IJBPAS, January, 2024, 13(1): 303-323

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF METFORMIN HCL AND CANAGLIFLOZIN AND ITS RELATED IMPURITIES IN PHARMACEUTICAL DOSAGE FORM

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Received 25th Nov. 2022; Revised 26th Dec. 2022; Accepted 1st May 2023; Available online 1st Jan. 2024

https://doi.org/10.31032/IJBPAS/2024/13.1.7717

ABSTRACT

A simple, economic, selective and precise RP-HPLC method has been developed and validated for the estimation of related impurities of Metformin HCl and Canagliflozin in combined tablet. RP-HPLC method with gradient elution analysis was performed on Hypersil BDS C18 column (250mm X 4.6mm, 5µm) using mobile phase 0.05M Potassium Dihydrogen Phosphate buffer pH-5.0 and Acetonitrile in the ratio of (70: 30 v/v) at a flow rate of 1.0 ml/min and the detection wavelength was 290nm. The analytical method is validated according to ICH guidelines. The linearity was observed in the range of LOQ-

15.0µg/ml for Metformin HCl and its related impurity A. Similarly the linearity was observed in the range of LOQ-7.5µg/ml for Canagliflozin and its related Impurity 2. The correlation coefficient was observed NLT than 0.99 for all the analyte. The % recovery value was found minimum of 98.128% and maximum of 101.996% for Metformin HCl Impurity A. Similarly the % recovery value was found minimum of 98.472% and maximum of 101.150% for Canagliflozin Impurity 2. The % recovery values observed between the range of 98.128% to 101.996% for Metformin Impurity A and 98.472% to 101.150% for Canagliflozin Impurity 2. The LOD value was found 0.495µg/ml for Metformin HCl and 0.098µg/ml for its related impurity A. The LOD value was found 0.248µg/ml for Canagliflozin and 0.050µg/ml for its related Impurity 2. The LOQ value was found 1.500µg/ml for Metformin HCl and 0.297µg/ml for its related impurity A. The LOQ value was found 0.753µg/ml for Canagliflozin and 0.151µg/ml for its related Impurity 2. The results indicate that the developed method is accurate, precise, simple and rapid.

Keywords: Stability-indicating RP-HPLC; Metformin HCl; Canagliflozin; ICH guidelines; related substance; and validation

INTRODUCTION

The prevalence of diabetes is increasing worldwide. As per studies 536.6 million people are living with diabetes either diagnosed or undiagnosed in 2021, and this number is projected to increase by 46%, reaching 783.2 million by 2045 **[1]**. Diabetes mellitus type 1 is a disease causing due to the lack of insulin however Diabetes mellitus type 2 is a disease causing due to insulin resistance by cells. Anti-Diabetic drugs used to treat diabetes mellitus by lowering the glucose level in the blood **[2]**. The type-2 diabetes mellitus needed a lifetime treatment with ant diabetic drugs **[3]**. During the treatment the basic objective is to achieve the glycemic control & to reduce diabetes-associated cardiovascular risk **[4]**. The pathogenesis of type-2 diabetes mellitus is involving several organs, and treatments using a combination of drugs with different mechanisms of action effectively control the plasma glucose levels **[5]**.

Metformin hydrochloride (MET) is chemically N, N- dimethyl imidodicarbonimidic diamide hydrochloride (1, 1dimethylbiguanide hydrochloride). Metformin HCl is an effective biguanide anti diabetic agent that has been used to control blood glucose level of type II diabetic patients for decades and has been considered the first line treatment according to international guidelines. Mitochondrial inhibition and activation of AMPK are key molecular effects of Metformin HCl to inhibit hepatic

gluconeogenesis. Metformin HCl on the other hand can directly and indirectly improve skeletal muscle sensitivity towards insulin **[6, 7]**. It is also official in Indian Pharmacopoeia **[8]**, British Pharmacopoeia **[9]**, European Pharmacopoeia **[10]** and United States Pharmacopeia **[11]**.

Canagliflozin, chemically, (2S,3R,4R,5S,6R)-2-(3-{[5-(4-fluorophenyl)thiophen-2-yl]methyl}-4-methylphenyl)-6-(hydroxymethyl)oxane-3,4,5-triol, used for the treatment of type 2 diabetes mellitus. Canagliflozin inhibits the sodium-glucose co-transporter 2. This inhibition leads to lower reabsorption of filtered glucose into the body and decreases the renal threshold for glucose (RTG), leading to increased glucose excretion in the urine **[12]**. SGLT2 inhibitors approved by FDA and the Committee for Medicinal Products for Human Use of the European Medicines Agency alone or in combination with Metformin HCl **[13] (Figure 1)**.

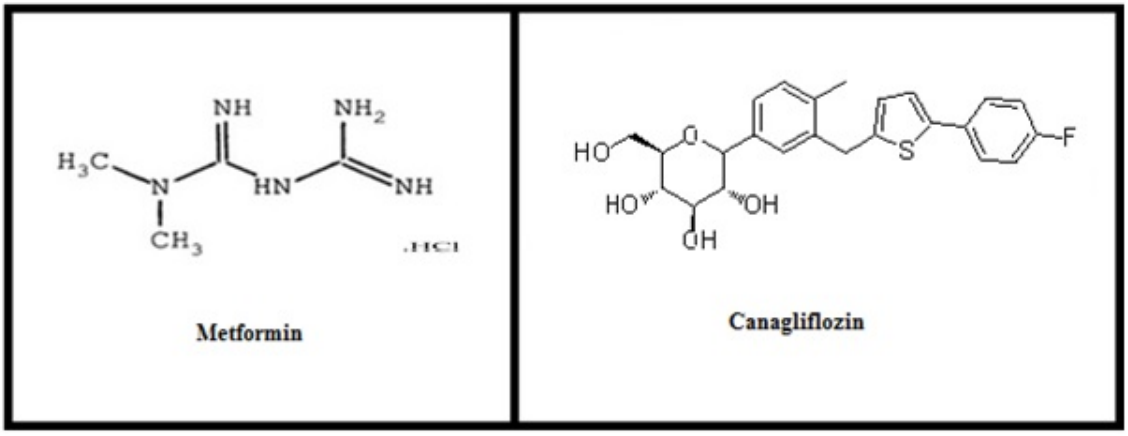

Figure 1: Chemical Structure of Metformin HCL and Canagliflozin

Impurity profiling is the group of analytical activities that aims the detection, identification/structure elucidation and quantitative determination of identified and unidentified (organic and inorganic impurities, residual solvents) impurities in bulk drugs and pharmaceutical formulations **[14, 15]**. Impurity profiling provide crucial data regarding quality, safety, efficacy, toxicity of drugs, various limits of detection (LOD) and limits of quantification (LOQ), structures of several organic and inorganic impurities, usually associated with bulk drugs and finished products and it is essential to develop analytical methods along with its impurities **[16]**. Various sophisticated analytical techniques are described in the literature to analyze Metformin HCL and Canagliflozin either individually or in combination with other drugs by UV Spectrometry method **[17]**, HPLC **[18-22]**, LC MS method **[23]**, Stability indicating HPLC method **[24-28]**. However, the RP-HPLC stability-indicating

