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DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF CANAGLIFLOZIN AND METFORMIN BY RP-HPLC IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Canagliflozin and Metformin RP-HPLC Bulk and Combined Dosage Form Analytical Method Development and Validation, Canagliflozin and Metformin concentrations were determined concurrently using a novel RP-HPLC technique. Using Symmetry ODS C18 (4.6mm 250mm, 5m) at a flow rate of 1.0 ml/min and a mobile phase ratio of (70:30 v/v), the optimum chromatographic conditions for the separation of Canagliflozin and Metformin were created. The detection wavelength was 250 nm, and the buffer was methanol: TEA at pH 3.8 (pH was modified with orthophosphoric acid). WATERS Alliance 2695 separation module with Empower 2, 996 PDA detector was utilized for this experiment. 2.246 and 5.461 minutes were calculated to be the retention times. Canagliflozin had a purity of 101.27 percent, whereas Metformin was determined to be 99.7 percent pure. Canagliflozin and Metformin both have a resolution of 2.97, whereas other system appropriateness metrics such as theoretical plates and tailing factor are 5387 and 0.97, respectively. According to ICH rules (ICH, Q2 (R1)). Canagliflozin and metformin were both shown to be linear throughout a concentration range of 30–70 mg and 60–140 mg, respectively, with correlation coefficients (r2) of 0.999 and 0.999, respectively.

Results showed a recovery rate of 100.14%, a repeatability error of 0.14%, and an intermediate precision error of 0.14%. Accurate, solid, and reproducible results were found in the precision investigation. Both the LOD and LOQ values were quite high: 0.56 and 1.2, and 1.7 and 3.6, respectively. Therefore, Canagliflozin and Metformin in API and Pharmaceutical dosage form may be routinely analyzed with the proposed RP-HPLC approach.

Keywords: Methodological Improvement, Validation, and Reliability with Canagliflozin and Metformin.

NTRODUCTION

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Canagliflozin1, also known as Invokana, is a sodium-glucose co transporter 2 (SGLT2) inhibitor used in the management of type 2 diabetes mellitus along with lifestyle changes including diet and exercise. It was initially approved by the FDA in 2013 for the management of diabetes and later approved in 2018 for a second indication of reducing the risk of cardiovascular events in patients diagnosed with type 2 diabetes mellitus. Canagliflozin is the first oral antidiabetic drug approved for the prevention of cardiovascular events in patients with type 2 diabetes. Cardiovascular disease is the most common cause of death in these patients. The Chemical Structural of Canagliflozin is

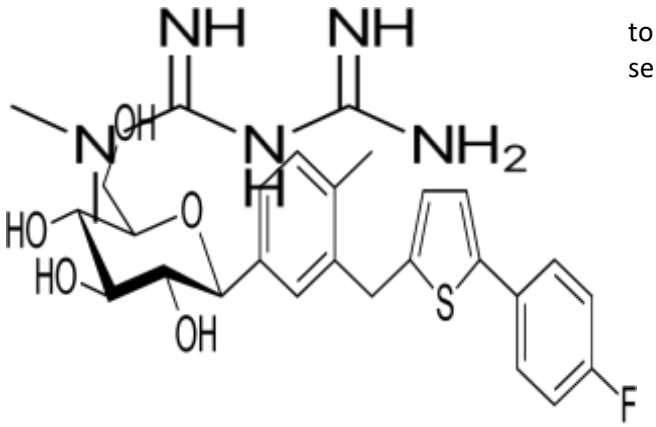

Fig-: Chemical Structure of Canagliflozin

Metformin2 is a biguanide antihyperglycemic drug used for patients with type 2 diabetes. At least 120 million individuals throughout the globe are now using Metformin for the treatment of type II diabetes. Metformin is an antihyperglycemic because it reduces blood glucose levels, especially in those with type 2 diabetes.

to avoid low blood sugar. As an insulin sensitizer, metformin lowers insulin resistance and fasting plasma insulin levels to a clinically relevant degree. Some people use this medication to help them shed a few pounds, and this is another perk that's often advertised. To treat type II diabetes in people who are overweight, metformin is often used. In 1972, metformin gained approval in Canada; in 1995, it was accepted in the United States. There are both immediate-release and extended-release versions of this medication.

