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DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR THE DETERMINATION OF ANTIDIABETICS (CANAGLIFLOZIN AND METFORMIN) IN API AND PHARMACEUTICAL DOSAGE

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ABSTRACT

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Canagliflozin & Metformin API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Canagliflozin & Metformin indicated that the developed method is specific for the estimation of Canagliflozin & Metformin. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

Keywords: RP-HPLC, Canagliflozin, Metformin, method development, validation parameters

INTRODUCTION[1,2,3]

The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements of the method. Method development usually requires selecting the method requirements and deciding on what type of instrumentation to utilize and why.

There are several valid reasons for developing new methods of analysis:

- There may not be a suitable method for a particular analyte in the specific sample matrix.
- Existing methods may be too error, artifact, and/or contamination-prone, or they may be unreliable (have poor accuracy or precision).

Reversed phase mode is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non polar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and non polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are octa decyl silane (ODS) or C_{18}, C_8, C_4, etc., (in the order of increasing polarity of the stationary phase).

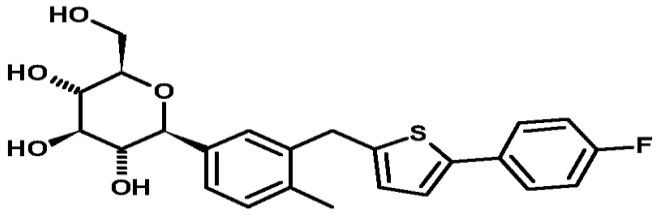
There may be a need for an alternative method to confirm, for legal or scientific reasons, analytical data originally obtained by existing methods.

DRUG PROFILE

CANAGLIFLOZIN

Description: 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.

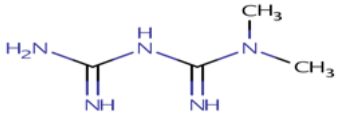
Structure :



Chemical formula : **$C_{24}H_{25}FO_5S$**
Molecular mass : **444.516 g/mol**
Physical appearance : Canagliflozin hemihydrate drug substance is a white to off white powder
Solubility : It is Soluble in DMSO (88 mg/ml at 25 °C), water (<1 mg/ml at 25 °C), ethanol (<1 mg/ml at 25 °C), and methanol.
Category : Antidiabetic drug.
Mechanism of action : Sodium-glucose co-transporter 2 (SGLT2), expressed in the proximal renal tubules, is responsible for the majority of the reabsorption of filtered glucose from the tubular lumen. Canagliflozin is an inhibitor of SGLT2. By inhibiting SGLT2, canagliflozin reduces reabsorption of filtered glucose and lowers the renal threshold for glucose (RTG), and thereby increases urinary glucose excretion.

METFORMIN HCL

Structure:



Chemical Formula: $C_4H_{11}N_5$
Description: This compound belongs to the class of organic compounds known as biguanides. These are organic compounds containing two N-linked guanidines.
Solubility: Slightly soluble in ethanol and also slightly soluble in alcohol, and insoluble in substances such as acetone, ether, and chloroform.
Indication: For use as an adjunct to diet and exercise in adult patients (18 years and older) with NIDDM. May also be used for the management of metabolic and reproductive abnormalities associated with polycystic ovary syndrome (PCOS).

MATERIALS AND INSTRUMENTS

Drug Sample: Were obtained from Canagliflozin and .Metformin - Syncorp Clincare Technologies Pvt. Ltd., Hyderabad.
Chemicals and Solvents Used:1.Water –HPLC grade, 2. Methanol – HPLC grade 3. Acetonitrile - HPLC grade, 4. Tri Ethyl Amine -HPLC grade, 5. Potassium Dihydrogen Phosphate, 6. Dipotassium Hydrogen Phosphate
All the above chemicals and solvents were supplied by S.D. Fine Chemicals Ltd., India; Qualigens Fine Chemicals Ltd., Mumbai, India and Ranbaxy Chemicals Ltd., New Delhi, India.
Instruments Used:1. Shimadzu ATY224 Digital Electronic Balance 2. Wensar Pvt. Limited, India, pH meter, 3. Vacuum Pump Filter (PCI) 4. Life Care Sonicator5. Elico SL 210 Double beam UV/Vis Spectrophotometer 6. Hitachi Elite Lachome HP LC model Containing Pump: L2130, Detector: UV, L2400, Software: D2000 Elite.