chromatographic method for the determination of Metformin HCl and Canagliflozin with its related impurities in dosage form is not available. So Precise, accurate, and sensitive method for stability-indicating chromatographic method for the determination of Metformin HCl and Canagliflozin with its related impurities was planned and validated as per Q2 (R1) guideline.

EXPERIMENTAL

Materials

The Reference standard of Metformin HCl and its Related Impurity A as well as Canagliflozin and its Related Impurity 2 were obtained as gift sample from Cadila Pharmaceuticals Limited, Dholka, Ahmedabad. Water, Methanol, ACN in HPLC grade, Potassium Dihydrogen Phosphate and Ortho phosphoric acid of analytical reagent grade from Merck, Mumbai were used for the present study. The commercially available Metformin HCl and Canagliflozin tablet formulation 'Vokanamet' (Johnson & Johnson Ltd) with label claim Metformin HCl 500 mg and Canagliflozin 50mg was procured from market for use.

Instrumentation

Shimadzu LC-20 AT HPLC chromatographic system, Shimadzu digital weighing balance (ATX 224) and Sartorius BT224S, Systronic 119 UV Visible spectrophotometer, Frontline Ultrasonic Cleaner ultra-Sonicator, Ana lab Scientific Pvt. Ltd pH meter, and Thermolab India hot air oven were used for the development of the method. A 0.45µ Millipore filter was used for filtration due to Broad chemical compatibility and Low extractable prevent sample contamination.

Chromatographic conditions

This describes a specific and precise HPLC method for the quantification of Metformin HCl, Canagliflozin and there reference selected impurities. The procedure included an efficient solid phase extraction, and the HPLC analysis used a phosphate buffer/acetonitrile mobile phase with a C18 column and ultraviolet detection at 290nm.Colum oven temperature and sample cooler temp was kept 25ºC. Seal wash, needle wash and for rinsing water: methnol solution used (50:50). The separation of Canagliflozin and Metformin HCl was achieved using BDS Hypersil C_{18} column (250mm X 4.6mm, 5µm), 0.05M Phosphate buffer pH 5.0. Acetonitrile (70:30%, v/v), flow rate of 1 ml/min, an injection volume of 20.0µl, at a λ max of 290 nm, and runtime was 10 minutes.

Preparation of Mobile Phase

Buffer Preparation: (0.05M Phosphate buffer pH 5.0):

Weigh and dissolved 6.8 gm of potassium Dihydrogen phosphate into 1000ml of water. Adjust the pH of solution to 5.0 with dilute 1.0% Orthophosphoric acid solution.

Filtered the buffer from 0.45µ Membrane filter. Mixed the buffer solution and Acetonitrile in ratio of 70 : 30% v/v. Mixed well and sonicate the solution for 10 minutes to degass and the resulting solution filtered through 0.45µ Millipore filter..

Standard Solutions Preparation

Preparation of Standard Stock Solution of Metformin HCl (100 ppm):

Standard stock solution of Metformin HCl prepared by accurately weighing 10mg of Metformin HCl bulk drug into a 100ml volumetric flask. Add 60ml of solvent and sonicated the solution for 5 minutes to dissolve. Allowed to stand at room temperature and mark the volume up to the mark.

Metformin HCL Working Standard Solution: (10 ppm)

Pipette out and transferred 1 ml of Metformin HCL Standard stock solution (100 ppm) into 10 ml volumetric flask. Mark up to volume with mobile phase

Preparation of Standard Stock Solution of Metformin HCl Impurity A (100 ppm):

Standard stock solution of Metformin HCl Impurity A prepared by accurately adding 10mg of Metformin HCl Impurity A into a 100ml volumetric flask and dissolved with methanol up to get 100ppm of Metformin HCl Impurity A Standard Stock Solution.

Metformin HCl Impurity A Working Standard Solution: (10 ppm)

Pipette out and transferred 1 ml of Metformin HCl Impurity A Standard stock solution (100 ppm) into 10 ml volumetric flask. Mark up to volume with mobile phase.

Preparation of Standard Stock Solution of Canagliflozin (50 ppm):

Standard stock solution of Canagliflozin prepared by accurately adding 5mg of Canagliflozin bulk drug into a 100ml volumetric flask and dissolved in methanol up to mark get 50ppm of Canagliflozin standard stock Solution.

Canagliflozin Working Standard Solution: (10 ppm)

Pipette out and transferred 1 ml of Canagliflozin Standard stock solution (100 ppm) into 10 ml volumetric flask. Mark up to volume with mobile phase.

Preparation of Standard Stock Solution of Canagliflozin Impurity 2 (50 ppm):

Standard stock solution of Canagliflozin Impurity 2 prepared by accurately adding 5mg of Canagliflozin Impurity 2 into a 100 ml volumetric flask and dissolved with methanol up to the mark to get 50ppm of Canagliflozin Impurity 2 solution.

Canagliflozin Impurity 2 Working Standard Solution: (10 ppm)

Pipette out and transferred 1 ml of Canagliflozin Impurity 2 Standard stock solution (100 ppm) into 10 ml volumetric flask. Mark up to volume with mobile phase

Preparation of sample solution from Pharmaceutical Marketed Tablets

About 10 tablets of 'Vokanamet' were weighed and average weight of 10 tablets determined and powdered finely in a mortar. Tablet powder equivalent to 10mg of Metformin HCl and 5mg of Canagliflozin was weighed and transferred in to a 100ml volumetric flask and dissolved completely by sonicating for 10 minutes using 60ml of mobile phase. After ensuring complete solubilisation of drugs after sonication final volume was made up with mobile phase and filtered through 0.45 micron membrane filter and the final filtrate is collected. Transfer 1ml of this solution into 10ml volumetric flask and make up the volume with mobile phase to obtain the final test solution.