Fig-: Chemical Structure of Metformin

EXPERIMENTAL INSTRUMENTS USED

Table-: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius

4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra Sonicator	Enertech

CHEMICALS USED:

Table-: Chemicals Used

S. No.	Chemical	Brand Names
1	Canagliflozin	Sura labs
2	Metformin	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Triethylamine	Merck

Accurately weigh and transfer 10 mg of Canagliflozin and Metformin working standard into a 10 ml of clean, dry volumetric flasks; add roughly 7 ml of Methanol; sonicate to dissolve and removal of air entirely; and bring volume up to the mark with the same Methanol. Pipette 0.5 ml of the Canagliflozin stock solution and 1 ml of the Metformin stock solution into a 10 ml volumetric flask, and then fill the flask with Methanol until it reaches the desired concentration.

Take an average weight of one tablet, smash it with a pestle and then weigh out 10 mg equivalent weight of Canagliflozin and Metformin into a 10 mL clean dry volumetric flask. Add around 7 mL of Diluent, sonicate to dissolve it, and then fill the flask to the mark with more of the same solvent. Add 1 ml of the Metformin stock solution and 0.5 ml of the Canagliflozin stock solution to a 10 ml volumetric flask and dilute to the appropriate concentration with Methanol.

Method: Inject samples under varying chromatographic settings, record chromatograms, and make note of the optimal peak elution conditions for validation in accordance with ICH recommendations.

BUFFER AND MOBILE PHASE PREPARATION:

In order to make a Triethylamine (TEA) buffer (pH-4.2), you will need to: Achieve a pH of 3.8 by dissolving 1.5 ml of Triethyl amine in 250 ml of HPLC water. You may filter and sonicate the solution by using a vacuum filter and an ultrasonicator.

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Mobile Phase was prepared by mixing precisely measured 360 ml (36% Methanol) and 640 ml (64% buffer) in a computerized ultra Sonicator for 15 minutes, followed by degassing and filtering through a 0.45 filter under vacuum.

The mobile phase was utilized as the diluent throughout the preparation process.

Optimizing the Mobile Phase: At first, several mixtures of methanol, water, acetonitrile, phosphate buffer, and acetonitrile were tested as mobile phases. In the end, a mobile phase of 30% methanol and 70% TEA buffer (pH 3.8) proved to be optimal.

Column optimization: the procedure was tried with other columns, including the C18 column, the symmetry column, and the zodiac column. At 1ml/min flow, the optimum particle size was determined to be Symmetry ODS C18 (4.6mm 250mm, 5m).

CRITERIA FOR EVALUATING METHODS

In order to test the suitability of the system, 10 mg of Canagliflozin and 10 mg of Metformin working standard should be weighed and transferred to a 10 mL clean, dry volumetric flask. About 7 mL of Diluents should be added, and the mixture should be sonicated to dissolve the drug thoroughly. It's a stock answer. Pipette 0.5 ml of the Canagliflozin stock solution and 1 ml of the Metformin stock solution into a 10 ml volumetric flask, and then fill the flask with Methanol until it reaches the desired concentration.

HPLC was used to measure the area of five separate injections of the reference solution. Five duplicate injections' percent relative standard deviation (%RSD) was determined to be well within the allowed range.

SPECIFICITY3:

Accurately weigh and transfer 10 mg of Canagliflozin and 10 mg of Metformin working standard into a 10 ml of clean dry volumetric flasks; add around 7 mL of Diluents; sonicate to dissolve fully; and bring volume up to the mark with the same solvent.

solvent. (Readily available answer). Pipette 0.5 ml of the Canagliflozin stock solution and 1 ml of the Metformin stock solution into a 10 ml volumetric flask, and then fill the flask with Methanol until it reaches the desired concentration.

