A Stability Indicating RP-HPLC Method Validation for Simultaneous estimation of Metformin HCl and Canagliflozin in Pharmaceutical Dosage Form

Darshak Patel [a], Ujashkumar Shah [a*], Jayvadan Patel [a], Hirak Joshi [a], Darshana Patel [a] and Pavan Patel [b]

[a] *Department of Quality Assurance & Pharm Chem, Faculty of Pharmacy, Nootan Pharmacy College, Sankalchand Patel University, S. K. Campus, Visnagar-384315, Gujarat, India.*
[b] *Restech Pharmaceuticals, Plot No. 407 New Ahmedabad Industrial Estate, Sarkhej-Bavala Road, Moraiya-382 210, Dist-Ahmedabad, Gujarat, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Author DP designed the study, wrote the protocol, performed statistical analysis and wrote first draft of manuscript. Authors US and HJ managed the final review and analysis of data. Authors JP, DP and PP managed the literature search. All authors read and approved final manuscript.

Original Research Article

ABSTRACT

Aims: Canagliflozin and Metformin HCl is a new drug combination for the treatment of Diabetes Mellitus which is one of the oldest and lethal diseases of the mankind. Aim of the research work was to develop and validate novel, rapid, sensitive, specific, robust stability indicating analytical method for the simultaneous estimation of Canagliflozin and Metformin HCl in the pharmaceutical dosage form as fixed dose formulation.
Study Design: Method development and validation was performed as recommended in ICH guideline "Validation of analytical procedures: Test and Methodology Q2(R1)".
Methodology: Method develop with chromatographic parameters as C_{18} column (250mm×4.6 mm, 5mm particle size), HPLC system with PDA detector and mobile phase contained a mixture of Phosphate Buffer pH 5.0 and Acetonitrile (60:40 v/v). The flow rate was set to 1ml/min with responses measured at 290 nm, injection volume was 20 µl, and run time of 15 mins.
Results: The retention time of Metformin Hydrochloride and Canagliflozin was 5.4 min and 7.6 min

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Corresponding author: E-mail: shahujashkumar@gmail.com;

respectively with resolution of 7.0. Linearity was established in the range of 10-30 μg/ml for Metformin Hydrochloride and 0.5-1.5 μg/ml for Canagliflozin with correlation coefficients more than 0.999. The percentage recoveries were between (98.62-101.22%) and (98.68-101.27%) for Metformin Hydrochloride and Canagliflozin respectively. Validation parameters were evaluated according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The forced degradation studies were performed by using HCl, NaOH, H_2O_2, thermal and UV radiation. The developed method was successfully applied for the quantification and hyphenated instrumental analysis.

Conclusion: Significance of developed method is that it can be utilize for routine or unknown sample analysis of assay of Metformin HCl and Canagliflozin in pharmaceutical dosage form developed by various Pharmaceutical Industry.

1. INTRODUCTION [1-11]

Diabetes Mellitus (DM) is an endocrinological disorder resulting from an irregularity in insulin secretion and insulin action or both. Absence or reduced insulin in turn leads to persistent abnormally high blood sugar and glucose intolerance. It is probably an oldest disease known to human. It is also referred as black-death from the 14th century. Diabetes mellitus mainly classified into two categories, Insulin dependent diabetes mellitus when the pancreas does not produce enough insulin to properly control blood sugar levels, in this condition the patient completely depends parental formulation of insulin another one Non-insulin dependent diabetes mellitus, the cells of the body become resistant to insulin which are treated with oral antidiabetic agents such as Sulfonylureas, Biguanides, Thiazolidinediones derivatives, carbohydrate analogue and DPP-4 inhibitors. Fixed dose combination therapy (FDC) is called as a combination of two or more actives in a fixed ratio of doses. The International Diabetic Federation (IDA) and American Diabetic Federation (FDA) tend to suggest if the monotherapy fails along with lifestyle modification the patient should followed by combination therapy. Combination therapy based on the rationale of a multi targeted approach and it helps to achieve and maintain the desired therapeutic targets. The advantages of Fixed Dose Combination are easy of administration, convenience, synergistic effect, complementary mechanism of action, with low dose less side effects, economical, reduce the pill burden and thereby, improve adherence to treatment, improve tight glycemic control, decrease the incidence/severity of Adverse Drug Reactions, delay the need for insulin therapy. Sodium-glucose cotransporter 2 (SGLT2) inhibitors, such as Canagliflozin and Biguanides, such as Metformin, exert antidiabetic effects via different mechanisms of action. SGLT2 inhibitors inhibit renal glucose reabsorption, resulting in increased urinary excretion of glucose and thereby reducing plasma glucose levels in an insulin independent manner. Biguanides does not have clear mechanism of action but it decreases glucose production and increases body's response to insulin. Because of the complementary mechanisms of action of SGLT2 and Biguanides, dual therapy improves glycemic control in patients with Type II Diabetes Mellitus without increasing the risk of hypoglycemia or weight gain.

FDC combination of Canagliflozin and Metformin is available with brand name of INVOKAMET 50 mg /1000 mg respectively.