Chromatographic Separation

Standard solutions of Metformin HCl and Canagliflozin along with its related impurities were injected in column with 20 µl micro-syringe. The chromatogram was run for appropriate 10 minutes with mobile phase. The detection was carried out at wavelength 290nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc. were recorded using software.

Forced Degradation Study [29]

A forced degradation study is an essential step in the design of a regulatory compliant stability program for both drug substances and products, and was formalized as a regulatory requirement in ICH Guideline Q1A. A single analytic method that is capable of separating the degradants peaks from the drug substance/drug.

Forced degradation studies are undertaken to degrade the active drug deliberately. These studies are used to evaluate an analytical method's ability to measure an active ingredient and its degradation products without interference. Samples or drug product (spiked placebo) and drug substance are exposed to acid, base, oxidizing agent, reducing agent, and water. The degraded samples were then analyzed using the method to determine if there are interferences with the active. Thus, stability-indicating property was evaluated.

Acid degradation

1.0 ml Metformin HCl and Canagliflozin stock solution transferred into the 10 ml volumetric flask further 1.0 ml of 0.1 N HCl solution added and mixed well and kept for 6 hours at room temperature. After completing 6 hours, the volume adjusted with mobile phase. After making final solution run into HPLC and the peak area and shape observed under optimized chromatographic conditions.

Base degradation

1.0 ml of Metformin HCl and Canagliflozin stock solution transferred into the 10 ml volumetric flask further 1.0 ml of 0.1 N

NaOH solution added and mixed well and kept for 8 hours at room temperature. After completing 8 hours volume adjusted with mobile phase. After making final solution run into HPLC and the peak area and shape observed under optimized chromatographic conditions.

Oxidative degradation

1.0 ml Metformin HCl and Canagliflozin stock solution transferred into the 10 ml volumetric flask further 1.0 ml of 3.0% H_2O_2 solution added and mixed well and kept for 4 hours at room temperature. After completing 4 hours volume adjusted with mobile phase. After making final solution run into HPLC and the peak area and shape observed under optimized chromatographic condition

Thermal degradation

Thermal degradation performed by taking 1.0g of standard powder drugs and further kept in a Petri dish and the Petri dish placed in oven at 110ºC for 24 hours. After that stock solution prepared. Further withdrawn 1ml from stock solution and make up the volume with mobile phase. After making final solutions, it is injected into HPLC and the peak area and peak shapes were analyzed under the optimized chromatographic conditions.

Photolytic degradation

Photolytic degradation performed by taking 1.0g of standard powder drugs kept in a Petri dish and the Petri dish placed in UV chamber for 24 hours. After 24 hours stock solution prepared. Further withdrawn 1ml from stock solution and make up the volume with mobile phase. After making final solutions, it is injected into HPLC and the peak area and peak shapes were analyzed under the optimized chromatographic conditions.

Method Validation

The developed RP-HPLC method was validated as per ICH guidelines. The parameters validated are Specificity, System suitability, Linearity & Range, (Intraday precision, Inter-day precision), Accuracy, Limit of detection (LOD), Limit of quantitation (LOQ), Robustness and System suitability.

System suitability

The System Suitability was calculated from different parameters like retention time, theoretical plates, resolution, tailing factor.

Specificity

The effect of excipients and other additives usually present in the combined tablet dosage form of Metformin Hydrochloride and Canagliflozin in the determination under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the blank and placebo solution into the HPLC system.

Linearity & Range

The linearity of an analytical procedure is its ability (within a given range) to obtain

test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity for Metformin HCl and its Impurity and Canagliflozin and its impurity were assessed by analysis of combined standard solution in range of LOQ-1.5µg/ml and LOQ-0.297µg/ml respectively. LOQ, 5, 7.5, 10, 12.5, 15.0 ml solutions were pipette out from the Stock solution of Metformin HCL and its Impurity A (100µg/ml) and transfer to 10 ml volumetric flask and make up with mobile phase to obtain LOQ, 5, 7.5, 10, 12.5, 15ml. for Metformin HCl and its Impurity A respectively. Similarly LOQ, 2.5 ,3.75, 5, 6.25, 7.5ml solutions were pipette out from the Stock solution of Canagliflozin and its Impurity 2(50µg/ml) and transfer to 10 ml volumetric flask and make up with mobile phase to obtain LOQ, 2.5, 3.75, 5, 6.25 and 7.5µg/ml for Canagliflozin and its Impurity 2 respectively. Each mixed standard solution was injected and chromatograms were recorded in term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Precision

System precision was performed by injecting six replicates of a mixed standard solutions containing Metformin HCl and its Impurity A (10.0µg/ml) and Canagliflozin and its Impurity 2 (5.0 µg/ml) and chromatograms were recorded and areas of peaks were measured to calculate results of repeatability. Standard solution containing (LOQ, 10 µg/ml, 15µg/ml) of Metformin HCl and its Impurity A as well as standard solution containing (LOQ, 5.0 µg/ml, 7.5µg/ml) of Canagliflozin and its Impurity 2 were analyzed three times on the same day in intraday precision and on different day in interday precision and % RSD was calculated.

Accuracy

To check the accuracy of the proposed method for determination of Metformin HCl Impurity A & for Canagliflozin Impurity 2, recovery studies were carried out at LOQ, 80%, 100% and 120% of the test concentration according to ICH guidelines. The recovery study was performed three times at each level.

Robustness

The robustness study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions, which have been described in the Chromatographic conditions section. The factors chosen for this study, which were critical sources of variability in the operating procedures such as flow rate of mobile phase was changed (± 0.2 ml/min), mobile phase pH (± 0.2) and ratio of Mobile phase was changed (±2) were identified.

Optimization of chromatographic conditions

Chromatographic parameters were preliminary optimized to develop a stability indicating Related Substances method for Metformin HCl and Canagliflozin. Metformin HCl and Canagliflozin having one impurity each. So these impurities need to separate from each other and also from main analyte to show the stability indicating Related Substances method. Method development process was carried out by examining different conditions like mobile phase compositions like Water: Methanol, Water: Acetonitrile, Buffer: Acetonitrile with different ratios were used. The Metformin HCl, Canagliflozin, Metformin HCl Impurity A and Canagliflozin Impurity 2 were found showing a significant UV absorbance at 290 nm, so this wavelength was chosen for UV detection. By use of a C18 column it was found that the mobile phase consisting of Buffer [Buffer (0.05 M KH_2PO_4, pH 5.0): Acetonitrile (70:30) provided well defined peak shape with good resolution. The peaks with retention time (RT) 3.470 minutes and 5.370 minutes for Metformin HCl and Canagliflozin and the retention time of Metformin HCl impurity A and Canagliflozin Impurity 2 were found to be 4.517 minutes and 7.253 minutes respectively. The representative chromatograms **(Figure 2)** showing good peak shapes and a significant amount of resolution with selected mobile phase.

