concentration of 100µg/ml of Metformin Hydrochloride and 10µg/ml of Canagliflozin.

RESULTS AND DISCUSSION:

Optimization of Chromatographic Conditions: Proper selection of the method depends upon the nature of the sample (ionic or ionisable or neutral molecule), its molecular weight, and solubility. Metformin Hydrochloride and Canagliflozin were dissolved in polar solvent, so the developed method of estimation was carried out on reverse phase high performance liquid chromatography. To develop a rugged and suitable HPLC method for the quantitative determination of Metformin and Canagliflozin, the analytical conditions were selected after the consideration of different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition, and other chromatographic conditions.

Preliminary trials were taken with different composition of buffer and organic phase of mobile phases with pH range of 2.5–5. The column selection has been done by backpressure, resolution, peak shape, theoretical plates, and day-to-day reproducibility of the retention time and resolution between Metformin and Canagliflozin peak. After evaluating all these factors, a Kromosil C_{18} column was found to be giving satisfactory results. The selection of acetonitrile and buffer were based on chemical structure of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates, and peak shape of both components. Best results were obtained with 0.1% O-phosphoric acid pH adjusted to 2.8 with sodium hydroxide solution that improved the peak shapes of Metformin Hydrochloride and Canagliflozin.

For the selection of organic constituent of mobile phase, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape. Therefore, final mobile phase composition consisting of a mixture of buffer - pH 2.8 (0.1% ortho phosphoric acid): Acetonitrile (60:40 v/v). Flow rates between 0.5 to 1.2ml/min were tried. Flow rate of 1ml/min was observed to be enough to get both the drugs eluted within less than 10 min.

The column temperature was set at 30 °C. Optimized method was providing good resolution and peak shape for Metformin Hydrochloride and Canagliflozin. Under above described experimental conditions, all the peaks were well defined and free from tailing.

The concern of small deliberate changes in the mobile phase composition, flow rates, and column temperature on results were evaluated as a part of testing for methods robustness.

Validation of Method Developed: The proposed method was validated according to the ICH guidelines [15] for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

System Suitability: The Retention time of Metformin Hydrochloride and Canagliflozin using optimum conditions was 2.13 min and 2.66 min respectively. For two of them, the peak symmetries were < 1.5 and the theoretical plate's numbers were > 2000 and % RSD of areas of six standard injections of Metformin Hydrochloride and Canagliflozin was less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in **Table 1.**

TABLE 1: SYSTEM SUITABILITY RESULTS OF METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN

Parameter	Metformin Hydrochloride	Canagliflozin
Peak area	924091 (0.61%)*	187002 (0.72%)*
Theoretical plates	2599.3±0.861	7491.83±0.641
Retention time	2.13±0.031	2.66±0.057
Tailing factor	1.22±0.04	1.28±0.06

*RSD (%)

Specificity: The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized Chromatogram of Metformin Hydrochloride and Canagliflozin is shown in **Fig. 1** clearly shows the ability of the method to assess the analyte in the presence of other excipients.

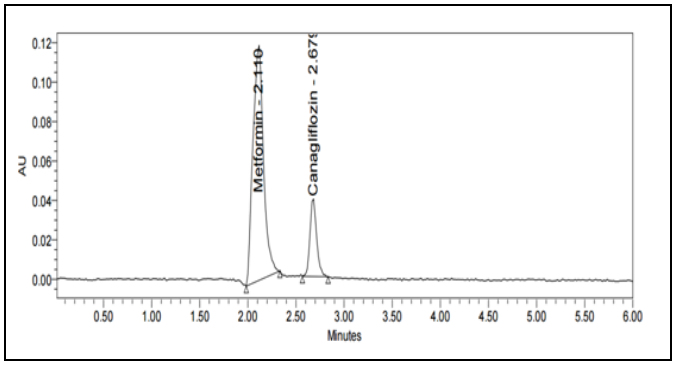

FIG. 1: OPTIMIZED CHROMATOGRAM OF METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN

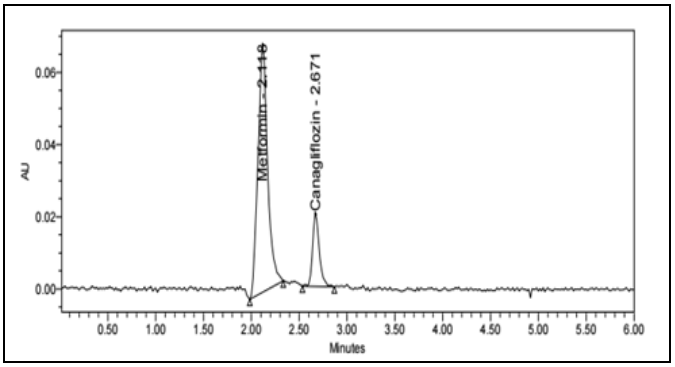

FIG. 2: CHROMATOGRAM OF LEVEL 1

Linearity and Range: Linearity was assessed for the two oral anti diabetic drugs at concentration ranges 25-150µg/ml for Metformin Hydrochloride and 2.5-15µg/ml for Canagliflozin. The Chromatograms of level 1 and level 6 are shown in **Fig. 2** and **Fig. 3**. A linear relationship was established at these ranges between Area under the peak (AUP) and concentration. Good linearity was proved by high values of coefficient of determinations **(Fig. 4** and **Fig. 5)**. The results were tabulated in **Table 2.**

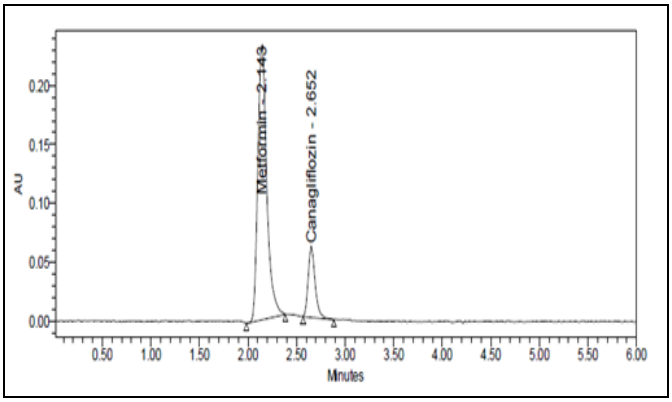

FIG. 3: CHROMATOGRAM OF LEVEL 6

TABLE 2: LINEARITY DATA OF METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN

Level	Concentration of Metformin Hydrochloride (µg/ml)	Peak area	Concentration of Canagliflozin (µg/ml)	Peak area
1	25	265031	2.5	54588
2	50	490445	5	98368
3	75	724680	7.5	143504
4	100	938891	10	189612
5	125	1162631	12.5	238251
6	150	1382624	15	284210

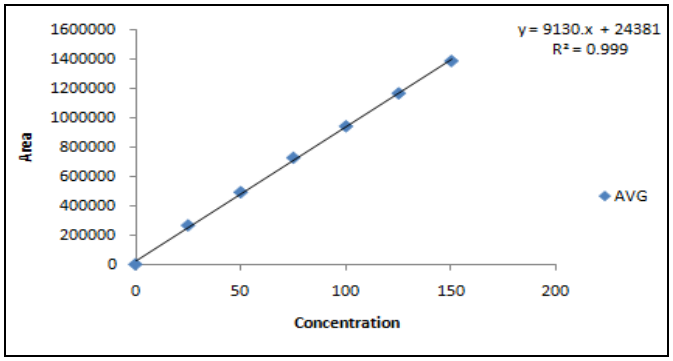

FIG. 4: LINEARITY GRAPH OF METFORMIN HYDROCHLORIDE

Limit of Detection (LOD) / Limit of Quantitation (LOQ): The LOD was determined on the basis of signal to noise ratios and was determined using analytical response of three times the background noise. LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injections. Both LOQ and LOD were calculated on the peak area using the following equations:

$$LOQ = 10 \times N / B$$

$$LOD = 3 \times N / B$$

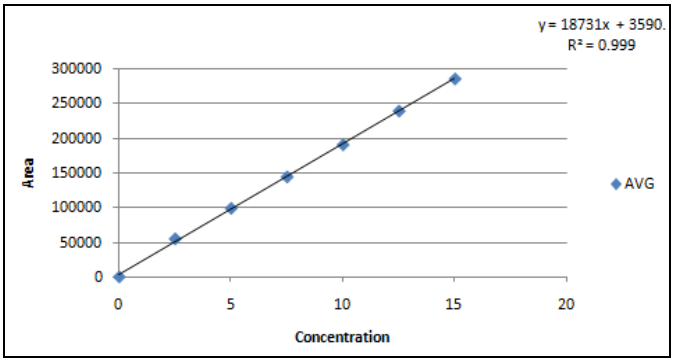

FIG. 5: LINEARITY GRAPH OF CANAGLIFLOZIN

The limit of detection and limit of quantification were evaluated by serial dilutions of Metformin Hydrochloride and Canagliflozin stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Metformin Hydrochloride and Canagliflozin was found to be 0.17µg/mL and 0.01µg/mL, respectively, and the LOQ value 2.20µg/mL and 0.50µg/mL, respectively.