Structure of Canagliflozin and Metformin are presented as Fig. 1 and Fig. 2 respectively [12-13]
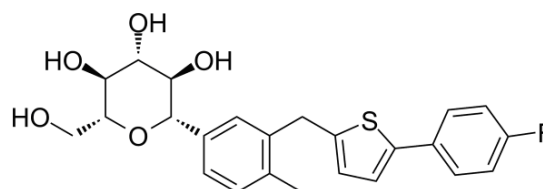
Fig. 1. Chemical Structure of Canagliflozin

After reviewing the literature it can be said that very limited techniques are available for simultaneous estimation of Canagliflozin and Metformin HCl in FDC [14-24]. The previous methods are not that much of economic or sensitive so it was thought to develop simple, novel, sensitive and selective method.

Developing Stability indicating RP-HPLC method for simultaneous estimation of Canagliflozin and Metformin HCl in FDC is both requirement and challenge. In present work, an attempt was made to develop specific, stability indicating, linear, precise, accurate and robust analytical method for simultaneous estimation of both drugs in FDC.
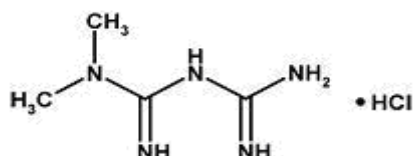

Fig. 2. Chemical Structure of Metformin HCl

2. MATERIALS AND METHODS

2.1 Chemical

The laboratory (working) standard of Canagliflozin and Metformin HCl were received as gift sample from M/s Zydus Cadila. FDC product of Canagliflozin and Metformin HCl was procured from market. Solvents like Water, methanol and Acetonitrile were HPLC grade and reagent such as Potassium dihydrogen phosphate and potassium hydroxide were of analytical grade

2.2 Analytical Method Development

2.2.1 Chromatographic conditions and instrument

Instrument: HPLC equipped with Photodiode-Array detector
Stationery Phase / Column: C_{18} Column, 250 mm X 4.6 mm, 5 µ
Mobile Phase: Phosphate Buffer pH 5: Acetonitrile (60:40)
Diluent: Methanol
Flow Rate: 1 ml / min
Injection Volume: 20 µl
Detection Wavelength: 290 nm
Column Temperature: Ambient
Run Time: 15 mins
Other Instruments: Analytical balance, sonicator

2.2.2 Preparation of solutions

2.2.2.1 Preparation of phosphate buffer pH 5

Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 ml of water and adjust pH to 5.0 with 10M potassium hydroxide.

2.2.2.2 Preparation of mobile phase

Prepare a required volume of degassed mixture of Phosphate Buffer pH 5 and Acetonitrile in the ratio of 60:40 v/v.

2.2.2.3 Preparation of stock and standard preparation

Standard Stock Solution for Metformin: Weigh 50 mg of Metformin in 100 ml clean dry volumetric flask. To this add 100 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix well.

2.2.2.4 Standard stock solution for canagliflozin

Weigh 10 mg of Canagliflozin in 100 ml clean dry volumetric flask. To this add 100 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix well.

2.2.2.5 Standard solution

Transfer 4 mL of Standard Stock solution for Metformin and 1 mL of Standard Stock Solution Canagliflozin in 100 ml clean and dry volumetric flask, dilute to volume with diluent and mix well. Filter the solution with 0.45 µ PVDF / Nylon filter presaturated with diluent followed by discarding about 5 ml of initial solution. Final Concentration: Metformin (20 ppm) & Canagliflozin (1 ppm).

2.2.2.6 Preparation of sample solution

Weigh about 20 intact tablets of Metformin and Canagliflozin 1000/50 mg Tablet FDC. Crush and transfer powder equivalent to 1000 mg Metformin and 50 mg Canagliflozin in to clean and dry 500 ml volumetric flask. Add about 200 ml of diluent and sonicate at room temperature for about 15 mins with intermittent shaking at 5 min time interval. Further add about 100 ml diluent and sonicate at room temperature for 15 mins with intermittent shaking at about 5 min intervals. Dilute to volume with diluent and mix well. Equilibrate the solution to room temperature. Further dilute 1 ml of solution to 100 ml with diluent and mix well. Filter the solution through 0.45 µ PVDF / Nylon filter presaturated with diluent followed by discarding about 5 ml of initial solution. Final Concentration: Metformin (20 ppm) & Canagliflozin (1 ppm).

2.3 Analytical Methods Validation

The analytical method validation for was performed as per ICH guideline on "Validation of analytical procedures: Text and Methodology Q2 (R1)"[25]. Parameters such as System Suitability specificity, forced degradation, linearity, precision, and accuracy, stability of solution and filter study were validated.

2.3.1 Criteria for system suitability

%RSD of area for replicate injections of standards, tailing factors, theoretical plates, retention time and resolution criteria were chosen as parameter for system suitability. Six replicate injection of standard were evaluated and chromatograms were recorded as part of system suitability study.

2.3.2 Specificity

Specificity was established by injecting blank, individual standard solution, combine standard solution and sample solution. Retention time and peak purity data were evaluated for individual analyte peak to confirm any interference.

2.3.3 Forced degradation study

To observe the effect of forced degradation and establish stability indicating nature of method sample preparation were kept under acid hydrolysis, base hydrolysis, oxidation, Photolytic exposure and Thermal exposure. After completing degradation study sample preparation was injected and studied for peak purity of individual peak of analyte in sample preparation.

