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DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CANAGLIFLOZIN IN TABLET DOSAGE FORM BY RPHPLC

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ABSTRACT

RP-HPLC was developed and validated for the measurement of Canagliflozin in tablet dosage forms. Canagliflozin, a type 2 diabetes mellitus (T2DM) inhibitor, requires precise analysis in order to ensure quality and consistency. Based on the optimal method, methanol, water, and acetonitrile were used as mobile phases in a ratio of 40:40:20, with 225 nm detection. There was excellent linearity, with a correlation coefficient (r2) of 0.999, precision (intra-day RSD = 0.435%, inter-day RSD = 1.033%), and accuracy (recovery between 99.1% and 100.2%). Limits of detection (LOD) and limits of quantification (LOQ) were 0.34 µg/mL and 1.03 µg/mL, respectively. Flow rate, composition of the mobile phase, and wavelength of the detection were not sensitive to changes in the method. As a routine quality control and stability test method for Canagliflozin in bulk and finished dosage forms, this RP-HPLC method is suitable. As a result of the validation, the system is confirmed to be capable of conforming to regulatory standards for pharmaceutical analysis.

Keywords: Canagliflozin, RP-HPLC, SGLT2 Inhibitor, Tablet Dosage Forms.

INTRODUCTION

Canagliflozin is a new type of antihyperglycemic agent by the means of the class of sodium glucose co transporter 2(SGLT2) inhibits [1]. It is currently marketed as an approved treatment against type 2 diabetes mellitus (T2DM) by preventing SGLT2 receptor expression in proximal kidney tubules, voluntarily limiting glucose uptake and increasing the concentration of glucose in the urine. This insulin independent effect adds to glycemic control with relatively low risk of hypoglycemia when used in monotherapy, and the add-on effect of modest weight loss and systolic blood pressure reductions, which are consistent with the emerging paradigm of multifactorial risk reduction in managing diabetes. Taking into consideration the burden and significant morbidity and mortality by T2DM in worldwide populations, optimal antidiabetic treatment is still a population health priority [2]. The effectiveness of canagliflozin in lowering of the glycated hemoglobin (HbA1c) coupled with cardiovascular and renal protective effects seen in massive clinically trials explains its importance in treatment regimes more so to patients with elevated cardiovascular risk. It is therefore essential to maintain uniformity in dosage strength and quality of the products by strong analyses [3]. Canagliflozin is a new innovative oral antihyperglycemic agent that contains sodium-glucose co-transporter 2 (SGLT2) inhibitor. It has been authorized to treat type 2 diabetes mellitus (T2DM) which is a systemic metabolic disease including the presence of insulin resistance and defective glucose control. The mechanism of action of canagliflozin is selective inhibition of the SGLT2 receptors located in kidney proximal tubules, which consequently decreases glucose reabsorption by the kidneys and increases the urinary excretion of glucose (glucosuria). The mechanism is also independent of insulin and so the mechanism has potential as an effective treatment option in a wide spectrum of patients including those with insulin resistance or on insulin-sparing regimens. Canagliflozin has several clinical benefits as none of the

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effects are dependent on insulin. Of note, its risk of hypoglycemia is low both as a monotherapy and in combination with drugs that are not affected with insulin secretion stimulation. As well as glycemic control, canagliflozin has secondary advantages, including modest weight reduction, which is probably rooted in caloric loss via glucosuria, and lowered systolic blood pressure, which is probably attributable to a mild diuretic effect. These extended consequences comply with the current management direction of active treatment of diabetes, where the focus is on the multifactorial risks that prevent the development of long-term complications of the disorder and increase the quality of life among patients.