Forced degradation studies

Sample was injected under various stress conditions. The acidic degradation, base degradation, oxidative degradation, thermal degradation & photo degradation performed as per procedure and % degradation calculated from the chromatographic peaks. Metformin HCl & Canagliflozin in standard as well as sample mixture in acid degradation, base degradation& oxidative degradation. The details of % degradation are given in below **Table 1**.

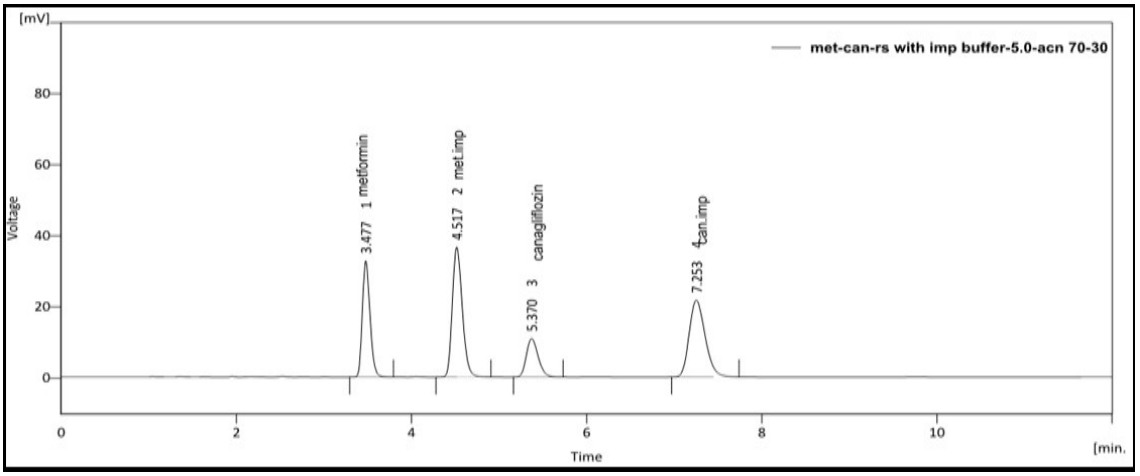

Figure 2: Optimized Chromatogram of Metformin HCl, Canagliflozin, Metformin HCl Impurity A and Canagliflozin Impurity 2 in Buffer (pH 5.0): Acetonitrile (70:30v/v)

Table 1: Results of forced degradation

Parameters (Degradation)	Standard				Sample			
	Area	SD	%RSD	% Degradation	Area	SD	%RSD	% Degradation
Metformin HCl								
Acid	9843.56	38.80	0.40	17.25	9928.582	52.42	0.53	16.53
	9823.99			17.41	9832.651			17.34
	9768.75			17.88	9917.235			16.63
Base	9935.038	62.13	0.63	16.48	10135.11	55.73	0.55	14.80
	9826.069			17.40	10226.63			14.03
	9932.267			16.50	10235.98			13.95
Oxidative	9780.057	79.06	0.81	17.78	10033.47	119.11	1.18	15.65
	9856.652			17.14	10265.13			13.70
	9698.565			18.47	10101.21			15.08
Photolytic	10697.44	117.66	1.11	10.07	10805.97	96.93	0.89	9.16
	10598.11			10.90	10923.64			8.17
	10463.02			12.04	10998.23			7.54
Thermal	10398.76	68.29	0.65	12.58	10305.57	69.44	0.67	13.36
	10412.12			12.47	10405.23			12.53
	10523.15			11.54	1039.17			12.24
Canagliflozin								
Acid	5489.99	79.38	1.47	14.14	5577.692	48.34	0.86	12.76
	5378.85			15.87	5669.754			11.33
	5336.25			16.54	5598.123			12.45
Base	5678.577	68.55	1.21	11.19	5613.664	118.53	2.07	12.20
	5572.654			12.84	5703.898			10.79
	5701.01			10.84	5848.623			8.53
Oxidative	5277.866	26.27	0.49	17.45	5240.957	83.84	1.57	18.03
	5326.965			16.69	5368.256			16.04
	5318.623			16.82	5399.135			15.56
Photolytic	5761.442	94.30	1.66	9.89	5962.286	82.73	1.41	6.75
	5577.342			12.77	5854.213			8.44
	5704.887			10.78	5799.741			9.29
Thermal	5619.602	99.43	1.77	12.11	5596.619	49.18	0.89	12.47
	5524.203			13.60	5536.129			13.41
	5723.019			10.49	5499.198			13.99

Chromatogram for Acid Degradation:

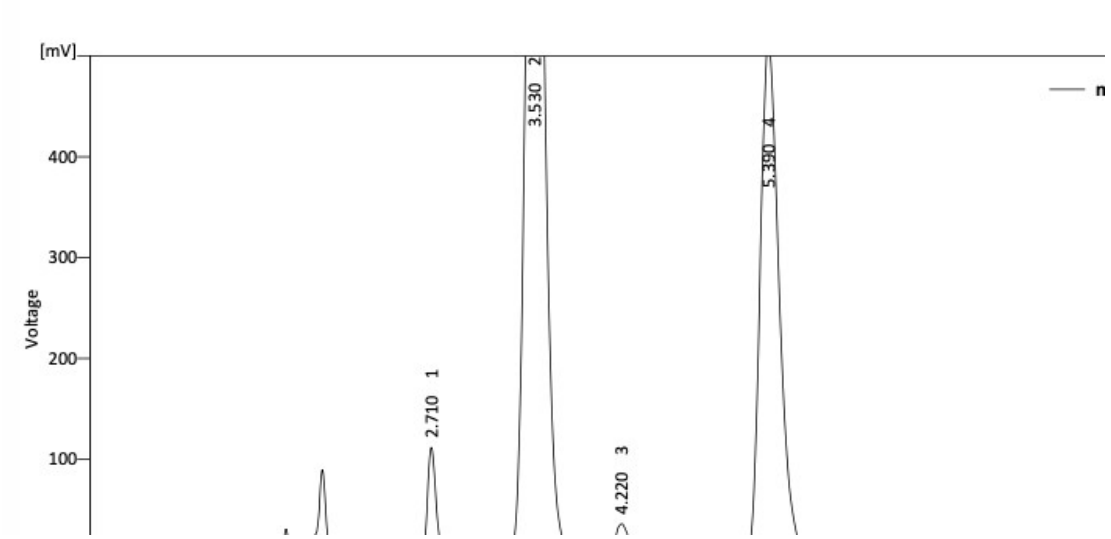

Figure 3: Metformin HCl and Canagliflozin Acid Degradation Sample

Chromatogram for Base Degradation:

