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Analytical method development and validation for the simultaneous estimation of canagliflozin by using rp-hplc technique

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ABSTRACT

A simple and selective LC method is described for the determination of CANAGLIFLOZIN dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Methanol:ACN:H_2O (30:50:20v/v/v), with detection of 250 nm. Linearity was observed in the range 20-60 µg /ml for CANAGLIFLOZIN(r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2.

Keywords: Canagliflozin, chromatographic, linearity

INTRODUCTION

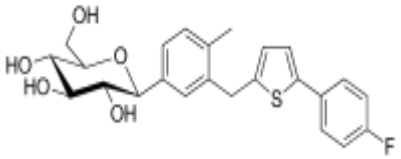
A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals and manufactured exclusively in accordance with the formulae mentioned in authoritative books.

Qualitative analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in each sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half-life etc.Quantitative analysis techniques are mainly used to determine the amount or concentration of analyte in a sample and expressed as a numerical value in appropriate units.

INTRODUCTION TO DRUG

Canagliflozin belongs to a new class of anti-diabetic drugs that works by inhibiting the sodium-glucose transport protein (SGLT2). This transport protein is found in the kidney and is responsible for reabsorbing glucose that has been filtered. FDA approved on March 29, 2013.

Structure:



IupacName: (2S,3R,4R,5S,6R)-2-(3-{[5-(4-fluorophenyl)thiophen-2-yl]methyl}-4-methylphenyl)-6-(hydroxymethyl)oxane-3,4,5-triol

Chemical Formula: $C_{24}H_{25}FO_5S$

Molecular Weight: 444.516

AIM

To develop new RP HPLC method for the estimation of CANAGLIFLOZIN pharmaceutical dosage form.

MATERIALS AND METHODS

Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC	Shimadzu(LC 20 AT VP)
HPLC	Agilent 1200 series
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	INERTSILcolumn,C18(150x4.6 ID) 5µm

Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen ortho Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate	AR Grade
Tetra Hydro Furan	AR Grade
Dipotassium hydrogen phosphate	AR Grade
Triethyl amine	HPLC Grade
Orthophosphoric acid	HPLC Grade

Drug used

Canagliflozin	Gift Samples obtained from Chandra labs, Hyd.
Canagliflozin (INVOKANA- 100mg)	Obtained from local pharmacy

METHOD DEVELOPMENT AND VALIDATION

Introduction to Method Development

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias.

Method Development Using HPLC

In method development, an attempt to select the best chromatographic conditions like the best column, the best mobile phase, the detection wavelength etc. to be used for routine analysis of any drug is done. For the method development by HPLC method some information about the sample is very essential.

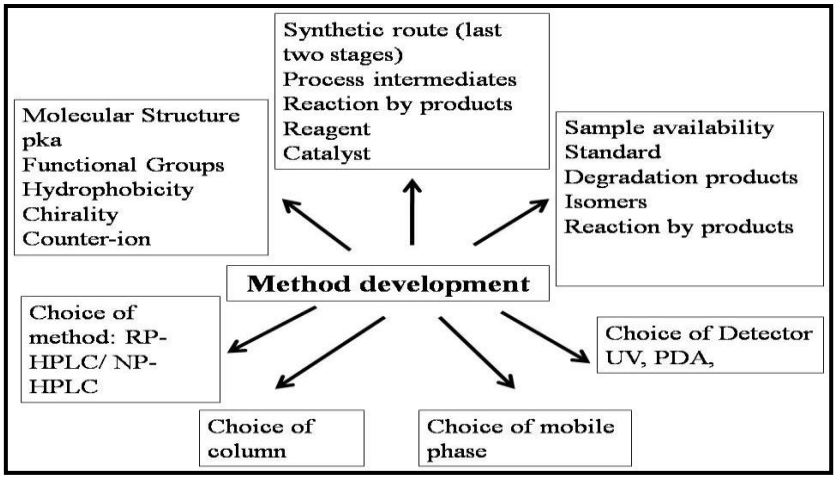


Fig 1: Outline of the process involved in method development

Method Validation (ICH Guidelines)

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy should be established across the specified range of the analytical procedure.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Specificity
Specificity is the ability to assess accurately the analyte in the presence of components which may be expected to be present in the sample matrix. Typically, these might include impurities, degradants, matrix, etc. it is a measure of the degree of interference from such other things such as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to a single component only.

Limit of Detection (LOD)
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. It is a limit test that specifies whether or not an analyte is above or below a certain value.

Limit of Quantitation (LOQ)
The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

Linearity and Range
Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Range is the (inclusive) interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the method.

Robustness
Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated varying method parameters such as percent organic solvent, pH, ionic strength, or temperature and determining the effect (if any) on the results of the method.