Precision:

System Precision: System Precision was carried to ensure analytical system is working properly. One dilution of both the drugs in six replicates was injected into HPLC system and was analyzed and the results were found within the acceptance limits (RSD < 2) as shown in the **Table 3** below.

TABLE 3: SYSTEM PRECISION DATA FOR METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN

	Metformin Hydrochloride			Canagliflozin		
S. No	Concentration (µg/ml)	Retention time (min)	Peak Area	Concentration (µg/ml)	Retention time (min)	Peak Area
1	100	2.104	918397	10	2.665	185725
2	100	2.114	926677	10	2.668	188192
3	100	2.103	928636	10	2.664	187958
4	100	2.108	919625	10	2.672	186091
5	100	2.116	933152	10	2.674	185409
6	100	2.111	921164	10	2.675	187565
	Average		924609	Average		186823
	SD		5810	SD		1221
	% RSD		0.6	% RSD		0.7

Method Precision (Repeatability): Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of sample preparation of Metformin (100µg/mL) and Canagliflozin (10µg/mL) have been analyzed by injecting them into a HPLC column on the same day and on consecutive days. From the results obtained, % RSD was calculated and was found to be within the limits (< 2). The results of precision are given in **Table 4**.

TABLE 4: METHOD PRECISION DATA FOR METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN

	Metformin Hydrochloride			Canagliflozin		
S. No	Concentration (µg/ml)	Peak Area	% Assay	Concentration (µg/ml)	Peak Area	% Assay
1	100	911508	99.3	10	189490	99.22
2	100	939016	100.2	10	187952	100.54
3	100	908096	100.4	10	181626	100.41
4	100	940019	99.4	10	186148	99.41
5	100	924217	100.9	10	187708	99.05
6	100	921693	99.6	10	189087	100.2
	Average	924092	99.96	Average	187002	99.8
	SD	13390	0.63	SD	2883	0.65
	% RSD	1.45	0.63	% RSD	1.5	0.651

TABLE 5: RUGGEDNESS DATA FOR METFORMIN HYDROCHLORIDE

	Laboratory-1 (% Assay)-HPLC-1				Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Concentration (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
100	99.28	99.68	99.67	99.45	99.43	99.64	100.02	99.86
100	99.46	100.21	99.41	99.42	99.54	99.43	99.42	100.02
100	99.54	99.62	99.46	99.34	99.28	99.01	99.27	99.52
100	99.89	99.46	100.14	99.86	99.45	99.45	99.48	99.54
100	100.29	99.84	100.07	100.23	100.23	100.08	99.59	100.05
100	100.03	100.14	99.21	100.14	100.03	100.12	99.76	99.92
Average	99.75	99.83	99.66	99.74	99.66	99.62	99.59	99.82
SD	0.38	0.30	0.38	0.39	0.38	0.42	0.27	0.23
% RSD	0.39	0.30	0.38	0.39	0.38	0.43	0.27	0.23

Ruggedness: Intermediate precision was accessed injecting sample preparation of Metformin (100 µg/mL) and Canagliflozin (10µg/mL) in six replicates in to HPLC column on the same day and on consecutive days and in different laboratories by different analysts. Results were found within the acceptance limits (RSD < 2) as shown in the **Tables 5** and **6** below.