Take an average weight of one tablet, smash it with a pestle and then weigh out 10 mg equivalent weight of Canagliflozin and Metformin into a 10 mL clean dry volumetric flask. Add around 7 mL of Diluent, sonicate to dissolve it, and then fill the flask to the mark with more of the same solvent. Pipette 0.5 ml of the Canagliflozin stock solution and 1 ml of the Metformin stock solution into a 10 ml volumetric flask, and then fill the flask with Methanol until it reaches the desired concentration.

The three duplicate injections of standard and sample solutions4 are injected, and the assay is calculated using the following formula.

$$\%ASSAY = \frac{\text{Sample area of sample}}{\text{Standard area of sample}} \times \frac{\text{Weight of standard}}{\text{Purity}} \times \frac{\text{Dilution of standard}}{\text{Weight of tablet}} \times \frac{\text{Dilution}}{\text{Label claim}} \times 100$$

Carefully measure 10 mg of Canagliflozin and 10 mg of Metformin working standard into 10 ml of clean, dry volumetric flasks. Add around 7 mL of Diluents, sonicate to dissolve completely, and bring volume up to the mark with the same solvent. As-is answer

Using a pipette, transfer 0.3 ml of Canagliflozin stock solution and 0.6 ml of Metformin stock solution to a 10 ml volumetric flask, and dilute to the appropriate concentration with diluent, resulting in a concentration of 30 ppm

Canagliflozin and 60 ppm Metformin, respectively.

Level II (40 ppm Canagliflozin and 80 ppm Metformin) was prepared by adding 0.4 ml of Canagliflozin stock solution and 0.8 ml of Metformin stock solution to a 10 ml volumetric flask and diluting to the appropriate volume with diluent.

Prepare Level III (50 ppm Canagliflozin and 100 ppm Metformin) by adding 1 ml of Metformin stock solution and 0.5 ml of Canagliflozin stock solution to a 10 ml volumetric flask, respectively, and then filling the flask to the mark with diluent.

Level IV (60 ppm Canagliflozin and 120 ppm Metformin) was prepared by adding 0.6 ml of Canagliflozin stock solution and 1.2 ml of Metformin stock solution to a 10 ml volumetric flask and diluting to the appropriate concentration with diluent.

To make Canagliflozin/Metformin Concentrate, Level V (70 ppm/140 ppm), 0.7 mL of Canagliflozin stock solution and 1.4 mL of Metformin stock solution were pipetted into a 10 mL volumetric flask, respectively, and diluted to the appropriate concentrations with diluent.

Measure the peak area after injecting a known volume of each concentration into the chromatographic system5. Determine the correlation coefficient6,7 by plotting a graph of peak area vs concentration (with concentration along the X-axis and peak area along the Y-axis).

RELIABLE EXACTNESS

Precise Canagliflozin with Metformin Treatment: In a 10 mL clean, dry volumetric flask, accurately weigh and transfer 10 mg of Canagliflozin and 10 mg of Metformin working standard; add around 7 mL of Diluents; sonicate to dissolve; then, bring volume up to the mark using the same solvent. (Readily available answer). Canagliflozin 0.5 mL and Metformin 1 mL stock solutions should then be pipetted into a 10 mL volumetric flask, respectively, and diluted to the appropriate concentrations with Diluent. Five separate HPLC injections of the reference solution were each assessed for area. Five duplicate injections' percent relative standard deviation (%RSD) was determined to be well within the allowed range.

INTERMEDIATE PRECISION:

Precision was run on various days under the same circumstances to assess the method's intermediate precision (also called Ruggedness).

Procedure:

On Day 1, HPLC measured the area of six separate injections of the reference solution. The percent relative standard deviation (%RSD) for the area of six sets of duplicate injections8 was determined to be acceptable.