Acid Hydrolysis: Sample was treated with 2mL 0.1N HCl at 85°C for 120 mins neutralize with 2mL 0.1N NaOH.

Base Hydrolysis: Sample was treated with 2mL 0.1N NaOH at 85°C for 120 mins neutralize with 2mL 0.1N HCl.

Oxidation: Sample was treated with 2 mL 30% H_2O_2(Hydrogen peroxide) at 85°C for 120 mins.

Thermal Degradation: Sample was treated at 105°C for 5 Days.

Photo Degradation: Sample was treated for 1 day in UV chamber.

2.3.4 Precision

The precision was established based on three types of studies. i.e System Precision, Method Precision and Intermediate precision.

2.3.5 System precision

After establishing system suitability system precision was established by injecting six replicate injection of standard solution. The relative standards deviation for peak area should not be more than 2.0% for six replicate injections.

2.3.6 Method precision

After establishing system suitability Method precision was established by preparing and injecting 6 sample preparations at 100% concentration level. The % RSD of 6 sample preparation at 100% concentration level should not be more than 2.0%.

2.3.7 Intermediate precision

After establishing system suitability Intermediate precision was established by preparing and injecting 6 sample preparations at 100% concentration level. (from same lot as that of method precision). The % RSD of 6 sample preparation at 100% concentration level should not be more than 2.0%. The % RSD of result of method precision and intermediate precision (Total 12 sample preparation at 100% concentration level) should not be more than 2.0.

2.3.8 Linearity

Linearity is established on a minimum of 5 concentrations level across the range of 50-150% of standard solution concentration in duplicate. The standard solution at 5 different concentration levels in concentration range of 10-30 µg/ml for Metformin and 0.5-1.5 µg/ml for Canagliflozin were prepared and injected in duplicate for each concentration level. The Linearity correlation coefficient obtains from the graph should not be less than 0.995.

2.3.9 Accuracy

The accuracy was established using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range from 50-150% (e.g., 3 concentrations/3 replicates). % recovery at all recovery level should be within the range of 98.0-102.0%. The %RSD at all recovery level should not be more than 2.0%.

2.3.10 Stability of solution

Stability of standard solution and sample solution were established by injecting same standard and sample solution at specific time intervals and comparing the peak area of the individual analyte over the time intervals.

2.3.11 Robustness

Robustness of the method was established by varying flow-rate, Buffer pH of mobile phase and Composition of mobile phase. System suitability was studied at each varied condition. System suitability criteria should be complied at each varied condition to prove robustness of method.

3. RESULTS AND DISCUSSION

3.1 Method Development

UV spectra of Metformin HCl and Canagliflozin show good absorption at common wavelength of 290 nm. Therefore 290 nm was selected as detection wavelength for both drugs. The retention time of Metformin and Canagliflozin were found 5.4 and 7.6 minutes respectively at flow rat of 1.0 ml/minutes in isocratic mode. The well resolved peak with resolution factor was found to be around 7.2. (Figs. 3 to 8).

