Analytical Method Development And Validation Of Canagliflozin Hemihydrate In Bulk And Pharmaceutical Dosage forms

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ABSTRACT

New selective and sensitive high-performance liquid chromatography (HPLC) method with UV detection at 260 nm for the quantification of canagliflozin hemihydrates (CFH) in pharmaceutical dosage form. Chromatographic separation was achieved on a Kromasil C18 (100 mm x 4.6 mm 5 µm) column kept at 30°C with an isocratic mixture of mobile phase (acetonitrile: water ph 2.5 adjusted with orthophosphoric acid , 50 : 50 v/v) at a flow rate of 1.0mL/min. The method was validated to fulfill International Conference on Harmonization (ICH) requirements and this validation included specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness. The calibration curve was linear over the concentration range from 10 to 200 µg/ml. All results were acceptable and this confirmed that the method issue table forits intended use in routine quality control and assay of drugs.

Keywords:. Method Validation, HPLC, Canagliflozin, Diabetes drug

1. INTRODUCTION

Canagliflozinhemihydrate1S)-1,5-anhydro-1-[3-[[5-(4-fluorophenyl)-2-thienyl]methyl]-4methyl pheny l]-D-glucitol hemihydrates a medication used to treat type 2 diabetes.[1] It was approved by the U.S.[1].it was the 192nd most commonly prescribed medication in the United States. [2,3] Food and Drug Administration (FDA) on Mar 29, 2013, then approved by European Medicine Agency (EMA) on Nov 15, 2013, and approved by Pharmaceuticals and Medical Devices Agency of Japan (PMDA) on Jul 4, 2014. Canagliflozin is an inhibitor of subtype-2 sodium-glucose transport protein (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney (SGLT1 being responsible for the remaining 10%). Anagliflozin reduces reabsorption of filtered glucose and lowers the renal threshold for glucose (RTG), and thereby increases urinary glucose excretion (UGE). It is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. Analytical methods keep on updating with time as per the requirements so as to develop a simple, reliable, cost effective, reproducible and above all a method bearing a high level of accuracy and precision. Our study aimed to develop a rapid, robust, selective, sensitive, and precise HPLC method for the determination of DS. The assay method was validated using by USP 26 (United States Pharmacopeia Convention, 2003) or by the ICH guidelines (CPMP/ICH/381/95, 1994). The linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ) and used for in determination of drug content of the DS in different pharmaceutical commercial products. The review of literature reveals that methods including LC/MS[4], UV[5], HPTLC[6], HPLC[7], Volta metric methods[8] etc. have been reported for the estimation of drugs individually/simultaneously mixture in biological specimen but no method has been reported so far in literature for estimation of canagliflozin hemihydrate solution dosage form by RP-HPLC method with UV detector.

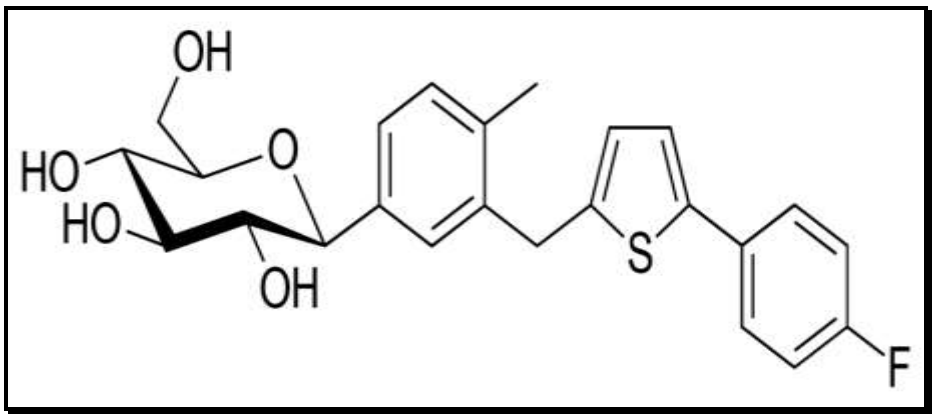


Fig. 1 Canagliflozin

2. MATERIALS AND METHODS

Instruments and reagents: A Shimadzu-1800 UV/Vis double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements Distilled water was used as solvent for dilution. Authentic drug sample of canagliflozin hemihydrate was given as a gift sample by Aurobindo pharmaceuticals (Hyderabad, India) inhibitor of subtype 2 sodium-glucose transport proteins (Invokana as Brand name), Janssen cilag Pharmaceutical Limited,Mumbai, India was procured from a local pharmacy with labelled amount (100mg/ml).