Because of the prevalence of T2DM worldwide-increasing prevalence, financial burden, and related complications, including cardiovascular disease, nephropathy, and neuropathy, an emphasis is placed upon the optimization of treatment interventions. Clinical trials have shown not only canagliflozin improves the levels of HbA1c but also provides cardiovascular and renal protection and therefore is a pivot stone in managing diabetic patients with comorbidities. These results identify its usefulness in high-risk groups and to its increasing adoption to evidence-based diabetes medication algorithms. As the application of canagliflozin in the clinical practice becomes more and more frequent, it becomes particularly important to achieve the correct dosing and ensure quality of the products. In the quality control of pharmaceutical formulation, there is a need to develop strong and valid analytical procedures. Trustworthy procedures can be used to establish drug content, identify wantonness or matters of decay, and check distribution duplicate-to-duplicate incorporate, all of which are critical in maintaining therapeutic possession and divergent safety. Analytical research, therefore, behind the estimation of canagliflozin in tablet dosage forms, is rather important in the pharmaceutical sciences.

Challenges in Pharmaceutical Analysis of Canagliflozin Tablets

The dosage form of tablets has inbuilt limitations of analysis due to the complicated formulation matrix [4]. Detection and quantification of the active pharmaceutical ingredient (API) can be interfered by excipients that include binders, fillers, disintegrants and coatings. In addition, the chemical nature of canagliflozin (such as its low aqueous solubility and chromophores, which absorb UV rays) requires a cautious choice of solvents, mobile phases and detection modes. HPLC method development with canagliflozin analysis of tablets thus need to consider the following factors: extracting canagliflozin completely in this sample; obtaining sufficient chromatographic separation between canagliflozin and possible excipient peaks, degradation products or impurities; and choice of detection conditions (UV, PDA or MS) with the specificity, sensitivity and linearity over the therapeutic concentration range. The dosage form as a tablet also poses some analytical difficulties since many inactive ingredients are found in the form of complex mixture. This can contain binders, fillers, disintegrants, lubricants and film coatings, which may possibly mask the reliable detection and quantification of the active pharmaceutical ingredient (API). Analysis and timed testing Analysis and timed testing of fine chemicals such excipients may result in the creation of overlapping chromatographic peaks in addition to solubility and extraction problems with the API, which cause an error in the results of the assay.

Choice of Chromatographic Technique: HPLC / UHPLC and Detection Modes

HPLC - A product of high-performance liquid chromatography (HPLC) continues to be a stalwart analytical instrument in pharmaceutical testing because of its flexibility in separation ability, compatibility with a variety of detectors, and durability. In the case of canagliflozin, UV-visible absorption is usually used as a detector- taking advantage of the chromophoric structure of the drug.

METHODOLOGY

Chemicals and Reagents

The Milli-Q water, methanol (HPLC grade), potassium dihydrogen phosphate monohydrate (AR grade) and orthophosphoric acid (GR grade) were obtained from Qualigens Ltd., Mumbai. Any other analytical grade chemicals were obtained locally unless otherwise stated. Standard class-A volumetric glassware was used on all dilutions.

Instrumentation

Its chromatographic analysis was done in a Waters LC, which consisted of 2695 pump, 2996 photodiode array detector, and Rheodyne injector. The Waters Empower 2 software was used in recording the data. The mobile phase consisted of a mixture of pH 3.0 buffer, methanol and acetonitrile with a ratio of 40:40:20 v/v and separation was performed via a Symmetry RP-18 column (150 mm x 4.6 mm, 5) column. The mobile phase underwent filtration using a 0.45 -m membrane filter and also de-gassing prior to usage. The flow rate was set 1.2 mL/min whereas the detection was also performed at 225 nm under ambient temperature [5].

Selection of Stationary Phase

The correct stationary phase selection is dependent upon the chemical nature of the analyte. As a polar compound, canagliflozin was fractionated by reverse-phase chromatography, which is appropriate in such analysis [6]. A C18 column (Symmetry RP-18, 150

mm x 4.6 mm, 5 µm) was selected based on initial trials and looking up in literature because it provided the best possible resolute chemistry system of Canagliflozin.

Selection of Wavelength

The wavelength of detection was selected on the basis of Canagliflozin absorption spectrum. It was observed that the 225 nm wavelength provided the drug with the best sensitivity and response, thus the best method to use in the quantification of the drug.

Selection of Mobile Phase

The optimisation of the mobile phase helped to obtain the optimal separation of Canagliflozin. MIxture of solvents such as methanol, water and acetonitrile has been tried. The optimal mixture of the mobile phase to give the optimal resolution and peak shape was methanol: water (90:10 v/v), its use resulted in sharp, symmetrical peak and short retention time of the analyte.