3.1.1 System suitability

For the system suitability to pass few criteria were set according to which the theoretical plate for Metformin and Canagliflozin peak should not be less than 2000, a tailing factor should be between 0.8 to 2.0, % RSD of six replicate injection should not be more than 2.0 and resolution factor between two peaks should be more than 5.0. Results of System Suitability observed for proposed method were summarized below in Table-1.
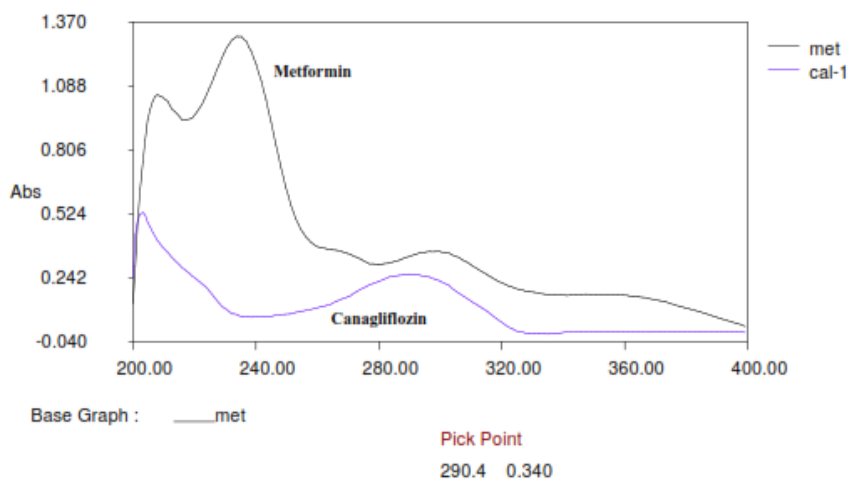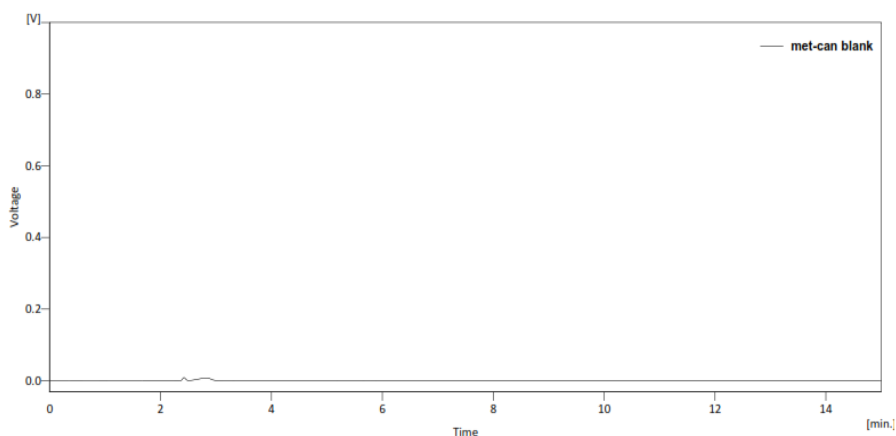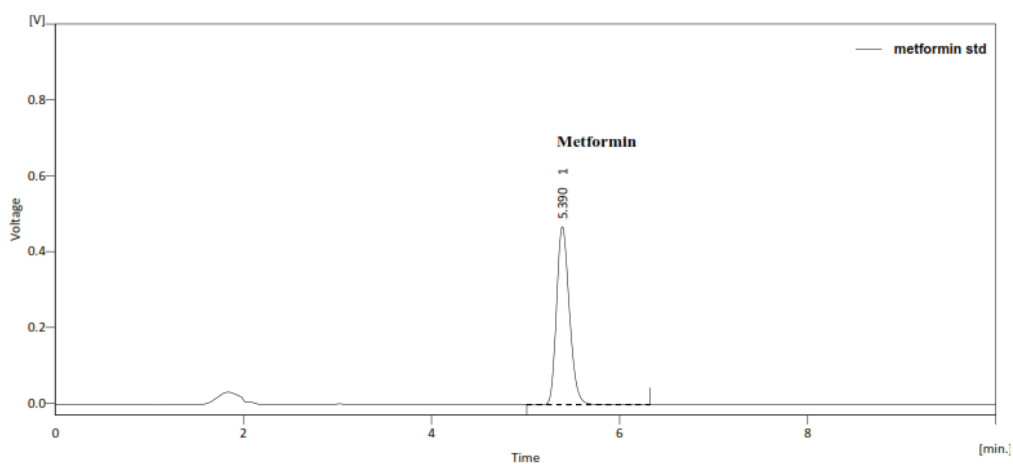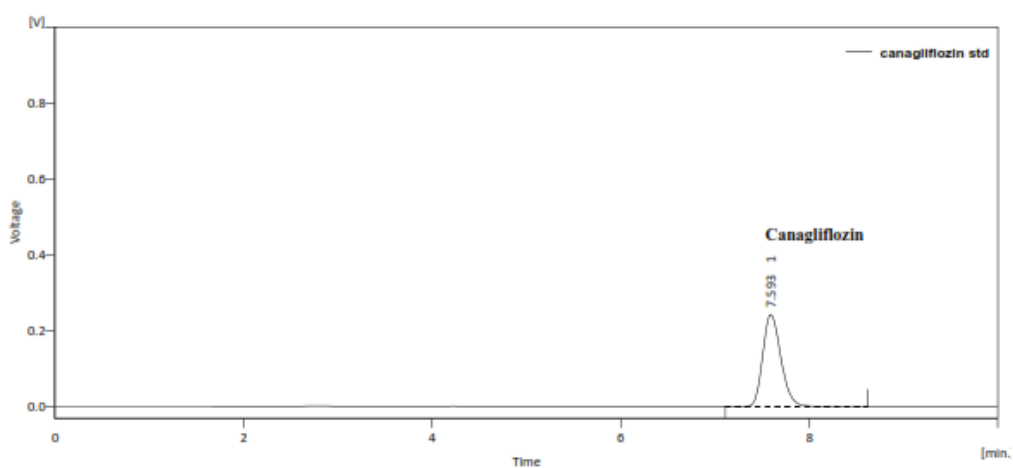

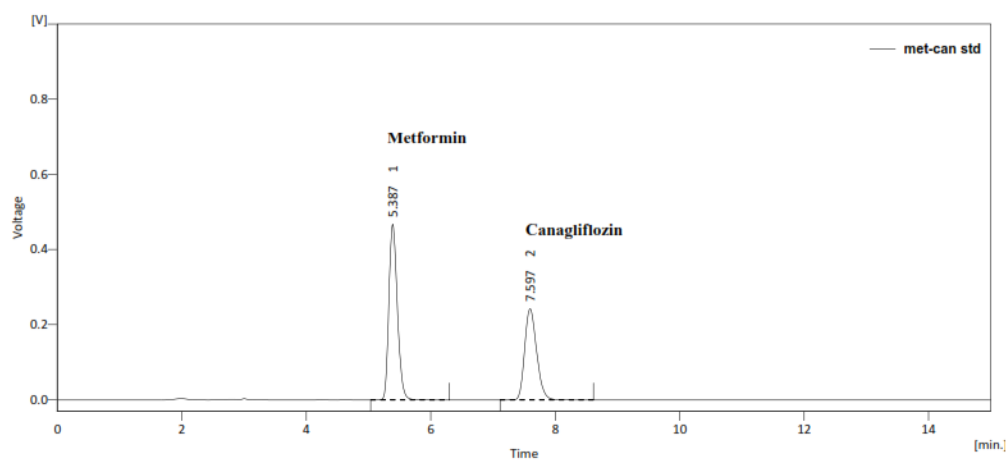**Fig. 3. Overlay US spectra of metformin HCl and canagliflozin**