Preparation of working standard drug solution: The stock solution (1 mg/ml) of Canagliflozin Hemihydrate (CFH) was prepared by dissolving 100 mg of it in 100 ml of methanol. A portion of this stock solution was diluted stepwise with the ethanol to obtain final concentration of 1000 µg/ml and the resulting solution was used as working standard solution.

Analysis of marketed formulation: An accurately weighed amount of formulation equivalent to 100 mg of drug was dissolved in 20 ml of ethanol, sonicated for 5 min and filtered. The filtrate was further diluted to 100 ml with ethanol to get 1 mg/ml solution of drug in formulations. One ml of this solution was furthered diluted to 25 ml to get 40 µg/ml solution. The absorbance of the solution was determined 260nm . The quantity of the drug was computed from the Beer's law plot of the standard drug in ethanol.

Calibration curve:

Method 1

To a series of 25 ml calibrated tubes, aliquots of standard CFH solution (10.0 –100.0 ml, 20µg/ml) was transferred and then solutions of Fe (III)(1.5ml) and *o*-phenanthilein 2.0ml was added successively. The total volume in each flask was brought to 10.0 ml with distilled water and heated for 30 min in a water bath. After cooling to room temperature, 2.0 ml of o-phosphoric acid was added, the volume in each tube was made up to the mark with distilled water. The absorbance of the colored complex solution was measured after 5 min at λ_{max} 510 nm against a reagent blank prepared similarly. The content of the drug was calculated from its calibration graph.

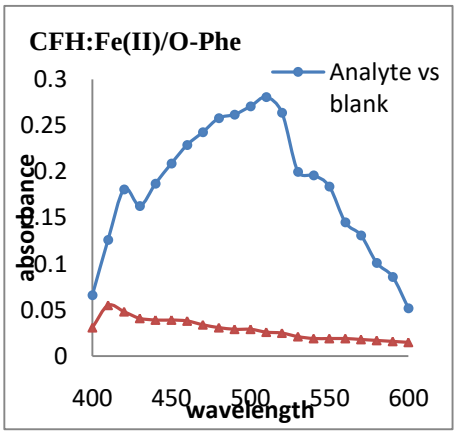


Fig:1 Absorption spectra

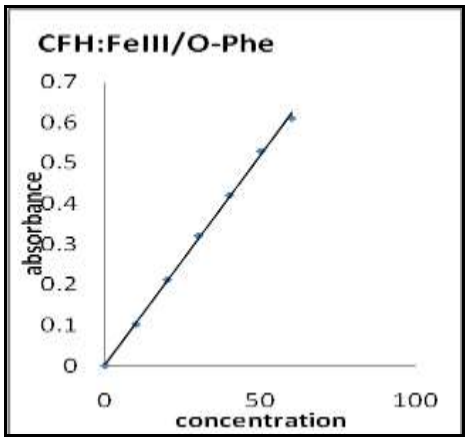
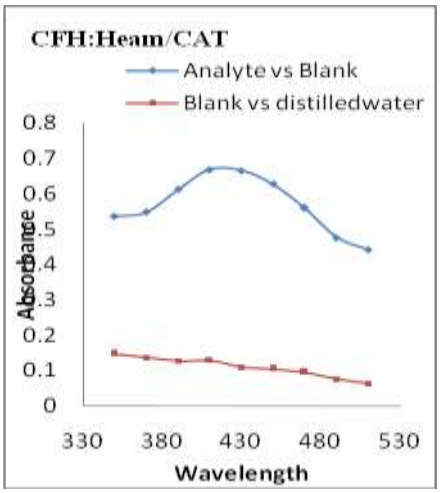