Method Development

Development of method was mainly to attain the best separation and resolution of Canagliflozin via the HPLC. Several experiments with varying combinations of solvents proved that a 90:10 v/v methanol: water combination as the mobile phase was the best to separate the drug. The mobile phase was developed in dissolving 2.72 grams of potassium dihydrogen phosphate monohydrate in 1000 mL of purified water and raising the pH to 3.0 by adding orthophosphoric acid. The solution was filtered with the use of a filter of 0.45µm prior to use. Subsequent experiments with the direct addition of acetonitrile to the mobile phase (40:40:20 ratio of buffer: methanol: acetonitrile) also improved the separation, but produced a desirable peak shape and retention time (2.85 minutes) with Canagliflozin [7].

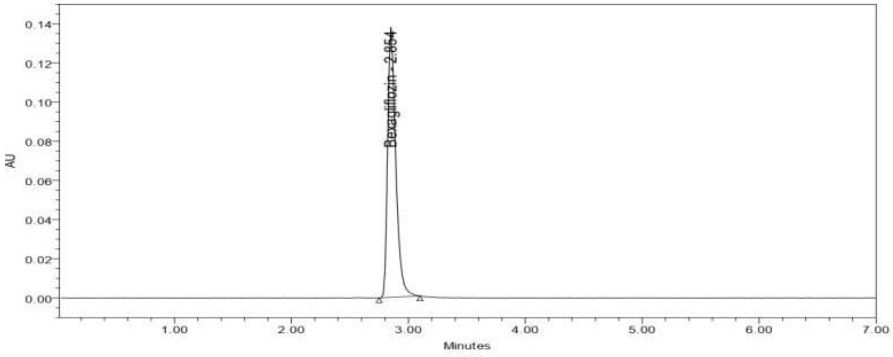
Preparation of Sample

The pre-paration of samples involved weighing and powdering 20 tablets finely. About 100 mg of the powdered material of Canagliflozin was introduced into a 200 mL volumetric flask, and 160 mL mobile phase was poured in. Sonication of the solution was carried out in 30-minute intervals or shaking at an adjustable temperature then diluted to volume with mobile phase. Then the filtration of the solution was done using a 0.45 µm membrane filter. The solution was pipetted into a 50 mL volumetric flask and the volume added to the mobile phase [8].

Preparation of Standard Solution

To make the standard solution: 100 mg of Canagliflozin was dissolved in 160 mL of mobile phase inside the 200 mL volumetric flask. Upon sonication to dissolve the drug, the solution was left at room temperature. Afterwards, it was diluted using the diluent to achieve the desired concentration. The 5.0 mL of this solution was measured into a 50 mL volumetric flask and diluted to its volume using the diluent (the mobile phase) to obtain the appropriate concentration used in analysis.

Figure 1: A typical HPLC Chromatogram showing the peak of Canagliflozin



Method Validation

The validated HPLC procedure used in the quantitation of Canagliflozin followed the ICH guidelines. The following parameters are examined as part of method validation in accordance to ICH guidelines.

Preparation of Standard and Sample Solution

Preparation of standard

Weigh the correct quantity of Canagliflozin and pipette it to a 200 mL volumetric flask. Add 160mL mobile phase and sonicate the solution to make sure Canagliflozin is fully dissolved. Cool the solution to room temperature and dilute Vol to volume with diluent. Pipette 5.0 mL of this solution into a 50 mL volumetric flask and bring to 50 mL with diluent (mobile phase) to obtain a final concentration of 50 µg/mL Canagliflozin.

Preparation of Formulation:

Take at least 20 tablets and powder as much as you can. Add to a 200 mL volumetric flask an equivalent of 100 mg of Canagliflozin. To the mixture add 160 mL

of mobile phase and sonicate the mixture at a controlled temperature with intermittent shaking using 30 minutes in total. Dilute to volume and mix. Pass the solution through a 0.45 µm membrane filter. Thereafter add 5.0 mL of this filtered solution to a 50 mL volumetric flask and add diluent to dilute to volume to form a solution containing 50 µg/mL of Canagliflozin.