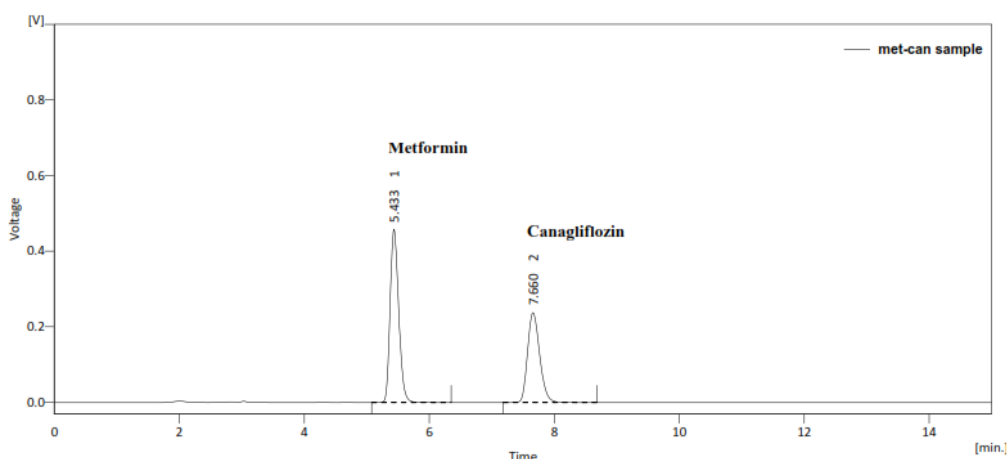**Fig. 4. Chromatogram of blank preparation**

Fig. 5. Chromatogram of metformin individual standard solution

Fig. 6. Chromatogram of canagliflozin individual standard solution

Fig. 7. Chromatogram of combined standard solution

Fig. 8. Chromatogram of sample solution

Table 1. Summary of system suitability results

Peak	Retention Time	Theoretical Plates	Tailing Factor	%RSD	Resolution
Metformin HCl	5.4	7100	1.3	1.70	-
Canagliflozin	7.6	7200	1.3	1.57	7.1
Acceptance Criteria	-	NLT 5000	Between 0.8-2.0	NMT 2.0%	NLT 5.0

Based on above summarized results it is evident that system suitability meets predefine acceptance criteria.

3.1.2 Specificity

Retention times of individual peak of drugs in standard solution were corresponding to that of sample solution. Peak purity angle was less than peak purity threshold for individual peak of drugs in standard solution and sample solution. Result evident that method is specific for its intended use.

3.1.3 Forced degradation study

During forced degradation study it was observed that Metformin and Canagliflozin were susceptible to acid, base, oxidation, Thermal and photo degradation. Summary of forced degradation study of Metformin HCl and Canagliflozin is summarized in Table-2. Purity Angle is less than Purity Threshold for individual peak of Metformin and Canagliflozin in all degradation samples. Based on this it is concluded that there is no interference with individual drug peak due to any other peak in all degradation samples. Hence, it is evident

that analytical method in stability indicating in nature.

3.1.4 Precision

System Precision: System precision was established by injecting six replicate injection of standard preparation. %RSD of six replicate injection of standard preparation was 1.71 and 1.57% for Metformin and Canagliflozin respectively.

Method Precision: Method precision was established by injecting 6 sample preparation at 100% concentration level. % RSD of 6 sample preparation at 100% concentration level was 1.3 and 0.93 for Metformin and Canagliflozin respectively.

Summary of result for Method Precision is summarized in Table-3.

Intermediate Precision: Intermediate precision was established by injecting 6 sample preparation at 100% concentration level. The % RSD of 6 sample preparation at 100% concentration level was below 0.95 and 1.07 for Metformin and Canagliflozin respectively. The % RSD of result of method precision and intermediate precision (Total 12 sample

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preparation at 100% concentration level) was 1.09 and 0.95 for Metformin and Canagliflozin respectively.

Summary of result for Intermediate Precision is summarized in Table-3.

3.2 Assay of Market Formulation

Market FDC of Metformin and Canagliflozin was analyzed and % Assay found 100.09% and 99.50% for Metformin and Canagliflozin Respectively.