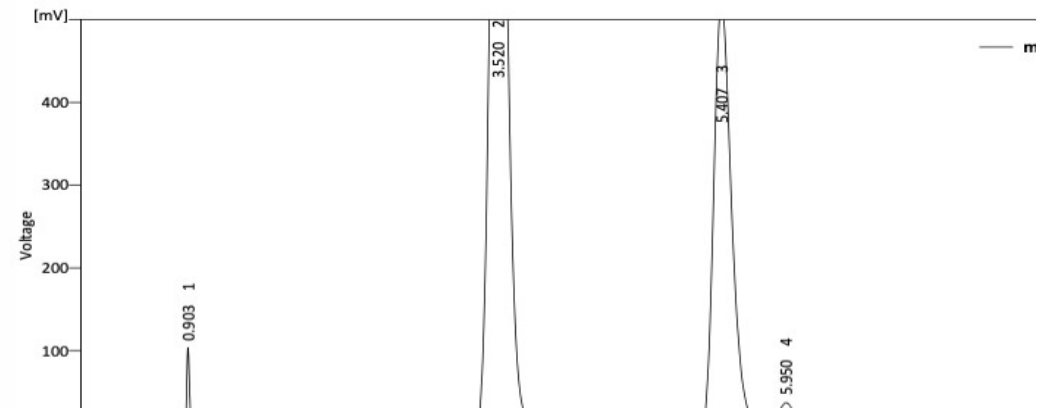

Figure 4: Metformin HCl and Canagliflozin Base Degradation Sample

Chromatogram for Oxidative Degradation

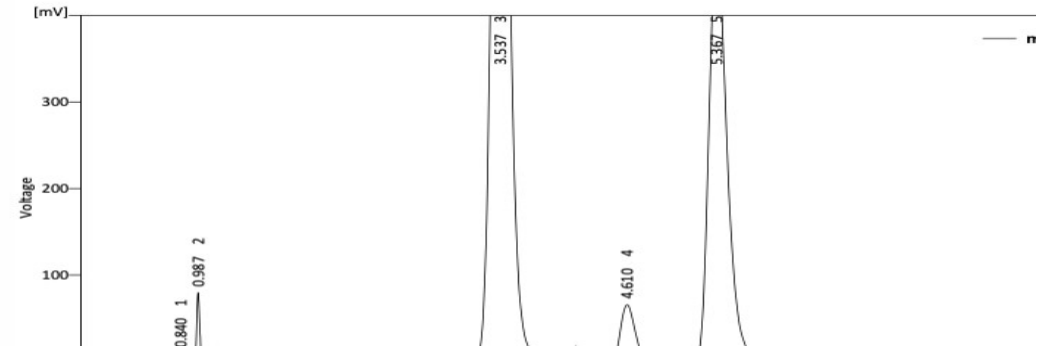

Figure no. 5 Metformin HCl and Canagliflozin Oxidation Degradation sample

Chromatogram for Thermal degradation

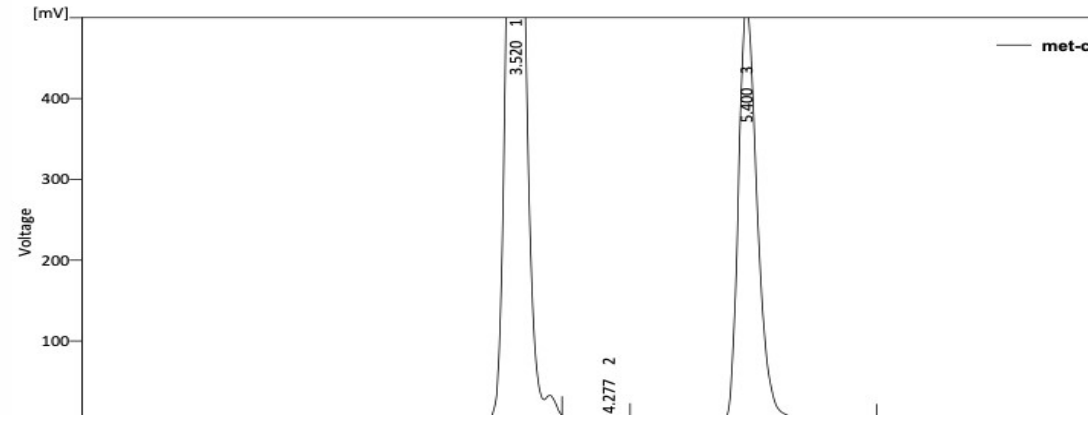

Figure 6: Metformin HCl and Canagliflozin Thermal Degradation sample

Chromatogram for Photolytic Degradation

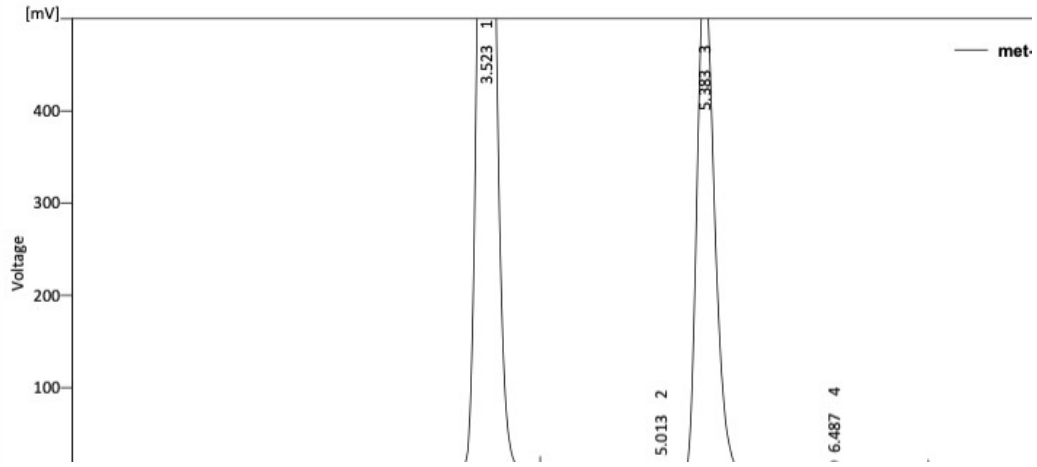

Figure 7: Metformin HCl and Canagliflozin photo Degradation sample

Method Validation

The proposed method was validated with the aspect of system suitability, specificity, linearity, LOD and LOQ, accuracy, precision and robustness.