Chromatographic Parameters:

Table 1 shows the chromatographic conditions which were applied in this analysis.

Table 1: Chromatographic conditions

Mobile phase	Mobile phase consisting of pH3.0 Buffer, Methanol and Mobile Phase acetonitrile in the ratio of 40:40:20 v/v respectively.
Column	Symmetry RP-18, 150mm x4.6mm 5µm particle size
Flow Rate	1.2 mL/min
Column Temperature	Ambient temperature
Wavelength	225 nm
Injection Volume	20 µL
Run Time	10 min
Retention Time	Canagliflozin peak about 4.0 minutes
Sample Preparation	Sample concentration of Canagliflozin is 50µg/mL.

System Suitability

System suitability testing is done to make sure that the system is performing as well as it can be just prior to or during the analysis of the unknowns. Before any validation experiment will be conducted, it is important that the HPLC and procedure can achieve the desirable quality of data. These tests are meant to ensure that the system has a resolution and repeatability which is adequate to the analysis being done. The idea of system suitability is that the experiments, equipment, electronics, analytical processes and samples comprise a system and so can be assessed as a system. The system suitability parameters and recommendation is shown in Table 2.

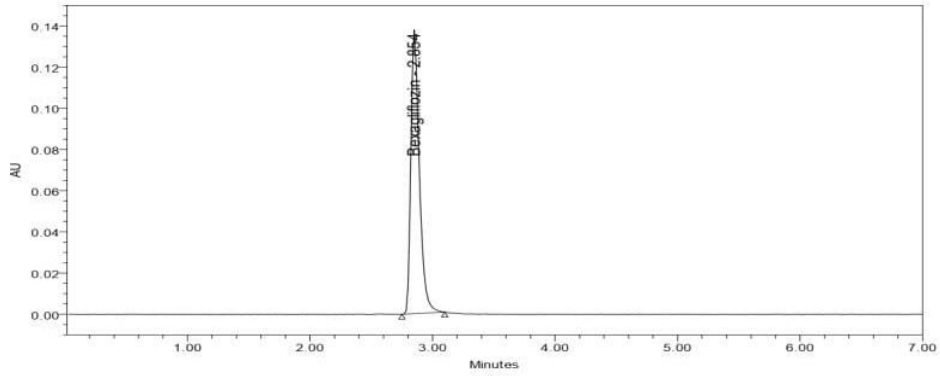
Table 2: System suitability parameters and recommendations

S. No	Parameters	Recommendations
1	Theoretical plates (N)	>2000
2	Tailing factor (T)	≤ 2
3	Resolution (Rs)	> 2 between peak of interest and the closest eluting potential interference
4	Repeatability	RSD ≤ 1% for N ≥ 5 is desirable
5	Capacity factor (k1)	> 2.0
6	Relative retention	Not essential as long as the resolution is stated

Procedure

Under the test method, Canagliflozin working standards were added to a standard solution and injected into a HPLC system six times. Based on the standard chromatograms, the % RSD of Canagliflozin retention times and peak areas were determined per 6replicate injections of the system suitability parameters. Figure 6.03 shows the resultant chromatogram.