Linearity: The calibration curve for Metformin and Canagliflozin was found linear over the concentration range of 10-30 µg/ml and 0.5-1.5 µg/ml for Metformin and Canagliflozin respectively. The regression coefficient value was found 0.9991 and 0.9994 for Metformin and Canagliflozin respectively. Calibration curve for Metformin and Canagliflozin are provided in Fig. 14 and Fig.15 respectively. Statistical Parameter observed during Linearity study is summarized in Table 4.
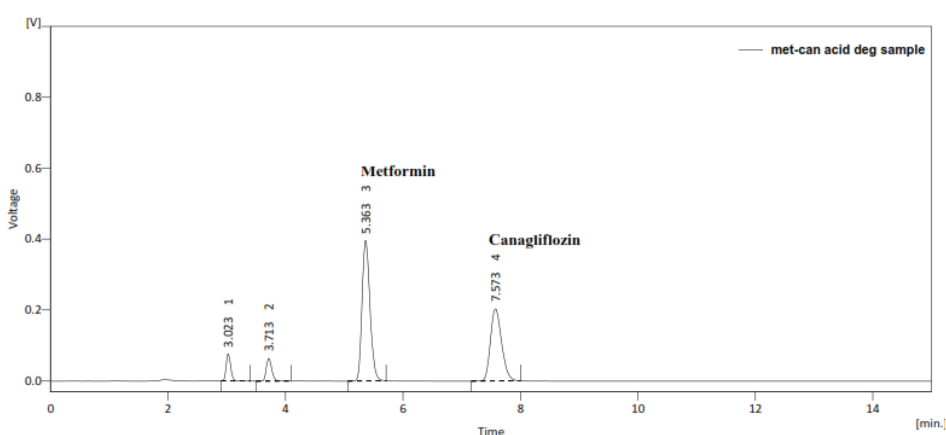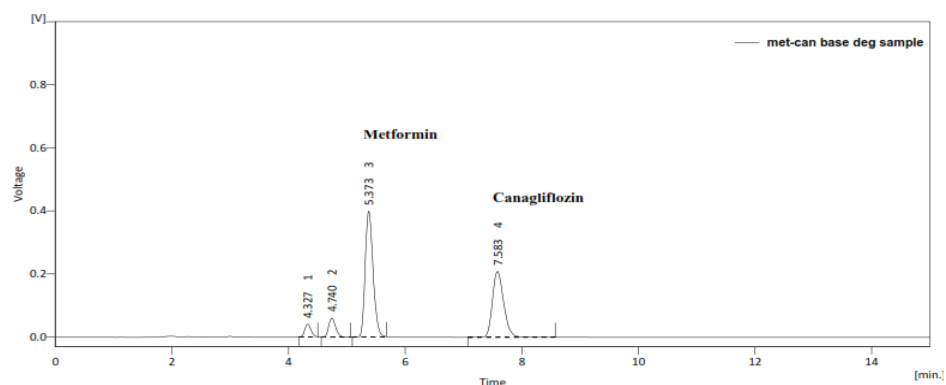

Fig. 9. Chromatogram of Acid degradation sample

Fig. 10. Chromatogram of base degradation sample

Table 2. Summary of Force degradation study of Metformin HCl and Canagliflozin

Degradation Condition	Metformin HCl		Canagliflozin	
	% Assay	% Degradation	% Assay	% Degradation
Undegraded Sample	100.08	-	99.50	-
Acid degradation	84.05	16.03	82.48	17.02
Base degradation	84.37	15.71	85.47	14.03
Oxidation degradation	83.28	16.80	77.56	21.94
Thermal Degradation	90.79	9.29	86.32	13.18

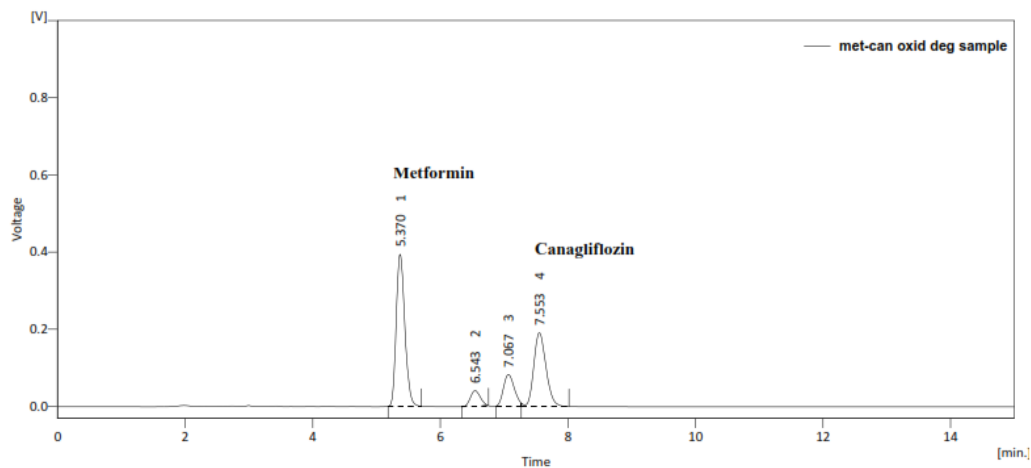| Photo Degradation	91.17	8.91	87.9	11.60

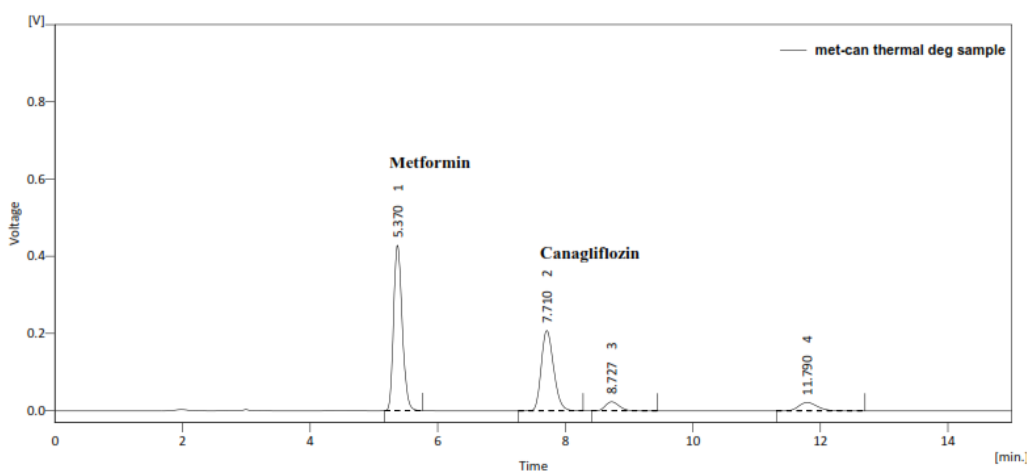**Fig. 11. Chromatogram of oxidation degradation sample**