On day 2, HPLC was used to measure the area of six separate injections of the standard solution. It was revealed that the percent relative standard deviation (%RSD) for the region of six duplicate injections was well within the allowed range.

ACCURACY:

Accurately weigh and transfer 10 mg of Canagliflozin and 10 mg of Metformin working standard into a 10 mL of clean dry volumetric flasks, add about 7 mL of Diluents9, sonicate to dissolve it completely, and make volume up to the mark with the same solvent. This will yield a 50% Standard stock solution. (Readily available answer). To finish, transfer 0.25ml of Canagliflozin and 0.5ml of Metformin stock solutions by pipette to a 10ml volumetric flask, and then fill the rest of the way with Diluent.

Accurately weigh and transfer 10 mg of Canagliflozin and 10 mg of Metformin working standard into a 10 ml of clean dry volumetric flasks, add about 7 mL of Diluents, sonicate to dissolve it completely, and make volume up to the mark with the same solvent. This will yield a 100% Standard stock solution. (Readily available

answer). Canagliflozin 0.5 mL and Metformin 1 mL stock solutions should then be pipetted into a 10 mL volumetric flask, respectively, and diluted to the appropriate concentrations with Diluent.

Accurately weigh and transfer 10 mg of Canagliflozin and 10mg of Metformin working standard10 into a 10 ml of clean dry volumetric flasks, add about 7 mL of Diluents, sonicate to dissolve it completely, and make volume up to the mark with the same solvent. This will yield a 150% Standard stock solution. (Readily available answer). Pipet 0.75 ml of the Canagliflozin stock solution and 1.5 ml of the Metformin stock solution into a 10 ml volumetric flask, and then bring to the desired concentration using Diluent.

Here's how it's done: Under the idealized conditions11, three sets of injections were made at different concentrations (50%, 100%, and 150% of the initial). Collecting chromatogram data and calculating peak responses. Find the individual recovery values and the mean recovery values for Canagliflozin and Metformin, as well as the amounts discovered and added.

TEST RESULTS VARIABILITY WAS FOUND13 by repeating the study under a variety of experimental settings, demonstrating ROBUSTNESS12. We look for deviations in outcomes under the following scenarios.

Accurately weigh and transfer 10 mg of Canagliflozin and 10 mg of Metformin working standard14 into a 10 mL of clean, dry volumetric flasks; add around 7 mL of Diluents; sonicate to dissolve fully; and bring volume up to the mark with the same solvent. This will serve as the Standard solution. (Field-tested method15). Canagliflozin 0.5 mL and Metformin 1 mL stock solutions should then be pipetted into a 10 mL volumetric flask, respectively, and diluted to the appropriate concentrations with Diluent.

The sample was examined at flow rates of 0.9 and 1.1 ml/min instead of 1 ml/min, with the other 16 variables held constant. The aforementioned sample (volume: 20 l) was injected, and chromatograms were taken.

In order to examine the material, we tried out different concentrations of the methanol used as the mobile phase. TEA Buffer17 was used, but instead of 30:70, the ratios used were 35:65 and 25:75. This material, which was 20 l in volume, was injected, and chromatograms18 were obtained.

RESULTS AND DISCUSSION

Method Development:

Optimized Chromatogram

Mobile phase : Methanol: TEA Buffer (pH-3.8) (30:70v/v)

Column : Symmetry ODS C18 (4.6mm×250mm, 5µm) particle size

Flow rate : 1 ml/min

Wavelength : 250 nm

Column temp : 37ºC Injection Volume : 20 µl
Run time : 10 minutes

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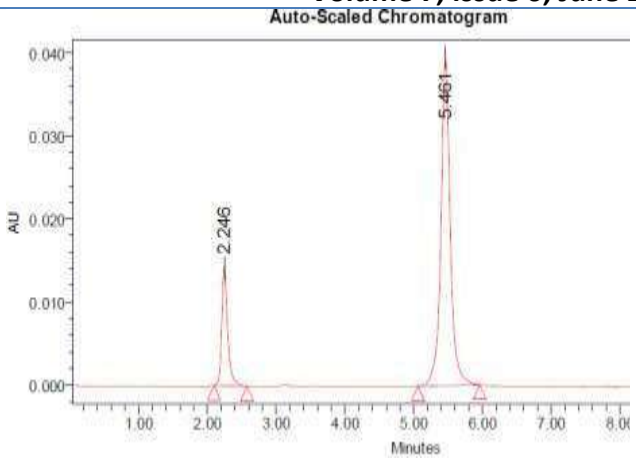