Fig :2 Beer's law plot of CFH/Fe(III)/o-pheCFH:$FeCl_3$/O-Phe

Method 2

To a series of 25 ml graduated test tubes, 1 ml each of Heamatoxylin and chloramines-T, 15 ml of buffer (pH 7.0) solutions were added successively. The mixture was kept aside for 20 min. Then added aliquots of CFH within the Beer's law limits (1.0- 5.0 ml, 500µg/ml) and kept in a water-bath at 70ºC for 20 min. The test tubes were removed from the water bath, cooled to room temperature. The contents in tube were diluted to 25 ml with distilled water and the absorbance was read at λ_{max} 410nm within the stability period (5 min). The amount of CFH wad deduced from its standard calibration curve



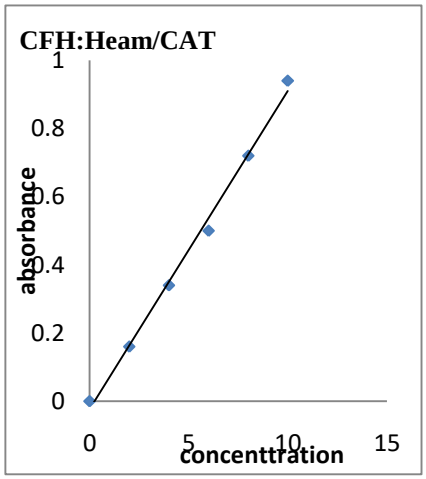


Fig.3: Absorption spectra of CFH Fig.4: Beer's law plot of CFH:Heam/CATCFH: Heam /CAT

Validation of the method: All these methods were validated according to ICH guidelines 8 in terms of linearity, precision, accuracy and ruggedness parameters.

Linearity: The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The linearity was found to be 3-24 µg/ml for zero, first and second order derivative spectrophotometric methods. Optimum conditions, Optical characteristics and Statistical data of the Regression equation in UV method are given in **Table1.**

Parameter	**M_1**	M_2
Λ_{max}(nm)	510	410
Beer's law limits (µg ml^{-1})	5.0-80.0	2.0-16.0
Detection limits(µg ml^{-1})	1.12	0.0677
Molar Absorptivity (1 mole cm^{-1})	6.65×10^4	$7.793x10^5$
Sandell's sensitivity (µg ml^{-2}/0.001 absorbance unit)	0.0069	0.00586
Regression equation(Y=a+bC)Slope (b)	0.010	0.093
Standard deviation of slope (S_b)	0.001	0.0027
Intercept (a)	0.003	0.023
Standard deviation of intercept(S_a)	0.0037	0.0021
Standard deviation of estimation (S_e)	0.0033	0.0022
Correlation coefficient (r^2)	0.998	0.995
Relative standard deviation (%)	1.334	0.4739
% Range of error (Confidence limits) 0.05 level	1.1406	0.4975
0.01 level	2.216	0.4975
% Error in bulk samples	0.277	0.742

Table 1: Optical and regression characteristics, precision and accuracy f the proposed methods for CFH

Accuracy: The accuracy of the method was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The values of standard deviation were satisfactory and the recovery studies were close to 100% . Hence these methods can be useful in routine analysis of canagliflozin hemihydrate in bulk drug and formulations.

Ruggedness: Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. The results of ruggedness testing are reported intable.

Precision: The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV).The results of inter-day and intra-day were reported in the **Table 2** .

Sample	Amount taken (mg)	Amount found by proposed methods		Reference methods	Percentage recovery by proposed methods	
		M_1	M2		M_1	M2
Tablet I	100	97.09 ±1.79 F=1.94 t = 1.16	98.42± 1.2 F=1.328 t = 1.191	98.1± 0.28 F=1.6 t =1.58	98.54±0.29	98.1± 0.78
Tablet II	50	49.0± 0.57 F=1.94 t =0.88	49.01± 0.02 F=1.669 t =1.416	49.06± .78 F=1.6 t =1.58	98.25±0.89	98.06± 0.78

