EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 4
333-340
Year: 2018

ANALYTICAL METHODS FOR CANGLIFLOZIN: REVIEW

Deepali Kailas Kadam*[1], Dr. Ravindranath Bhanudas Saudagar[1], Dr. Sarvesh Kumar Paliwal[2] and Dr. Swapnil Sharma[2]

[1]KCT's R. G. Sapkal College of Pharmacy, Anjaneri, Dist. Nashik, Pin-422212 (Maharashtra).
[2] Banasthali Vidyapith Banasthali, Dist. Tonk, Pin-304022 (Rajasthan).

***Corresponding Author: Deepali Kailas Kadam**
KCT's R. G. Sapkal College of Pharmacy, Anjaneri, Dist. Nashik, Pin-422212 (Maharashtra).

Article Received on 09/02/2018 **Article Revised on 01/03/2018** **Article Accepted on 22/03/2018**

ABSTRACT
Analytical methods development and validation play important roles in the drug discovery as it is continuous and inter-dependent task associated with development and Manufacture of pharmaceuticals. Method development is the process of proving that an analytical method is acceptable for use to measure the concentration of an API in a specific compounded dosage form which allows simplified procedures to be employed to verify that an analysis procedure, accurately and consistently will deliver a reliable measurement of an active ingredient in a compounded preparation. An effective analytical method development and its validation can provide significant improvements in precision and a reduction in bias errors. It can further help to avoid costly and time consuming exercises. Canagliflozin, being a Sodium Glucose co-Transporter type 2 (SGLT2) Inhibitor, as a new class for treatment of Type 2 diabetes mellitus, offer a novel mechanism of action, which has been recently approved by USFDA for use in type 2 diabetes mellitus, either alone or in combination with other oral hypoglycemic agents and insulin. The aim of this review is to focus on comprehensive update different analytical methods developed for Canagliflozin in bulk and in pharmaceutical preparations either alone or in combination with other hypoglycemic agent.

KEYWORDS: Canagliflozin, HPLC, HPTLC, UV, LC-MS/MS, UPLC-MS/MS.

INTRODUCTION

Canagliflozin is available commercially as INVOKANA and chemically known as (2S,3R,4R,5S,6R)-2-{3-[5-(4-fluoro-phenyl)-thiophen-2-ylmethyl]-4-methyl-phenyl}-6- hydroxymethyltetrahydro-pyran-3,4,5-triol Figure (1).

Canagliflozin having empirical formula is $C_{24}H_{25}FO_5S$, molecular weight of 444.52 g/mol. It is white to off white solid with a melting point of 95-105°C. It is soluble in many organic solvents (methanol, Dimethyl sulfoxide) and insoluble in aqueous media. Canagliflozin is the first Sodium glucose co-transporter-2 (SGLT-2) inhibitor which was used for the treatment of patients with type 2 diabetes. Canagliflozin reduces reabsorption of filtered glucose by inhibiting Sodium-glucose cotransporter 2 (SGLT2) and lowers the renal threshold for glucose (RTG) and thereby increases urinary glucose excretion.

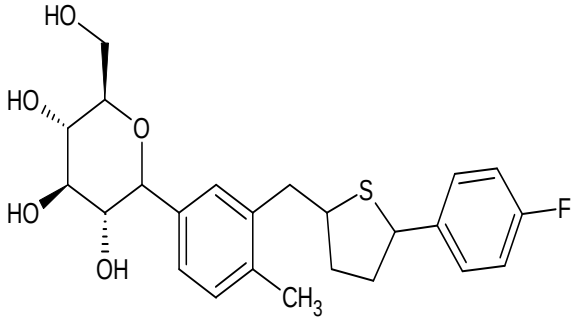

Figure (1)

Mechanism of action

Canagliflozin is an inhibitor of subtype 2 sodium glucose transport proteins (SGLT-2), which is responsible for at least 90% of renal glucose reabsorption (SGLT-1 being responsible for the remaining 10%). Blocking this transporter causes up to 119 grams of blood glucose per day to be eliminated through the urine, corresponding to 476 kilocalories. Additional water is eliminated by osmotic diuresis, resulting in a lowering of blood pressure. This mechanism is associated with a low risk of hypoglycaemia (too low blood glucose) compared to

other types of anti-diabetic drugs such as sulfonylurea derivatives and insulin.[1]

HPLC METHODS FOR DETERMINATION OF CANAGLIFLOZIN

Suneetha *et al.* has developed and validated a simple, specific, precise and accurate RPHPLC method for estimation of Canagliflozin in raw material and pharmaceutical dosage form. Chromatographic conditions consisted of a column (Hypersil BDS, C18 100 x 4.6 mm, 5µ) and a mobile phase composed of (0.1% ortho phosphoric buffer: acetonitrile (53:47), water and acetonitrile (50:50) as diluent in isocratic mode. The flow rate was 1.1ml/min and the detection wavelength was at 240 nm. The retention time (Rt) was around 3.3±0.2 min. The method was validated as per ICH guidelines. The assay method was linear within the range of 75-450 µg/ml with a Correlation coefficient (R^2) 0.9999. The percentage recovery of active pharmaceutical ingredient from tablet dosage form ranged 99.83-100.27%. The LOD and LOQ were 0.23µg/ml and 0.7µg/ml, respectively. Stress conditions of degradation in acidic, alkaline, peroxide, thermal and UV radiation were studied and found that Canagliflozin is sensitive to alkali degradation.[2]