System Suitability

The System Suitability was calculated from different parameters like retention time, theoretical plates, resolution, tailing factor. System suitability used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The system suitability parameters observed for Metformin HCl has retention time as 3.477, theoretical plates per column 7166 and tailing factor 1.400. The system suitability parameters observed for Metformin HCl impurity A has retention time as 4.517, theoretical plates per column 7430 and tailing factor 1.379. The system suitability parameters observed for Canagliflozin has retention time as 5.370, theoretical plates per column 7427 and tailing factor 1.412. The system suitability parameters observed for Canagliflozin Impurity 2 has retention time as 7.253, theoretical plates per column 7287 and tailing factor 1.340 **(Table 2)**.

Table 2: System suitability Data for Metformin HCl, Canagliflozin and its related impurities

Parameters	Metformin HCL	Canagliflozin	Metformin HCl Impurity A	Canagliflozin Impurity 2
Retention Time	3.477	5.370	4.517	7.253
Theoretical plates per column	7166	7427	7430	7287
Tailing factor	1.400	1.412	1.379	1.340

Specificity

The specificity of the method was established through study of resolution factor of drug peaks from nearest resolving peak and also among all other peaks. The specificity of the chromatographic method was determined to ensure separation of Metformin HCl, Canagliflozin, Metformin HCl Impurity A & Canagliflozin Impurity 2. The Chromatograms of Metformin HCl and Canagliflozin along with its Related Impurity standards and Metformin HCl and Canagliflozin sample show no interference with the Chromatogram of Metformin HCl and Canagliflozin Blank, so the Developed method is Specific **Figure 3**.

Linearity

The linearity for Metformin HCl and its Related Impurity A and Canagliflozin and its Related Impurity 2 were assessed by analysis of combined standard solution in range of LOQ-15.0µg/ml and LOQ-7.5µg/ml respectively. Correlation co-efficient for calibration curve of Metformin HCl and its Related Impurity A and Canagliflozin and its related Impurity 2 was found to be NLT 0.999 respectively **(Table 3)**.

Precision

Repeatability

The data for repeatability of peak area measurement for Metformin HCl and its Related Impurity A and Canagliflozin and its Related Impurity 2, based on six measurements of same solution. The mean area observed 200.520 for Metformin HCL & 298.964 for Metformin HCl impurity A at concentration 10µg/ml with % RSD 1.873 & 1.717 respectively while the mean area observed 98.284 for Canagliflozin and 278.70 for Canagliflozin Impurity 2 at concentration of 5.0µg/ml with % RSD 1.771 & 1.051 respectively. The repeatability shows that the % RSD values observed within acceptance limit of NMT 5%.

Intraday precision & Inter day precision

The data for intraday precision as well as inter day precision for Metformin HCl and its Related Impurity A and Canagliflozin and its Related Impurity 2 is shown in **Table 4**. The %RSD calculated and all values are within acceptance limit. Hence the method is précised.

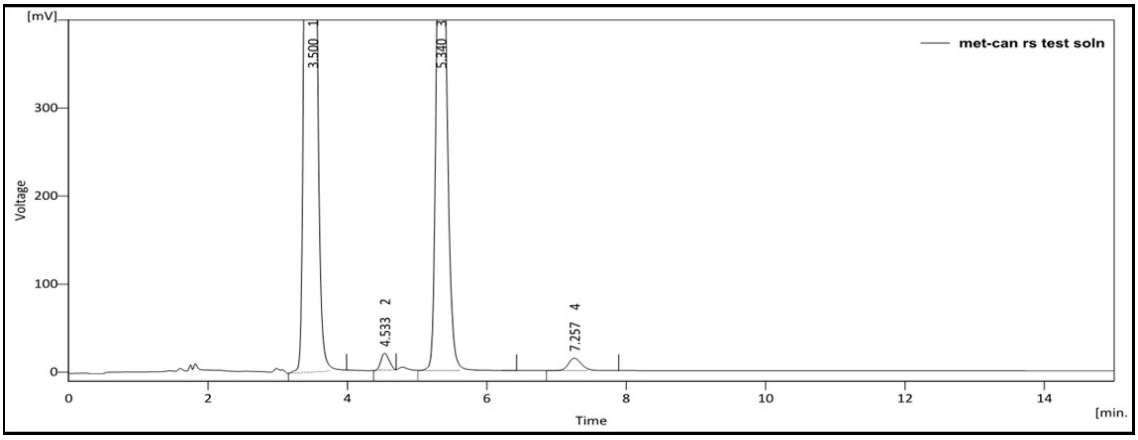

Figure 8: Chromatogram of Metformin HCl and Canagliflozin Sample

Table.3: Linearity Data for Metformin HCl, Canagliflozin and its related impurities

Sr. No.	Linearity Level	Concentration (µg/ml) Drug	Area of Drug	SD	% RSD	Concentration (µg/ml) Impurity	Area of Impurity	SD	% RSD
		Metformin HCl				Metformin HCl Impurity A			
1	LOQ	1.5	29.135 29.003 28.892	0.122	0.419	1.5	4.418 4.359 4.329	0.045	1.036
2	50%	5	98.991 98.469 99.024	0.311	0.315	5	147.191 142.983 146.363	2.229	1.532
3	75%	7.5	148.029 147.654 147.818	0.188	0.127	7.5	220.957 219.367 223.125	1.886	0.853
4	100%	10	203.819 202.451 202.957	0.692	0.341	10	292.884 295.368 291.244	2.076	0.708
5	125%	12.5	247.747 243.569 246.878	2.205	0.896	12.5	368.319 366.542 365.236	1.547	0.422
6	150%	15	297.514 295.666 297.235	0.996	0.336	15	441.806 440.265 441.200	0.776	0.176
		Canagliflozin				Canagliflozin Impurity 2			
1	LOQ	0.75	14.16 13.87 13.874	0.166	1.190	0.75	7.995 7.892 8.134	0.121	1.517
2	50%	2.5	48.622 47.44 47.12	0.791	1.658	2.5	137.622 135.978 136.212	0.889	0.651
3	75%	3.75	72.627 70.012 71.220	1.309	1.836	3.75	206.992 207.056 205.144	1.086	0.526
4	100%	5	100.165 100.364 102.342	1.204	1.192	5	274.373 275.142 276.005	0.816	0.297
5	125%	6.25	121.797 123.212 122.911	0.745	0.608	6.25	345.125 344.798 346.012	0.628	0.182
6	150%	7.5	146.298 144.855 145.253	0.745	0.512	7.5	414.152 412.336 414.003	1.008	0.244