Figure 2: Chromatogram of standard



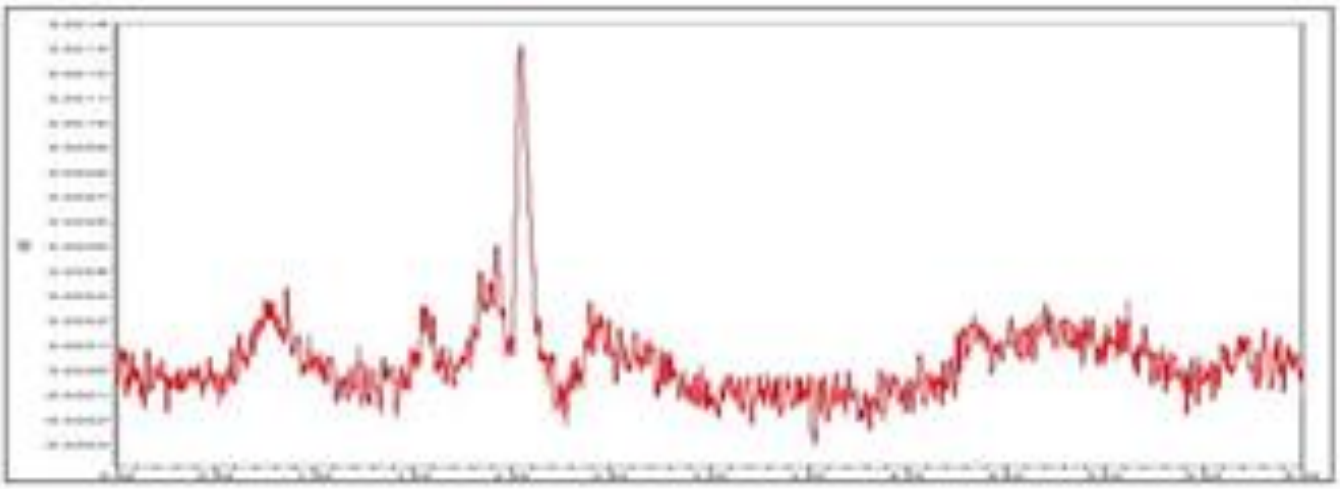
Specificity

Specificity is that property that allows the proper evaluation of the analyte that is present in the presence of other components that might be anticipated to occur. In the event that a method is not specific, other corroborative analytical processes may make up the deficiency. Blank, standard, Placebo, known related compounds, spiked samples and sample solvents were done and injected into the chromatographic system to find out any interference in Canagliflozin peak.

Placebo Interference

An experiment was performed to measure the confound of placebo. The assay method was placed in the same way as the test sample in the preparation of a placebo sample [9]. The placebo chromatogram showed no extra peaks which shows that the excipients used in the formulation did not interfere with Canagliflozin assay. The chromatogram obtained is as illustrated in figure 3.

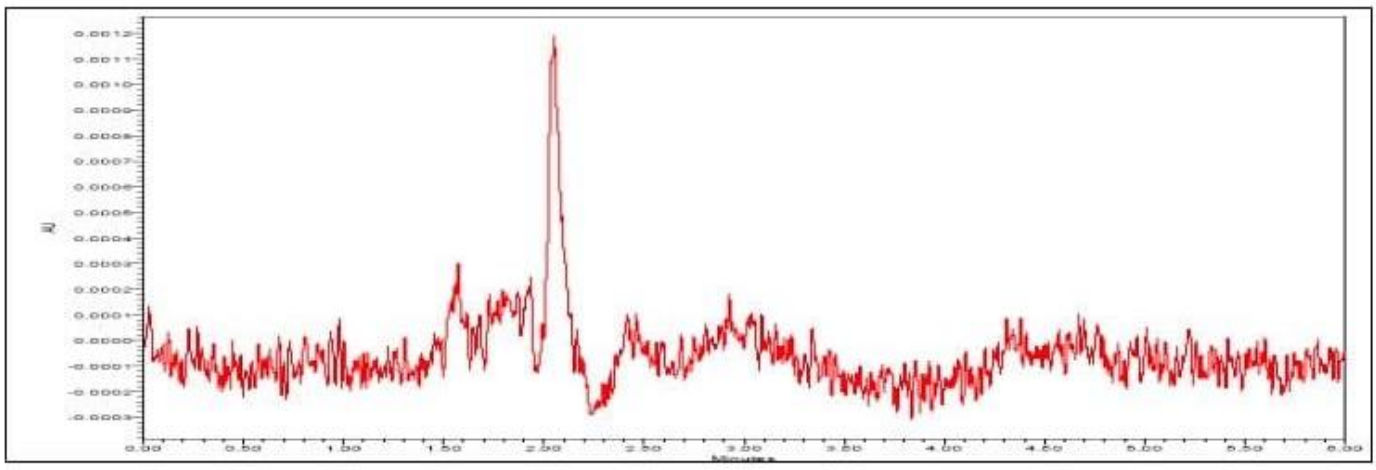
Figure 3: A typical HPLC chromatogram showing the no interference of placebo



Blank Interference

The interference of the blank was estimated in a study. The test procedure involved the injection of the mobile phase. Figure 4 shows the chromatogram. Placebo and blank interference with Canagliflozin.