Fig-: Optimized Chromatogram

Table-: Peak Results for Optimized Chromatogram

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Canagliflozin	2.246	765789	69584		0.97	5587.0
2	Metformin	5.461	2532158	190049	2.97	1.26	5398.0

Method Validation:

System Suitability: System suitability19 is an integral part of the method validation to evaluate the parameters like tailing factor, theoretical plates, resolution and %RSD for replicate injections. The results were within the limits and were presented in Table 2 shows the system suitability chromatogram.

Table-2: System Suitability Results

Parameter	Results		Limits
	Canagliflozin	Metformin	
RSD of Peak Area	0.060932	0.060932	<2.0
Retention Time	2.246	5.461	<1.0
USP Tailing Factor (T)	0.97	1.26	T < 2
USP Plate Count (N)	5587	5398	>2000
USP Resolution (R)	2.97		R > 2

Specificity: Peak purities for the sample solution >0.99 suggest the procedure is specific, and the placebo chromatogram lacked peaks during the retention periods of Canagliflozin and Metformin, as well as interference from degradants, as shown in degradation studies20. The chromatogram in Figure 3 is empty.

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The suggested method's accuracy was established by recovery trials, in which a known quantity of pure drug concentrations were spiked in placebo at three distinct levels (i.e., 50%, 100%, and 150%), and the percentage of false positives was then determined. The rate of recovery served as the quantitative measure of precision. The data was compiled in a table, which can be seen at

Table 3.

Table-: The Accuracy Results for Canagliflozin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	42594.67	25	25.070	100.280%	100.14%
100%	84867	50	49.965	99.930%	
150%	127654	75	75.164	100.218%	

Table-: The Accuracy Results for Metformin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2079124	50	50.445	100.890%	100.56%
100%	4082412	100	100.571	100.571%	
150%	6070195	150	150.309	100.206%	

Limit of Detection: The detection limit22 of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

LOD= 3.3 × σ / s

Slope of the calibration curve = / = Standard deviation of the response

An oral dose of canagliflozin is 0.56 micrograms per milliliter.

Level of metformin in plasma: 1.75 micrograms per milliliter

The lowest detectable concentration of an analyte in a sample is known as the quantization limit23 of a certain analytical method.

LOQ=10×σ/S

Where = Response Standard Deviation and S = Calibration Curve Slope

An oral dose of canagliflozin is 1.2 milligrams per milliliter.

Three point six milligrams per milliliter of metformin

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Six duplicate injections of 50 and 100 g/mL Canagliflozin and Metformin were performed to test three degrees of precision: repeatability, reproducibility, and intermediate precision24. Data on accuracy was calculated as a percentage of relative standard deviation.

Table 4.

Table-: Results of Repeatability for Canagliflozin

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Canagliflozin	2.269	766854	702564	5685	1.6
2	Canagliflozin	2.255	765884	698789	5584	1.4
3	Canagliflozin	2.252	765842	701235	5521	1.6
4	Canagliflozin	2.267	768985	700124	5525	1.9
5	Canagliflozin	2.260	765845	698986	5578	1.7
Mean			**766682**			
Std. Dev			**1357.973**			
% RSD			**0.177123**			

Summary and Conclusion

From the above experimental results it was concluded that, newly developed method for the simultaneous estimation of Canagliflozin and Metformin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost-effective and it can be effectively applied for routine analysis in research institutions, quality control department in pharmaceutical industries, approved testing laboratories.

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