METHOD DEVELOPMENT

HPLC Method Development: The parameters for the development were as follows:
1) Selection of detection Wavelength, 2) Selection and optimization of mobile phase, 3) Study of system suitability parameters, 4) Application of the method to the drug in pure form.
Validation Of Proposed Method: The parameters for the validation were carried out as follows:
1) System suitability 2) Linearity 3) Accuracy 4) Precision 5) Robustness 6) Ruggedness 7) Limit of detection 8) Limit of quantitation

Selection of chromatographic method for separation

Reverse phase chromatographic technique was selected since the drug is polar in nature.

HPLC METHOD DEVELOPMENT

Selection of wavelength

Selectivity of HPLC method that uses UV detector depends on proper selection of Wavelength. A wavelength which gives good response for the drug to be detected should be selected. From the UV spectra 238 nm was selected as the wavelength for Metformin & 275nm for Canagliflozin. The λ max selected for work was 238nm.

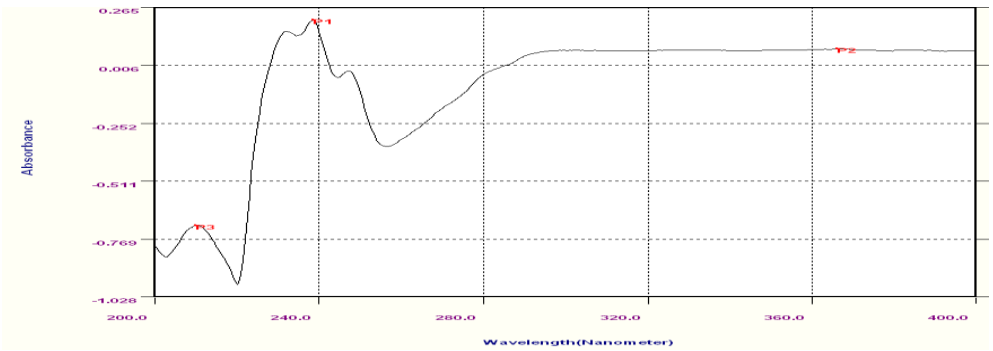


Fig 1: UV-Spectrum for Metformin (238nm)

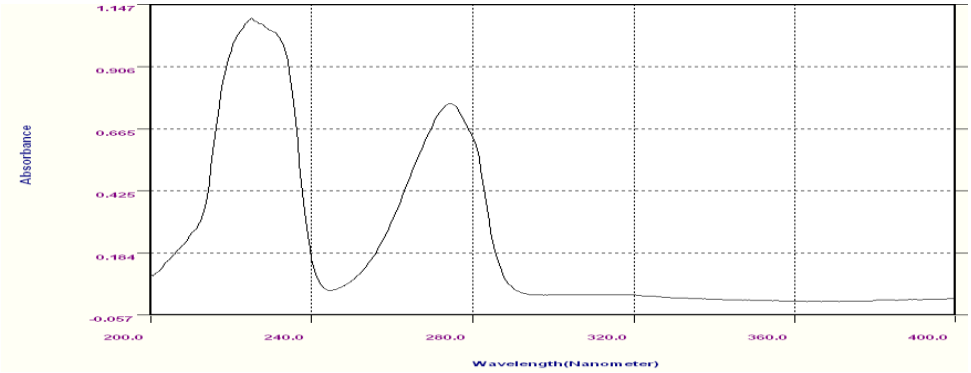


Fig 2: UV-Spectrum for Canagliflozin (275nm)

Selection of Mobile phase

Solvent selectivity (solvent type), solvent strength (percentage of organic solvent in the mobile phase), From the various mobile phase tried, mobile phase containing 0.01M Phosphate buffer (pH-3.6): Methanol (35:65) %v/v was selected, since it gave sharp peak with symmetry within limits and significant retention time.