Figure 4: A typical HPLC chromatogram showing the no interference of blank



Precision

Precision is the measure or the extent to which a given analytical method can be exactly replicated under the normal operational circumstances and in most cases it is presented in the form of % relative standard deviation (% RSD) of statistically significant samples.

Method Precision

Analytical Method Precision The proximity of the replicate outcomes of the analysis of a single homogenous sample measures the accuracy of an analytical methodology [10]. In precision, it was quantified at various degrees such as method precision, system precision, intraday precision and interday precision in this study.

An assessment of the accuracy of the developed method was performed through the six replicate injections of the 100 % test concentration. Precision at the intraday level was determined by comparing the sample today and interday precision evaluated by comparing the analysis taken on two consecutive days. The accuracy was assessed by computation of standard deviation and % RSD of analyte response. Table 3 shows the results of intra- and inter-day precision.

Table 3: Data for repeatability of Canagliflozin

Method Precision (Inter &Intra Day)	
100.2	100.2
99.8	100.2
100.1	99.6
100.2	99.4
Overall average	100.1
Over all standard deviation	0.32
Over all % RSD	0.3

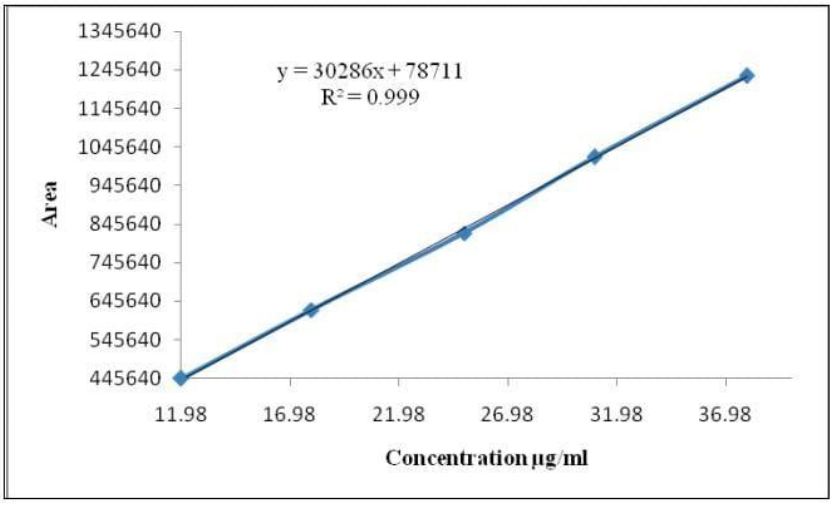
Linearity and Range

Linearity

Canagliflozin standard curve was plotted in the concentration range between 12-40 40 ug/ml. Linearity of the method was examined by linear regression. The slope, intercept and correlation coefficient (r^2) of the standard curve was determined which is shown in Figure 6.06 (in case of Canagliflozin) to show the linearity of the proposed method.

The HPLC system was injected with standard solutions, which varied between 50 and 150% of the standard solutions, with Canagliflozin. A linearity graph was used at the given range of concentration. Table 6.09 contains the data of linearity of Canagliflozin and Figure 6.06 shows a linearity curve.

Figure 5: Linearity curve of Canagliflozin



Acceptance criteria

a. The correlation coefficient (r2) must be NLT 0.997.
b. The RSD of replicate injections for lower and upper-level concentrations should not be more than 2.0 %.

LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found through the following formula:

LOD = 3.3 σ/S and LOQ = 10 σ/S,

where σ is the standard deviation of the responses and S is the slope of the calibration curves [11]. In this method, σ is the mean of the standard deviations of the y-intercepts from the three calibration curves, and S is the mean of the slopes of these calibration curves. The results are provided in Table 6.09.