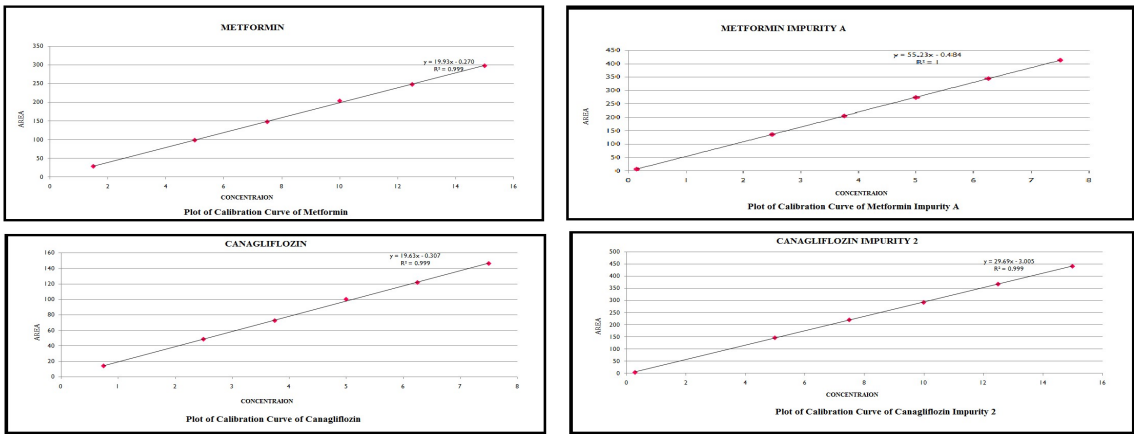

Figure 9: Plots of calibration curves of Metformin HCl, Canagliflozin and related impurities

Table 4: Intraday & Inter day precision data for Estimation of Metformin HCl, Canagliflozin and its related Impurities

Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Area Mean ± S.D. (n=3)	% R.S.D
Metformin HCl			Metformin HCl Impurity A		Canagliflozin			Canagliflozin Impurity 2	
Intraday Precision									
LOQ	29.348± 0.875	2.982	6.295± 0.111	1.770	LOQ	14.267± 0.429	3.005	8.655± 0.367	4.241
10	205.419± 2.102	1.023	291.909± 4.363	1.495	5	98.902± 1.149	1.161	272.549± 4.691	1.721
15	299.321± 3.149	1.052	442.959± 5.125	1.157	7.5	148.010± 2.502	1.690	413.951± 2.639	0.637
Inter day Precision									
LOQ	28.469± 1.331	4.674	6.060± 0.192	3.169	LOQ	13.774± 0.593	4.308	8.499± 0.214	2.518
10	199.063± 3.277	1.646	290.405± 4.060	1.398	5	98.741± 1.342	1.361	271.369± 2.943	1.085
15	296.676± 4.609	1.554	445.401± 5.799	1.302	7.5	144.316± 1.073	0.743	415.132± 4.584	1.104

Accuracy

To check the accuracy of the proposed method for determination of Metformin HCl Impurity A & for Canagliflozin Impurity 2, recovery studies were carried out at LOQ, 80, 100 and 120% of the test concentration according to ICH guidelines. Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The percentage recovery for Metformin HCl Impurity A was 98.128-101.996% and the Percentage recovery for Canagliflozin Impurity 2 was 98.472 to 101.150% **(Table 5)**.

LOD and LOQ

Limit of detection and limit of quantitation for both the drugs and their respective impurities were estimated using the linearity data. Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. The Limit of detection for Metformin HCl observed 0.495 µg/ml, for

Metformin HCl impurity A observed 0.098µg/ml, for Canagliflozin observed 0.248 µg/ml and for Canagliflozin Impurity 2 observed 0.050µg/ml. However the limit of quantitation for Metformin HCl observed 1.500µg/ml, for Metformin HCl impurity A observed 0.296µg/ml, for Canagliflozin observed 0.753µg/ml and for Canagliflozin Impurity 2 observed 0.151µg/ml.

Robustness

The robustness study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The chromatographic factors as flow rate of mobile phase (± 0.2 ml/min), mobile phase pH (± 0.2) and ratio of Mobile phase was changed (±2) without changing the mobile phase components and their effect observed on system suitability for standard preparation. The results shows the effect of changes was found to be within the acceptance criteria & the % RSD values observed within standard limit of not more than 5% **(Table 6)**.