Reagent and Pharmaceutical preparations

An analytically pure sample of Canagliflozin and Metformin was procured as gift sample from Syncorp Clincare Technologies Pvt. Ltd. (Hyderabad, India). HPLC grade Acetonitrile and HPLC grade Water procured from E. Merck (Ahmadabad). Orthophosphoric acid and Tri ethyl amine (AR grade, purity 99.5 %), dihydrogen potassium phosphate and Dipotassium hydrogen phosphate (AR grade) was procured from Qualigens.

Preparation of Phosphate buffer

About 1.36089 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. The pH was adjusted to 3.60 with diluted Orthophosphoric acid.

Preparation of mobile phase

650ml of Methanol and 350ml of Buffer are mixed. The solution was degassed in an ultrasonic water bath for 10 minutes and filtered through 0.45 µ filter under vacuum.

Preparation of stock solution

A stock solution of Canagliflozin was prepared by accurately weighing 25 mg of drug, transferring to 25 ml volumetric flask, Add about 15 ml of mobile phase and sonicated to dissolve it completely and make up volume up to mark with mobile phase (1000µg/ml). A stock solution of Metformin was prepared by accurately weighing 25 mg of drug, transferring to 25 ml volumetric flask, Add about 15 ml of mobile phase and sonicated to dissolve it completely and make up volume up to mark with mobile phase (1000µg/ml). From above stock solution final concentrations should be prepared. The final concentrations for Canagliflozin is 2 ppm & Metformin for 20 ppm

Chromatographic experiment

Different chromatographic conditions were tried to optimize the method, optimized method is following:

Table 1. Optimized Chromatographic Conditions

Stationary phase	C_{18} Hypersil, 150 x 4.6, particle size 3µ
Injector	Rheodyne
Mobile phase	Phosphate Buffer : Methanol (60:40%v/v) pH: 3.6
Flow rate	1ml/min
Injection volume	20µl
Wavelength	257nm
Temperature	250C
Run time	10 min