System Suitability
System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as a whole.

RESULTS AND DISCUSSION
Canagliflozin
It is slightly soluble in water. It is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol.

Determination of Working Wavelength (λmax)
In estimation of drug wavelength maxima is used.

Preparation of standard stock solution of Canagliflozin
10mg of Canagliflozin was weighed and transferred in to 25ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 40 µg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS
he wavelength of maximum absorption (λ_{max}) of the drug, 40 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. λ_{max} was found to be 250nm for Canagliflozin.

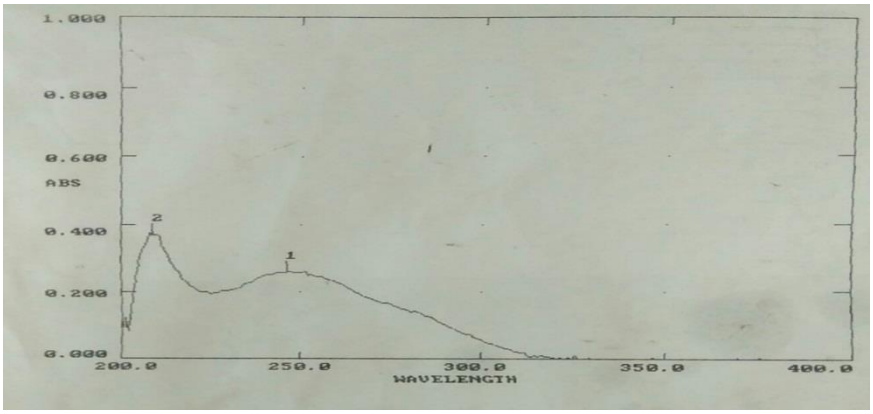


Fig 2: UV-VIS spectrum of Canagliflozin

METHOD DEVELOPMENT OF CANAGLIFLOZIN
Preparation of mixed standard solution
Weigh accurately 10mg of Canagliflozin in 25ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of Canagliflozin is prepared by diluting 1ml of Canagliflozin to 10ml with mobile phase. This solution is used for recording chromatogram.

Assay
Preparation of samples for Assay
Preparation of mixed standard solution
Weigh accurately 10mg of Canagliflozin in 25ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of Canagliflozin is prepared by diluting 1ml of Canagliflozin to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5 Tablets (each tablet contains 100mg of Canagliflozin) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of 400µg/ml were prepared by dissolving weight equivalent to 10mg of Canagliflozin dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 25ml with mobile phase. Further dilutions are prepared in 5 replicates of 40µg/ml of Canagliflozin was made by adding 1ml of stock solution to 10 ml of mobile phase. The amount of Canagliflozin present in the taken dosage form was found to be 99.52%.

VALIDATION

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The % RSD for the retention times of Canagliflozin Peaks from 5 replicate injections of each Standard solution should be not more than 2.0 %. The number of theoretical plates (N) for Canagliflozin peaks is not less than 2000. The % RSD for the retention times and peak area of Canagliflozin were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of CANAGLIFLOZIN in 25ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 40µg/ml of CANAGLIFLOZIN is prepared by diluting 1ml of CANAGLIFLOZIN to 10ml with mobile phase.

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Linearity and range

Preparation of mixed standard solution

Weigh accurately 10mg of CANAGLIFLOZIN in 25 ml of volumetric flask and from this, 1ml dissolve in 10ml of mobile phase and make up the volume with mobile phase. The relationship between the concentration of CANAGLIFLOZIN and area of CANAGLIFLOZIN should be linear in the specified range and the correlation should not be less than 0.999.

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of CANAGLIFLOZIN is 0.999. The relationship between the concentration of CANAGLIFLOZIN and area of CANAGLIFLOZIN is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. The % recovery of CANAGLIFLOZIN should lie between 98% and 102%. The percentage mean recovery of CANAGLIFLOZIN is 99.89%.

Method precision

Prepared sample preparations of CANAGLIFLOZIN as per test method and injected 5 times in to the column. The % Relative standard deviation of Assay preparations of CANAGLIFLOZIN should be not more than 2.0%. Test results for CANAGLIFLOZIN are the %RSD of Assay results are within limits.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision. The system suitability should pass as per the test method at variable conditions. It was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst-to-analyst variation by performing the Assay by two different analysts. The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%. % RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

DISCUSSION

A simple and selective LC method is described for the determination of CANAGLIFLOZIN dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Methanol:ACN:H_2O (30:50:20v/v/v), with detection of 250 nm. Linearity was observed in the range 20-60 µg /ml for CANAGLIFLOZIN(r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All

statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Canagliflozin 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 industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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