Table 5: Recovery data for Metformin HCl Impurity A & Canagliflozin Impurity2

Sr. No.	Conc. Level (%)	Area of Recovery Spiked with Test	Area of Impurity in Test	Net Area of Std	Area of Std	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery	SD	% R.S.D
Metformin HCl Impurity A										
1	LOQ	191.347	176.435	14.912	14.16	0.3	0.305	101.800	1.837	1.837
2		191.089	176.435	14.654	14.16	0.3	0.300	100.039		
3		190.809	176.435	14.374	14.16	0.3	0.294	98.128		
4	80 %	397.013	176.435	220.578	274.383	4	4.020	100.488	0.973	0.973
5		397.051	176.435	220.616	274.383	4	4.020	100.505		
6		393.334	176.435	216.899	274.383	4	3.952	98.812		
7	100 %	456.294	176.435	279.859	274.383	5	5.100	101.996	1.677	1.665
8		454.505	176.435	278.070	274.383	5	5.067	101.344		
9		447.581	176.435	271.146	274.383	5	4.941	98.820		
10	120 %	503.023	176.435	326.588	274.383	6	5.951	99.189	1.141	1.144
11		509.052	176.435	332.617	274.383	6	6.061	101.020		
12		502.157	176.435	325.722	274.383	6	5.936	98.926		
Canagliflozin Impurity 2										
1	LOQ	178.587	149.68	29.135	29.135	0.15	0.149	99.217	1.181	1.184
2		178.525	149.68	29.135	29.135	0.15	0.149	99.005		
3		179.15	149.68	29.135	29.135	0.15	0.152	101.150		
4	80 %	376.399	149.68	29.135	286.128	8	7.924	99.046	0.410	0.415
5		376.905	149.68	286.128	286.128	8	7.941	99.267		
6		375.085	149.68	286.128	286.128	8	7.878	98.472		
7	100 %	437.857	149.68	286.128	286.128	10	10.072	100.716	0.844	0.846
8		434.449	149.68	286.128	286.128	10	9.953	99.525		
9		433.187	149.68	286.128	286.128	10	9.908	99.084		
10	120 %	489.531	149.68	286.128	286.128	12	11.878	98.980	0.121	0.122
11		490.289	149.68	286.128	286.128	12	11.904	99.201		
12		490.208	149.68	286.128	286.128	12	11.901	99.177		

Table.6: Robustness data for Metformin HCl, Canagliflozin and its related impurities

Sr. No.	Area at Flow rate (+0.2 ml/min)	Area at Flow rate (-0.2 ml/min)	Area at pH (+0.2)	Area at pH (-0.2)	Area at Mobile phase (+2)	Area at Mobile phase(-2)
Metformin HCl						
1	165.466	220.049	211.195	207.551	173.441	211.609
2	169.417	224.517	204.836	214.702	174.882	219.416
3	171.690	218.267	207.411	211.161	177.628	217.135
SD	3.149	3.220	3.199	3.576	2.127	4.014
%RSD	1.185	1.457	1.539	1.693	1.213	1.858
Metformin HCl Impurity A						
1	263.085	317.773	293.194	290.231	260.850	302.240
2	261.066	327.144	298.836	299.074	259.287	304.514
3	266.322	328.995	289.661	290.246	261.536	298.228
SD	2.651	6.016	4.628	5.101	1.153	3.183
%RSD	1.006	1.853	1.575	1.740	0.442	1.055
Canagliflozin						
1	81.389	108.114	103.923	102.005	85.294	104.391
2	83.323	105.747	100.664	102.885	86.015	107.512
3	84.075	107.232	101.938	103.773	87.634	106.399
SD	1.386	1.196	4.628	0.884	1.198	1.582
%RSD	1.671	1.118	1.607	0.859	1.388	1.491
Canagliflozin Impurity 2						
1	246.522	297.642	274.659	271.868	244.381	283.097
2	245.610	306.420	277.383	275.115	237.078	277.738
3	249.523	299.497	271.343	271.889	245.086	279.394
SD	2.047	4.626	3.025	1.869	4.434	2.744
%RSD	0.828	1.536	1.102	0.685	1.831	0.980

Known and Unknown Impurities of Metformin HCl and Canagliflozin

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation Vokanamet. The results of known and unknown impurities are calculated in %RSD. The % RSD for known impurity, Metformin HCl impurity A observed 1.042% while Canagliflozin Impurity 2 observed 1.520%. The % RSD values observed within standard limit of not more than 5%.

The results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

RP HPLC method was validated as per ICH guidelines. The developed method was found to be linear within the range Correlation co-efficient for calibration curve of Metformin HCl and its Related Impurity A and Canagliflozin and its related Impurity 2 was found to be NLT 0.999 respectively. The accuracy of method was determined at 80%, 100%, 120% level. The Percentage recovery for Metformin HCl Impurity A was 98.128-101.996% and the Percentage recovery for Canagliflozin Impurity 2 was 98.472-101.150%. The LOD for Metformin HCl found 0.495µg/ml and for Metformin HCl Impurity A was 0.098µg/ml while for Canagliflozin was 0.248µg/ml and for Canagliflozin Impurity 2 was 0.050µg/ml. Also the LOQ of Metformin HCl found 1.500µg/ml and for Metformin HCl impurity A was

0.296µg/ml and for Canagliflozin 0.753 µg/ml and for Canagliflozin Impurity 2 was 0.151µg/ml indicates the sensitivity of the method. The developed method was found to be precise as the % RSD values for intra-day and inter-day were found to be less than 5.0%. The method was also found to be robustness indicated by the % RSD values which are less than 5 %.

CONCLUSION

There is no analytical work has been available regarding Related Impurities RP-HPLC method for Metformin HCl and Canagliflozin in a literature. Data regarding behavior of drug and its related impurities in chromatographic conditions and other relevant analytical properties are not available. It is the novel attempt in a field of research has been made to develop and validate Related Impurities method via RP-HPLC. Conclusively, the RP HPLC-method described in this paper is specific, sensitive, rapid and easy to perform. The proposed RP-HPLC method enables simultaneous estimation Metformin HCl and Canagliflozin along with its related impurities. This method provides good separation and resolution of the chromatographic peaks of the Metformin HCl, Canagliflozin and its related impurities. The 0.05 M Potassium Dihydrogen Phosphate (pH 5.0): Acetonitrile (70:30v/v) was used as mobile phase. The sample recoveries from all formulations were in good agreement with their respective label claims, which suggested non-interference of formulations excipients in the estimation. The method was successfully validated in terms of specificity, precision, linearity, accuracy and robustness as per ICH guidelines. It can be concluded that the proposed method can be used for routine analysis for estimation of related impurities of Metformin HCl and Canagliflozin in combined dosage form by RP-HPLC.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography; mm: millimetre; M: Molar; µm: Micrometer; pH: Potential of Hydrogen; ml: Milliliters; nm: nanometer; LOD: Limit of detection; LOQ: Limit of quantitation; µg: microgram; % RSD: Relative standard deviation; NMT: Not more than; NLT: Not less than; min: Minutes; ICH: International Conference on Harmonization; Rs: Resolution; SD: Standard deviation;°C: Degree Celsius; µg: Microgram;; mg: Milligrams; %: Percentage; v/v: Volume/volume; MET: Metformin HCl; AMPK: Adenosine monophosphate-activated protein kinase; UV: Ultra violet; pvt: private; g: gram; ppm: parts per million; fig: figure; RTG: Renal Threshold for glucose; SGLT2: Sodium-glucose transport protein 2.

COMPETING INTERESTS

The authors declare no conflicts of interest.

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