Method Accuracy

Accuracy of an analytical procedure is used to describe the closeness between the observed value and

the accepted true or reference value. Chromatogram of the sample in concentration 50, 100 and 150% was obtained and shown in Table 4.

Table 4: Recovery studies of Canagliflozin proposed method.

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	99.1 -100.2	0.1	0.6
100	100.3-100.4	0.4	
150	100.8-100.8	0.5	

Robustness

Robustness The capability of a method being insensitive to small, intentional changes in parameters, and gives some idea of how the method will behave in practice. The chromatographic system was injected using the given conditions in the method with a standard solution [8, 9]. It was again injected to the same standard but one parameter was changed at a time and the remaining parameters were kept constant. Method robustness was also determined by observing the capability of the method to be insensitive to slight variations in several parameters like the%age of the organic material, pH of the mobile phase, the level of the mobile phase buffers, temperature, volume of injection, and the flow rate. The 2 nm change in the detection wavelength, 0.2 mL/min change in flow rate and 5% change in the organic phase were tested separately. Standards injected using the faster conditions gave a set of system suitability data that could be compared to those correspondingly generated under the normal method conditions. Table 5 shows the results.

Table 5: Robustness studies for Canagliflozin by proposed method.

Parameters		%RSD
		Canagliflozin
Wavelength ±2	223 nm	0.48
	227 nm	0.43
Flow Rate mL /min	1.0 mL/min	0.52
	1.4 mL.min	0.76

Table 6: Assay of marketed samples for Canagliflozin by proposed method

Drug	Amount Claimed in mg per Tablet	Estimated Amount in mg/tablet	% Assay
Canagliflozin	20	20.21	100.04

RESULT AND DISCUSSION

RESULTS

The RP-HPLC procedure of the determination of Canagliflozin was logically optimized in terms of the maximum resolution, reproducibility and analysis precision. A set of experiment was carried out to determine how the different chromatographic variables such as column chemistry, solvent type and strength, mobile phase composition, and flow rate will affect Canagliflozin separation and its detection. The optimum condition developed in a methodical test way resulted in sharp symmetric peaks whose retention time was reproducible with minimal interference of the formulation excipients.

Chromatographic Optimization

In the optimization of the method, several pairs of aqueous and organic mobile phases were tried, moreover, the type of the buffer, its pH, and % components of organic solvent had variations. A mobile phase consisting of acetonitrile and ammonium acetate buffer (pH 4.0) in ratio of 40:60 (v/v) gave the most best chromatographic performance. The composition resulted in a clear Canagliflozin peak with minimal baseline noise and achieved the best resolution possible due to impurities. With these parameters, the retention time of Canagliflozin was noted to be 2.85 minutes which made it possible to analyze within a short time, and not affect peak resolution. The chosen parameters provided a very good peak symmetry, which revealed effective interaction among analyte, stationary phase, and mobile phase components.

System Suitability Studies

Tests involving system suitability were also carried out to determine whether the chromatographic system was optimally in place before analysis. The acquired data showed high efficiency of the column which was confirmed by the number of theoretical plates that was more than 2000. The tailing factor always had a value smaller than 2, proving that there was good peak symmetry and no over broadening of peaks. The

reproducibility of the system in the optimized conditions was further proven because the retention time was reproducible between replicate injections. All these observations concluded that the RP-HPLC system was tuned within acceptable ranges of analytical limits and suitable to the proposed analysis of Canagliflozin.

Specificity

Specificity testing was done to ensure that the method could determine Canagliflozin in the presence of other components of the formulation in an unequivocal manner. Injected blank mobile phases did not display any peak at the retentive period of Canagliflozin. Injections of standard Canagliflozin solutions and marketed formulation samples demonstrated a single, sharp peak at 2.85 minutes free of pricking excipients, degradants or other samples components. The specificity of the developed method was established since chromatographic profiles showed that Canagliflozin is separated at the baseline position with all other peaks that emerged during analysis.