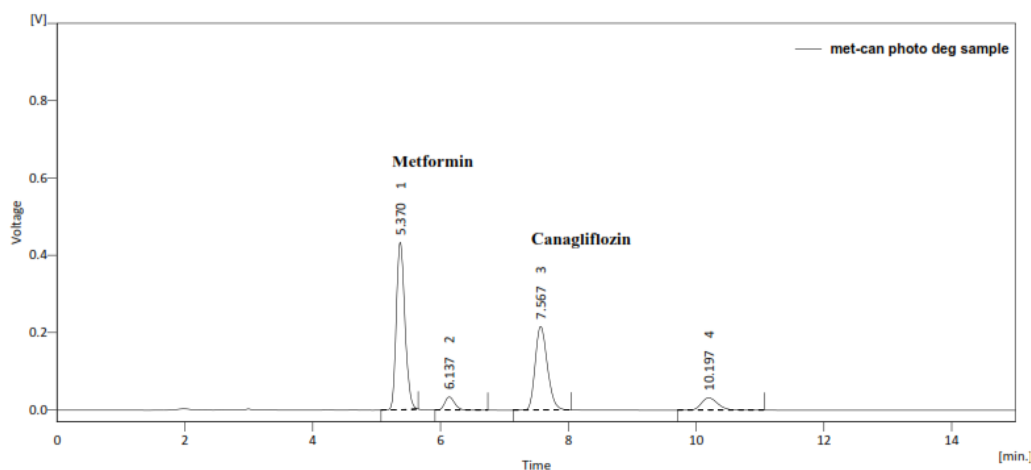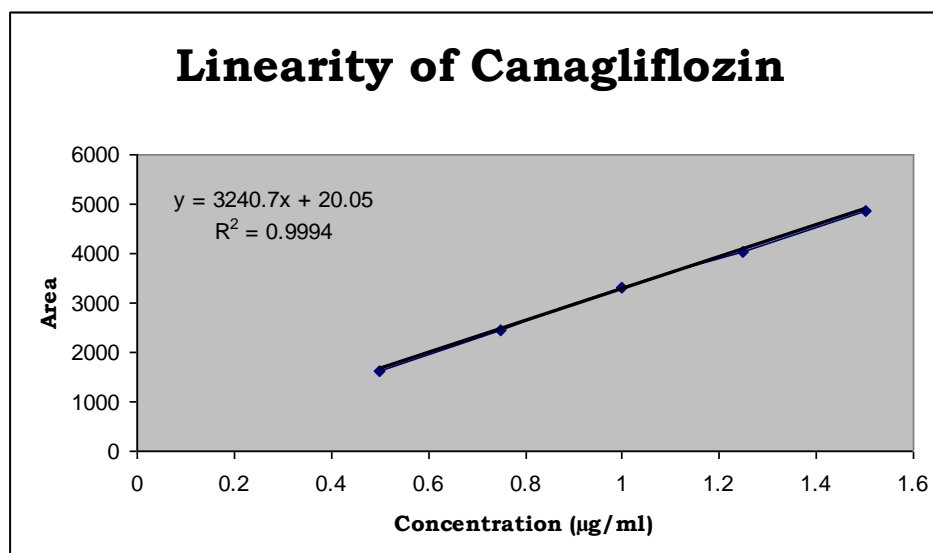**Fig. 12. Chromatogram of thermal degradation sample**

Fig. 13. Chromatogram of photo degradation sample

Table 3. Summary of method precision and intermediate precision study

Sample ID	Precision		Intermediate Precision	
	Metformin	**Canagliflozin**	**Metformin**	**Canagliflozin**
1	20.15	1.01	19.83	0.99
2	19.59	0.99	19.96	1.00
3	20.24	1.00	20.22	1.01
4	20.30	1.01	19.93	1.02
5	19.91	1.00	20.23	1.00
6	20.06	1.01	20.27	1.01
Mean	20.04	1.00	20.07	1.00
SD	0.26	0.01	0.19	0.01
%RSD	1.30	0.93	0.95	1.07
Overall %RSD	-	-	1.09	0.95