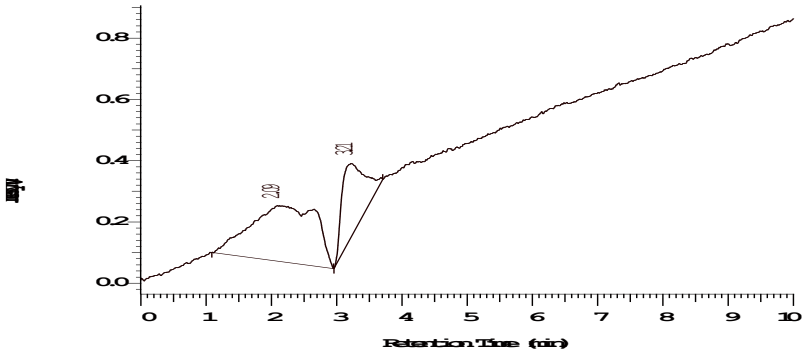


Fig 3: Chromatogram for Blank Solution

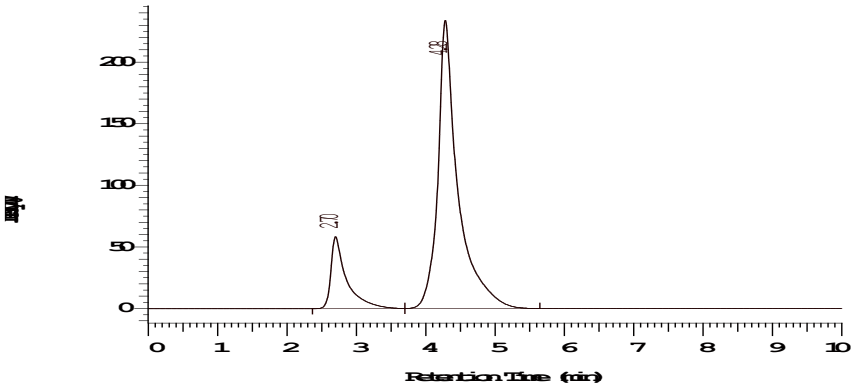


Fig 4: Chromatogram for Optimized Chromatographic Method

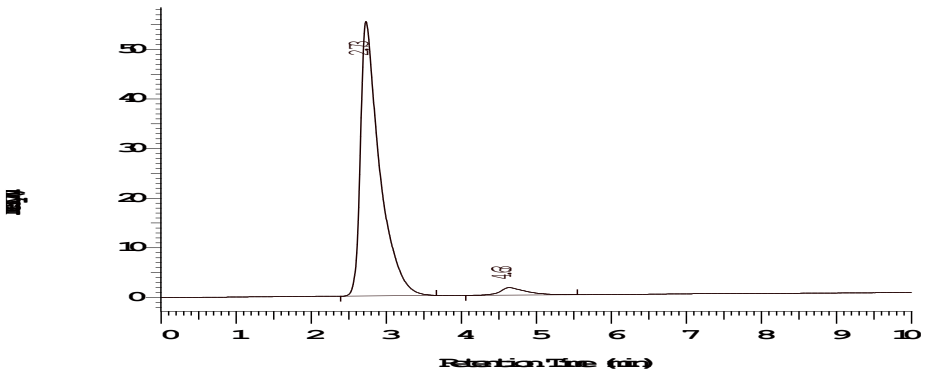


Fig 5: Chromatogram for Canagliflozin

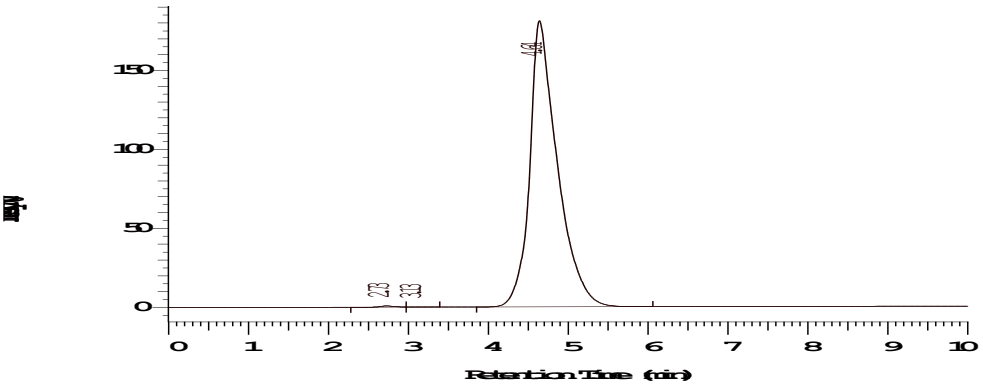


Fig 6: Chromatogram for Metformin

Assay of Metformin & Canagliflozin In Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (3.5 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table 2.

Table 2: Recovery Data for estimation Metformin and Canagliflozin

Brand name of Tablets (Invokamet Tab (Janssen Pharmaceuticals, Inc.)	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Assay + % RSD
Canagliflozin	50	49.895 (± 0.07)	99.864 (± 0.49)
Metformin	500	499.974 (± 0.07)	99.876 (± 0.46)

The amount of drugs in Invokamet tablet was found to be 99.876 (±0.46) mg/tab for Metformin and 99.864 (±0.49) mg/tab for Canagliflozin.