Linearity

Linearity of the procedure was evaluated using standard solutions of Canagliflozin at the concentration range of 12-40 µg/mL. Calibration curves were plotted by drawing curves between the area of peaks and corresponding concentrations. Regression analysis yielded a correlation coefficient (R 2) of 0.999, equivalent to a close direct proportionality relationship between the concentration and peak response over the ranges analyzed. Replicate calibration runs shared common values with regard to the slope and intercept, indicating high reliability of quantitation in this range. The statistics confirmed that the acquired RP-HPLC method has a good linearity and can be used to perform quantitative analysis.

Accuracy

Recovery studies using standard addition method were used to assess accuracy. Known concentrations of Canagliflozin standard were added at three points within the analysts working range to pre-analysed samples. Means of the % recoveries averaged between 98.9 and 101.0%, and the relative standard deviation (RSD) was low at all the concentration points tested. These findings validated the method to precisely estimate the actual quantity of Canagliflozin within bulk drug sample as well as formulated sample with low-level systematic bias.

Sensitivity: Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on standard deviation of the response and the slope of the calibration curve and provided the sensitivity of the method. LOD was found to be 0.34 µg/mL and the LOQ as 1.03 µg/mL. These values testify to the fact that the method is capable of measuring very low concentrations of Canagliflozin with high reliability, thus is applicable in analysis of traces and in stability studies where very low levels of detection are vital.

Precision

Accuracy of the procedure was tested under intraday condition and interday condition. To determine the day-to-day accuracy, injections were replicated at three concentrations per day on the same day and the results obtained had an RSD of 0.435%. To conclude on interday precision, I repeated the procedure three days morning to make 3 and found the RSD of 1.033%. The results of both values (2 samples) significantly satisfied the minimum acceptable value limit of 2%, thus, asserting a very high repeated and reproducible analytical test under different conditions of the activity.

Robustness

The stability of the method was assessed through the application of a small (but intentional) change in the critical chromatographic variables, namely the flow rate, the composition of the mobile phase and the detection wavelength. These alterations were meant to simulate possible change that may take place in the everyday laboratory application. In all modified conditions, retention time and the peak area of Canagliflozin was constant and RSD did not fall in between 2%. This showed that the method does not show much influence with minor operational modifications hence guaranteeing consistency in a normal application.

DISCUSSION

The RP-HPLC method developed to determine Canagliflozin is very effective as it gives good precise, accurate and reliable results [12]. Optimization of the method was essential to be sure that the conditions in which the method was run in terms of mobile phase composition and column would enable separation and accurate quantification of Canagliflozin to be clear. The selection of mobile phase acetonitrile and Ammonium acetate buffer (pH 4.0) at 40:60 v/v were an ideal choice to separate the peaks of Canagliflozin, and the retention time of 2.85 minutes was quite adequate with respect to the proposed purpose. Linearity analysis also supported the effectiveness of the methodology to some extent [13], given that the correlation coefficient was 0.999, implying that the relationship between the concentration of Canagliflozin and peak area was extremely linear. The sensitivity of the method was proved by the calculation of both the LOD and the LOQ that were found equal to 0.34 µg/mL and 1.03 µg/mL, respectively.

In robustness study, where the capability to be not influenced by minor alterations in experiment

conditions was assessed [14], it has been found that the method is highly robust. The findings ascertained the fact that the technique is forgiving to slight alterations in flow rate, composition of mobile phase and detection wavelength without affecting the slowness and exactness of examination. Lastly, the procedure worked effectively on the investigation of commercial Canagliflozin formulations. The average value of recovery that was 100.14% showed that the procedure can be of use in investigating an actual sample, thus justifies its accessibility in a day to day quality control laboratory used in pharmaceutical formulations.

CONCLUSION

The method of RP-HPLC developed in this work is simple, rapid, precise, accurate, sensitive, and robust and provides a validated procedure of the determination of Canagliflozin in bulk and finished pharmaceutical products. It provides high validation results, is stable to small changes in the analysis variables and has been demonstrated as applicable to commercial formulations, making it a useful tool in routine pharmaceutical analysis and stability testing. The efficiency and reliability of the method makes it suitable for use in industrial quality guarantee schemes and compliance with regulatory requirements.

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