TABLE 6: RUGGEDNESS DATA FOR CANAGLIFLOZIN

Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Concentration (µg/ml)	Day-1	99.24	99.42	99.43	Day-1	Day-2	Day-1	Day-2
100	99.42	99.46	99.84	99.86	99.84	99.29	99.41	99.21
100	100.01	100.1	99.56	99.45	99.49	99.45	99.64	99.48
100	99.78	99.89	100.04	99.62	99.34	99.81	99.21	99.42
100	99.83	100.23	99.48	100.23	99.89	99.42	99.89	99.61
100	99.65	100.43	99.46	100.42	99.94	99.19	100.21	99.49
100	100.14	99.89	99.63	99.84	99.44	99.12	99.37	100.21
Average	99.81	0.46	0.25	0.41	99.66	99.38	99.62	99.57
SD	0.26	0.46	0.25	0.41	0.26	0.25	0.37	0.34
% RSD	0.26	99.24	99.42	99.43	0.26	0.25	0.37	0.34

Accuracy: The percentage recovery was calculated by preparing standard drug concentrations of Metformin hydrochloride and Canagliflozin with concentration levels of 50%, 100% and 150%. A known amount of the standard drug was added to the blank sample at each level. Good recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of Metformin hydrochloride and Canagliflozin was achieved between 99.42–100.81 ± 0.753% and 99.53-100.16 ± 0.327. The results are given in **Tables 7** and **8**.

TABLE 7: RECOVERY DATA OF METFORMIN HYDROCHLORIDE

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Statistical Analysis
S1:50%	50	49.71	99.42	Mean=99.42% (n=3)
S2:50%	50	50.3	100.62	S.D=1.20
S3:50%	50	49.1	98.22	% RSD=1.2
S4:100%	100	100.69	100.69	Mean=100.62%(n=3)
S5:100%	100	101.65	101.66	S.D=1.08
S6:100%	100	99.49	99.5	% RSD=1.1
S7:150%	150	151.93	101.29	Mean=100.81%(n=3)
S8:150%	150	150.69	100.46	S.D=0.43
S9 :150%	150	151.01	100.68	% RSD=0.4

TABLE 8: RECOVERY DATA OF CANAGLIFLOZIN

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Statistical Analysis
S1:50%	5	4.98	99.63	Mean=100.16%(n=3)
S2:50%	5	4.96	99.30	S.D=1.21
S3:50%	5	5.07	101.54	% RSD=1.2
S4:100%	10	9.95	99.56	Mean=99.53%(n=3)
S5:100%	10	10.09	100.09	S.D=0.57
S6:100%	10	9.89	98.95	% RSD=0.6
S7:150%	15	15.03	100.24	Mean=100%(n=3)
S8:150%	15	14.80	98.69	S.D=1.20
S9 :150%	15	15.15	101.06	% RSD=1.20

Robustness: Robustness of the proposed analytical method is a measure of its capacity to remain unaffected, and it reflects the reliability of the analysis with respect to deliberate changes in the parameters such as flow rate (1.0 ± 0.2mL), column temperature (30 ± 5 °C), mobile phase ratio of the mobile phase. The result of robustness study of the developed assay method was established in **Table 9, 10** and **11**. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

TABLE 9: ROBUSTNESS (CHANGE IN FLOW RATE) FOR METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN

Drug	Change in Flow rate (ml/min)	Change in flow Rate (0.8ml/min to 1.2 ml/min)		
		%Assay	SD	% RSD
Metformin Hydrochloride	0.8	99.5	1.6	1.6
	1	100.62	1.08	1.1
	1.2	99.4	1.5	1.5
Canagliflozin	0.8	99.4	1.4	1.4
	1	99.53	0.57	0.6
	1.2	100.23	1.3	1.29

TABLE 10: ROBUSTNESS (CHANGE IN MOBILE PHASE COMPOSITION) FOR METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN

Drug	Change in mobile phase	Change in Mobile phase (0.8ml/min to 1.2 ml/min)		
		%Assay	SD	% RSD
Metformin Hydrochloride	10% less organic phase	99.3	1.4	1.4
	Actual	100.62	1.08	1.1
	10% more organic phase	99.6	1.5	1.5
Canagliflozin	10% less organic phase	99.8	1.3	1.3
	Actual	99.53	0.57	0.6
	10%more organic phase	100.13	1.2	1.1

TABLE 11: ROBUSTNESS (CHANGE IN COLUMN TEMPARATURE) FOR METFORMIN HYDROCHLORIDE AND CANAGLIFLOZIN

Drug	Change in column temperature	Change in Column temperature		
		%Assay	SD	% RSD
Metformin Hydrochloride	25°C	99.42	1.20	1.2
	30°C	100.28	1.06	1.06
	35°C	100.62	1.08	1.1
Canagliflozin	25°C	100.16	1.21	1.2
	30°C	99.53	0.57	0.6
	35°C	100.81	0.43	0.4

Forced Degradation Studies: The assay method was used to test the drug stability by conducting forced degradation studies for the drug substances under various stress conditions. Stress degradation studies were carried out for acid hydrolysis (1M HCl heated for 30 min at 60 °C), alkali hydrolysis (2N NaOH heated for 30 min at 60 °C), oxidative degradation (20% H_2O_2 heated at 60 °C for 30 min) and thermal degradation (samples placed in an oven at 105 °C for 6 hr). For photolytic stress studies, samples were exposed to UV light by keeping them in a UV chamber for 7 days. Results are shown in **Tables 12** and **13**.