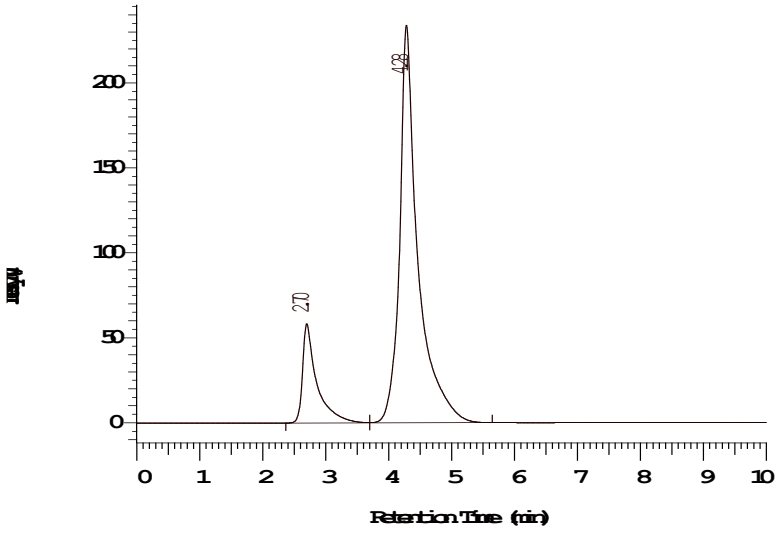


Fig 7: Sample Chromatogram-1

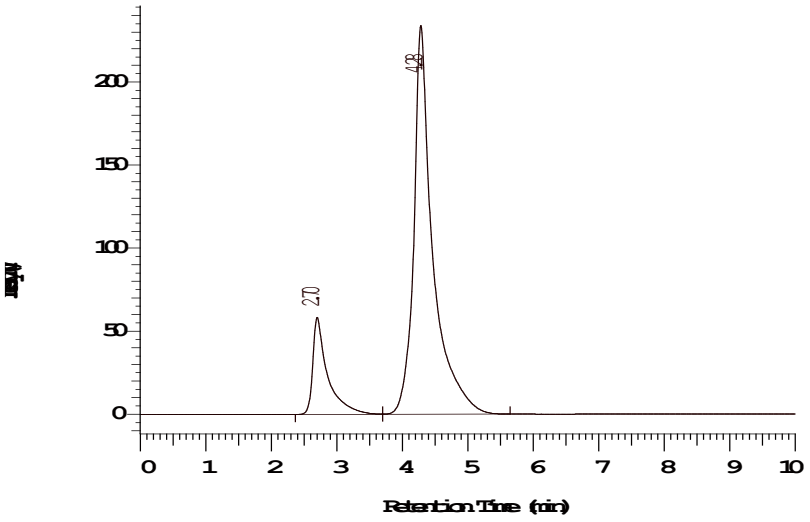


Fig 8: Sample Chromatogram-2

STABILITY STUDIES

Following protocol was strictly adhered to for forced degradation of Canagliflozin and Metformin Active Pharmaceutical Ingredient (API). The API (Canagliflozin and Metformin) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after along time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation.

Results of forced degradation studies

The results of the stress studies indicated the specificity of the method that has been developed. Canagliflozin and Metformin were stable in oxidation and thermal stress (wet heat) conditions. The result of forced degradation studies are given in the following Table 3.

Table 3: Results of forced degradation studies of Canagliflozin and Metformin API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	86.44	13.56	100.0
Basic Hydrolysis (0.I M NaOH)	24Hrs.	97.37	2.63	100.0
Wet heat	24Hrs.	87.06	12.94	100.0
UV (254nm)	24Hrs.	61.33	38.67	100.0
3 % Hydrogen peroxide	24Hrs.	89.07	10.93	100.0

RESULT & DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Canagliflozin and Metformin different chromatographic conditions were applied & the results observed are presented in the thesis. In case of RP-HPLC various columns are available, but here C_{18} Hypersil, 150 x 4.6, particle size 3µ column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). Canagliflozin was found to be Soluble in DMSO (88 mg/ml at 25 °C), water (<1 mg/ml at 25 °C), ethanol (<1 mg/ml at 25 °C), and methanol. Metformin was found to be freely soluble in water, Methanol slightly soluble in ethanol and also slightly soluble in alcohol, and insoluble in substances such as acetone, ether, and chloroform. Detection wavelength was selected after scanning the standard solution of drug over 200 to 800nm. From the U.V spectrum of Canagliflozin and Metformin it is evident that most of the HPLC work can be accomplished in the wavelength range of 215-290 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Canagliflozin and Metformin in different formulations.

CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Canagliflozin & Metformin API. Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Canagliflozin & Metformin indicated that the developed method is specific for the estimation of Canagliflozin & Metformin. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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