Linearity of Metformin

$y = 219.88x + 39.281$

$R^2 = 0.9991$
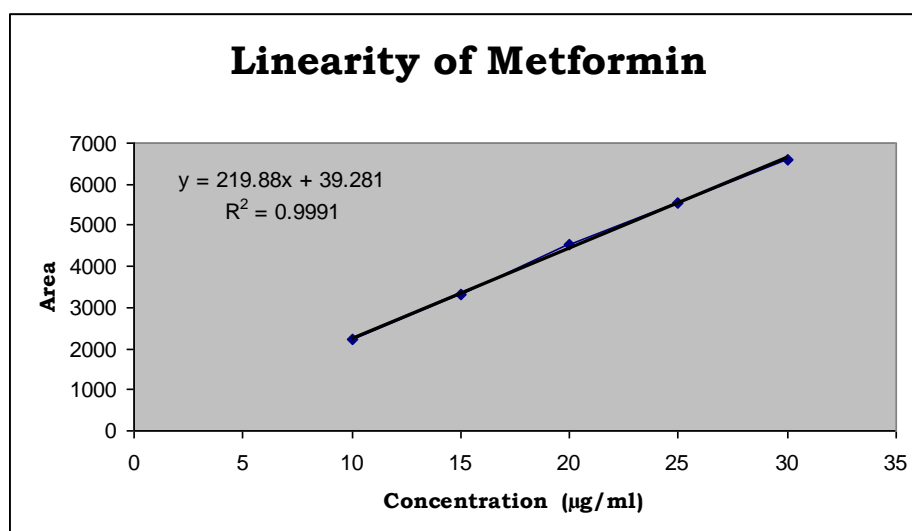
Fig. 14. Linearity calibration curve for Metformin HCl

Linearity of Canagliflozin

$y = 3240.7x + 20.05$

$R^2 = 0.9994$

Fig. 15. Linearity calibration curve for Canagliflozin

Table 4. Statistical parameter observed during linearity study

Regression analysis	Metformin	Canagliflozin
Linearity range	10-30 µg/ml	0.5-1.5 µg/ml
Regression equation	y = 219.88x + 39.281	y = 3240.7x + 20.05
Correlation co-efficient	0.9991	0.9994
Intercept	39.281	20.05
Slope	219.88	3240.7

Table 5. Summary of result of method validation parameters

Parameter	Metformin	Canagliflozin
Specificity	No Interference	No Interference
Range	10-30 µg/ml	0.5-1.5 µg/ml
System Precision (%RSD)	1.71	1.57
Method Precision (%RSD)	1.30	0.93
Intermediate precision (%RSD)	0.95	1.07
Overall Precision (n= 2*3) (%RSD)	1.09	0.95
Linearity (correlation co-efficient)	0.9991	0.9994
Accuracy (% Recovery)	98.62-101.22%	98.68-101.27
Robustness (%RSD)	Meets System Suitability Acceptance Criteria	
% Assay	100.09%	99.50%

Accuracy: The accuracy of the assay method was established three levels i.e. 50%, 100% and 150% of sample concentration. % Recovery observed between 98.62-101.22% and 98.68-101.27 for Metformin and Canagliflozin respectively. % RSD observed for accuracy was well below 2.0%.

Stability of Solution: Stability of standard and sample preparation was established by examining the peak area at 0 Hrs, 8 Hrs, 24 Hrs and 48 Hrs time intervals and % change in area of peak was monitored with time. No significant change in peak area was observed in peak area till 48 Hrs for standard and sample preparation.

Robustness: The robustness of method was established by varying flow-rate, pH of Mobile Phase buffer and ratio of Mobile Phase. System suitability was performed by varying the method parameters. System suitability criteria was in compliance to predefine limit of theoretical plate should not be less than 2000, a tailing factor should be between 0.8 to 2.0, % RSD of six replicate injection should not be more than 2.0 and resolution factor between two peak should be more than 5.0.

Summary of results for all method validation parameters are provided in Table-4. Based on result of method validation it is established that

proposed RP-HPLC is simple, specific, stability-indicating, Linear, Precise, Accurate and robust.

4. CONCLUSION

Based on above presented data it is clearly evident that retention time of Metformin HCl and Canagliflozin was 5.4 min and 7.6 min respectively with resolution of 7.0. Linearity was established in the range of 10-30 µg/ml for Metformin HCl and 0.5-1.5 µg/ml for Canagliflozin with correlation coefficients more than 0.999. The system precision, method precision and intermediate precision of the developed method was well within the proposed acceptance criteria of % RSD not more than 2.0%. The percentage recoveries were between (98.62-101.22%) and (98.68-101.27%) for Metformin HCl and Canagliflozin respectively. Developed method is robust as all system suitability acceptance criteria were comply with pre define acceptance criteria for all deliberate changes made in the method. All validation parameters were evaluated according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The forced degradation studies were performed by using HCl, NaOH, H2O2, thermal and UV radiation. A novel, rapid, sensitive, specific, robust stability indicating RP-HPLC method was developed and validated for Canagliflozin and Metformin fixed dose formulation. All method validation parameter lie

within its acceptance criteria as per ICH Q2 (R1) Guideline. So, it can conclude that method is selective, linear, accurate and precise. There is no co-elution of any degradation products with main peak and results obtained were found within the acceptance criteria. Hence, the method can be termed as selective. Therefore, the proposed RP-HPLC assay method can be applied for the estimation of Canagliflozin and Metformin HCL in pharmaceutical dosage form in the presence of degradation products. Significance of developed method is that it can be utilize for routine or unknown sample analysis of assay of Metformin HCl and Canagliflozin in pharmaceutical dosage form developed by various Pharmaceutical Industry. This will ensure the quality of the pharmaceutical product

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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