TABLE 12: FORCED DEGRADATION STUDIES OF METFORMIN HYDROCHLORIDE

Sample Name	Degradation (%)	Purity Angle	Purity Threshold
Unstressed Sample	---	0.345	0.452
Thermal Stress Sample	0.9	0.290	0.477
Photolytic Stress Sample	0.4	0.360	0.459
Water Stress Sample	0.2	0.355	0.456
Acid Degradation	2.5	2.045	3.090
Alkali Degradation	1.6	7.315	7.502
Peroxide Degradation	1.3	1.21	1.402

TABLE 13: DEGRADATION STUDIES OF CANAGLIFLOZIN

Sample Name	Degradation (%)	Purity Angle	Purity Threshold
Unstressed Sample	-----	1.112	1.571
Thermal Stress Sample	0.95	1.488	1.509
Photolytic Stress Sample	0.21	1.224	1.365
Water Stress Sample	0.11	1.122	1.572
Acid Degradation	3.39	1.424	1.556
Alkali Degradation	2.71	1.338	1.711
Peroxide Degradation	1.41	1.083	1.150

The retention time of Metformin Hydrochloride and Canagliflozin was found to be 2.112 min and 2.611 min respectively with resolution of 3.24. Linearity was established for Metformin Hydrochloride and Canagliflozin in the range of 25-150µg/ml for Metformin Hydrochloride and 2.5-15µg/ml for Canagliflozin with correlation coefficients (r^2= 0.999) and the percentage recoveries were between 98.22 % to 100.69% and 98.69% to 101.54% for Metformin Hydrochloride and Canagliflozin respectively, which indicate accuracy of the proposed method. The % RSD values of accuracy for Metformin Hydrochloride and Canagliflozin were found to be < 2 %. The % RSD values of method precision are 0.63% and 0.65% for Metformin Hydrochloride and Canagliflozin respectively and % RSD values of system precision are 0.6% and 0.7% for Metformin Hydrochloride and Canagliflozin.

The % RSD values of reproducibility are 0.41% and 0.34% for Metformin Hydrochloride and Canagliflozin respectively, reveal that the proposed method is precise. LOD values for Metformin Hydrochloride and Canagliflozin were found to be 0.17µg/ml and 0.01µg/ml respectively and LOQ values for Metformin Hydrochloride and Canagliflozin were found to be 2.20µg/ml and 0.50µg/ml respectively was shown in The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough was shown in (**Table 10, 11** and **12**). These data show that the proposed method is specific and sensitive for the determination of Metformin Hydrochloride and Canagliflozin.

CONCLUSION: RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Canagliflozin in their combine dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Metformin Hydrochloride and Canagliflozin in the range of 25-150µg/ml for Metformin Hydrochloride and 2.5-15µg/ml for Canagliflozin with correlation coefficients (r^2=0.999). The percentage recoveries of Metformin Hydrochloride and Canagliflozin were achieved in the range of 98.2-101.4% which was within the acceptance criteria. The percentage RSD was NMT 2% which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust.

ACKNOWLEDGEMENT: I express my sincere thanks to Dr. M. Bhagavan Raju, Principal for his support and encouragement throughout my research work. I am also thankful to Sri Venkateshwara College of Pharmacy for providing the chemicals and instruments and Hetero Pharma Pvt. Ltd., Hyderabad, India for providing the drug samples for research.

DISCLOSURE STATEMENT: The authors report no conflicts of interest. The authors alone are responsible for content and writing of this article.

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How to cite this article:
Kommineni V, Chowdary KPR and Prasad SVUM: Development of a new stability indicating RP-HPLC method for simultaneous estimation of metformin hydrochloride and canagliflozin and its validation as per ICH guidelines. Int J Pharm Sci Res 2017; 8(8): 3427-35.doi: 10.13040/IJPSR.0975-8232.8(8).3427-35.