Table 2. Assay of CFH In Pharmaceutical Formulations

Liquid chromatography conditions :
The HPLC system consisted of Waters 1525 binary pump separation module (Waters, USA) fitted with C_{18} column (300 mm 4.6 mm). The auto sampler injection system (Waters 2707) used was a 10 µl sample loop. A Millipore Swinnex type filter (pore size = 0.45 µm) was obtained from Millipore (Bangalore, India). AWatersHPLCsystemequippedwithaWaters484variable UV absorbance detector and a Waters 2707 plus auto sampler was used. Waters 515 solvent delivery system was used to operate the gradient flow through a symmetry C_{18}column (100 mm x 4.6 mm 5 µm 3.5 1m spherical particles). Acetonitrile: Water (pH-2.5 adjusted with *ortho* phosphoric acid) (50: 50 % V/V) respectively as a mobile phase at a flow rate of12.0 mL/min and the run time was 2 min. Degassing was achieved via filtration through a 0.45m Millipore membrane filter and sonication for 10 min. The injection volume was 20 µlanddetectionwasat235 nm. The HPLC system was operated at. 30 °C. Data were collected with a Breeze Chromatography Manager Data Collection System. A daily standard calibration curve (6stanards ranging from 10 to 200 µg/ml was prepared to determine the unknown CFH concentration.

Preparation of stock solutions
The stock standard solution of CFH was prepared in methanol at a concentration of 10.01 mg/mL Different working standard solutions of CFH (10–200 µg/ml) were prepared by diluting of the above mentioned stock solution in pure ethanol and were stored at 4 °C.

Validation of diclofenac HPLC assay

The RP-HPLC method for CFH assay was validated in term of accuracy, reproducibility, linearity, specificity, LOD, LOQ, and robustness according to ICH Harmonized Tripartite Guidelines. Three standard calibration curves were prepared at different times (at least three months) to evaluate the linearity, precision, accuracy and stability.

Specificity

The specificity of the HPLC method was evaluated to ensure that there was no interference from the excipients present in the formulations. The specificity was studied by injecting the excipients.

System specificity

The system suitability was assessed by six replicate analyses of CFH at a concentration of 01g/ml. The acceptance criterion was ±2% for the percent relative standard deviation (%RSD) for the peak area and retention times for CFH.

Linearity and range

Linearity is the ability to obtain test results that are directly proportional to the concentration of the analyte. Linearity was determined by three injections of seven different CFH concentrations (10, 20, 80, 120, 160 and 200 µg/ml).The average peak areas were plotted against concentrations. Then linearity was evaluated using the calibration curve to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r^2) > 0.998 is considered as the evidence of an acceptable fit for the data to the regression line.

Accuracy

The accuracy of an analytical method expresses the nearness between the expected value and the value found. It is obtained by calculating the percent recovery (R%) of the analyte recovered. In this case, to evaluate the accuracy of the developed method, successive analysis (n=3) for three different concentrations (200ng/ ml, 120 ng/ml and 20 µg/ml) of standard CFH solution were performed using the developed method. The data of the experiment were statistically analyzed using the formula [%Recovery = (Recovered conc. /Injected conc.) 100] to study the recovery and validity of the developed method. The mean recovery should be within 90–110% to be accepted.

Precision

Precision of a measurable technique is the degree of agreement among individual tests, when the technique is applied repetitively to analyze multiple replicates in three different occasions. The intraday precision was assessed by analyzing the calibration curves of six replicates of different concentrations of CFH within the same day. The inter-day precision was determined by analyzing of six replicates of different concentrations of DS on three different days. The total precision of the method was expressed as the relative standard deviation (%RSD). In the current method development and validation protocol, precision was determined by six replicate analyses at a concentration of 120µg/mL of standard CFH solution using the developed method and % RSD ≤ 2% was accepted.

Limit of detection and limit of quantification

LOD is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy. These two parameters were calculated using the formula LOD = 3.3 S D/S and LOQ = 10 SD/S, where SD = standard deviation of response (peak area) and S = slope of the calibration curve.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions.

Analysis of a marketed formulation

100 mg invokana ten tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 100 mg invokana was transferred into a 100 mL volumetric flask containing 60 mL ethanol, sonicated for 30 min and diluted up to 100 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (**1**g/mL). Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 µm filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of **1**g/mL. 1 mL volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak are as were measured at 235nm and concentration sin the samples were determine dusing multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation. In vitro results were expressed as mean ± SD of at least three replicates. The % RSD was calculated for all values. Student's t-test was used to inspect the concentration difference at each day and one-way analysis of variance (ANOVA) was used to assess the reproducibility of the assay using IBMSPSS Statistics [9]. The level of confidence was 97.85%.

3. RESULTS AND DISCUSSION

Linearity: Each of these solutions (1895, 1327,954, 636 and 382 mg/L) were injected into HPLC under the given conditions and the peak area was recorded and a graph of detector response (peak area) versus concentration in mg/L was plotted. The values for slope (m), intercept (b) and the linear regression coefficient (R^2) were calculated**.** The detector response to varying concentrations of Canagliflozin Hemihydrate was found to be linear (R^2 = 1.000) in the range of 382 to 1895 mg/L. The plot of concentrations versus detector response along with the regression parameters was attached and the results are presented .

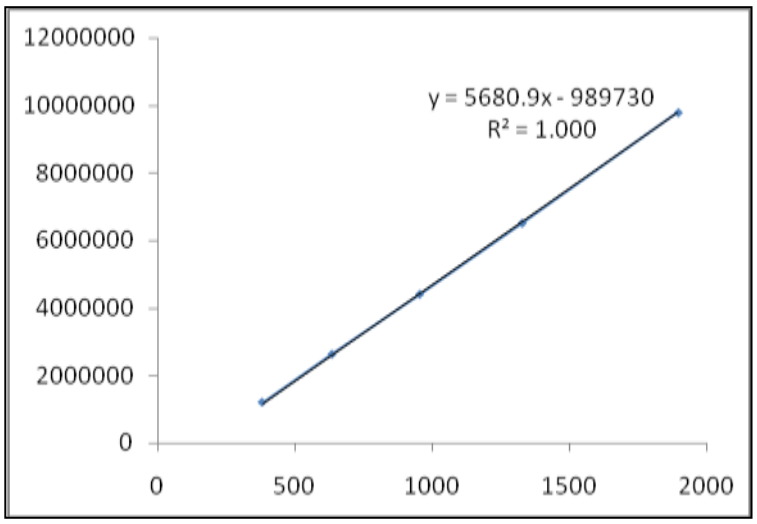


Fig 5 : Linearity curve of canagliflozin hemihydrate

Slope	5680.9
Correlation Co-efficient	1.000
Intercept	-989730

Precision: The precision of this method was calculated by injecting five replications of test item into High performance liquid Chromatography (HPLC) and the relative standard deviation was calculated. The precision (%RSD) 97.93±0.26 for canagliflozin Hemihydrate active ingredient content was also calculated for all five replications.

Accuracy : The above preparations were injected into HPLC and checked for accuracy (% Recovery is 100.37±1.18).The Accuracy was calculated by injecting five times of one replication of each fortified concentration in to HPLC

Active Ingredient Content: The above prepared Canagliflozin Hemihydrate standard solutions and sample solutions were injected into a High performance liquid chromatography (HPLC) by using the analytical conditions. Canagliflozin Hemihydrate active ingredient content, present in the sample was quantified by injecting reference standard and comparing the peak area of standard with the peak area of sample984.36±0.06

4. CONCLUSION

There were few reports on the visible spectrophotometric determination of CFH in the literature, but in the present investigation 2 spectrophotometric methods have been developed for CFH determination towards the following reagents such as Fe(II)/O-phe, Haematoxyline-CAT, The results presented above indicate that the proposed methods have good sensitivity, selectivity, precision and accuracy. Results of analysis of bulk form and formulations reveal that the proposed methods are suitable for the estimation of CFH in them, as the impurities and excipients present in them cause no interference virtually. The order of sensitivity among the methods M2> M1

Canagliflozin Hemihydrate was evaluated as per the guidelines of ICH 2. The method was validated for the determination of Active Ingredient Content in Canagliflozin Hemihydrate for the test item Canagliflozin Hemihydrate meets the acceptance criteria. The results obtained were within the specified limits The active ingredient content of the test Canagliflozin Hemihydrate was found to be 97.85%w/w) when the drug samples was analyzed by HPLC.

5. CONFLICTS OF INTEREST

The authors declare that there are no conflict of interest regarding the publication of this article.

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