Another study by Maddu *et al.* described a simple and sensitive RP-HPLC method for the determination of Canagliflozin in pharmaceutical dosage form. The chromatographic separation was achieved using ODS column (4.6 x150mm, 5µ particle size). Water and acetonitrile (55:45v/v) as a mobile phase, the flow rate was 1.0 ml/min. The eluent was monitored using PDA detection at 214 nm. Canagliflozin was resolved at 2.8 minutes. Validation parameters were studied as per ICH guidelines. The author concluded that this method can be employed for routine QC of Canagliflozin tablets in pharmaceutical industry.[3]

Kaur *et al.* developed and validated a simple and stability indicating HPLC method for determination of Canagliflozin in bulk and pharmaceutical dosage forms as per ICH Q2 R1 Guidelines. Chromatographic separation was achieved using C18 Column (250×4.6 mm, 5 µm particle size) with a mobile phase composed of Acetonitrile: orthophosphoric acid 55:45 v/v. the flow rate was 1 ml/min and the injected volume was 20 µL. The proposed method was validated with different parameters such as Linearity, Precision Accuracy, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ). The separation was achieved at a temperature of 30°C and the detection was observed by PDA detector at 290 nm. A linear range of 1-6 µg/ ml with a correlation coefficient of 0.998 unfolds good linear relationship between area and concentration in calibration curve. The retention time obtained was at 6.29 min. The LOD and LOQ were found to be 0.41 µg/ml and 1.24 µg/ml respectively. A recovery of Canagliflozin in tablet formulation was observed in the range of 99.6-99.8%. Percentage assay of Canagliflozin tablets (INVOKANA®) was 99.92%. The stability of the method was demonstrated by forced degradation studies of drug in which it was degraded under conditions of hydrolysis (acidic and alkaline), oxidation, photolytic and thermal stress as per ICH guideline Q1A (R2). Conclusion: The proposed method is definite, meticulous and reproducible and can be used for routine analysis of Canagliozin in bulk and pharmaceutical dosage form.[4]

Sonia *et al.* focused in their study on developing and validating an HPLC method to simultaneously estimation of Metformin hydrochloride and Canagliflozin in bulk using Grace Smart RP-18 column (250× 4.6mm, 5µ)at 30°C. Combination of acetonitrile (ACN) and ammonium acetate Buffer in the ratio of 45:55v/v with pH 4.5 was used as mobile phase with 1ml/min flow rate. It was detected by photo diode array detector at 252 nm. The retention Time observed for metformin hydrochloride and canagliflozin were found to be 4.00 and 5.76 min respectively. The method was developed and found to be linear with correlation coefficients R^2 of 0.9993 and 0.9992 for metformin hydrochloride and canagliflozin respectively within a concentration range of 1- 80µg/ml. Stability studies were performed by exposing the drugs to acidic, basic, oxidative, thermal and photolytic stress conditions with Samples with drawn at different time intervals. Analysis of the samples were done by the developed method. The method to estimate metformin hydrochloride and canagliflozin in bulk drug is easy, accurate, precise and less time consuming.[5]

Marell *et. al.* has developed a simple, specific and accurate reverse phase high performance liquid chromatographic method for the determination of Canagliflozin in bulk and pharmaceutical dosage forms. The method is optimized on an inertsil ODS-3(250×4.6mm, 5µ) column with a mobile phase combination of 0.02% Formic acid: Acetonitrile (40:60) at a flow rate 1.2ml/min and the eluents were monitored at 230nm. Under these LC conditions Canagliflozin peak was eluted at 4.4 min. The developed method was validated as per ICH guidelines. The calibration curve was linear over a concentration range of 10-50µg/ml (R^2 =0.999) and the mean percentage assay was found to be 98.2. The statistical data proved that proposed method is accurate, precise and reproducible.[6]

Bossunia *et. al.* Developed a comprehensive science and risk based stability indicating RP-HPLC Analytical method for the analysis of Canagliflozin Active Pharmaceutical Ingredient (API) according to Analytical Quality by Design (AQbD) concept. The method is consistent, reliable and cost effective method developed for the routine analysis of Canagliflozin in quality control laboratories.[7]

Ladva *et. al.* developed simple, specific, precise and accurate chromatographic method for estimation of canagliflozin in API and tablet dosage form by using

C18 column. The method was validated as per ICH guideline Q2 R1. All validation parameters were found to be within ICH guideline Q2R1.[8]

Sen *et. al.* developed a gradient reversed phase high performance liquid chromatography (RP-HPLC) method and validated for the determination for related substances of Canagliflozin drug substance. Good chromatographic separation of Canagliflozin from its process and degradation related substances was achieved on Ascentis Express RP-Amide, 150mm × 4.6mm 2.7 µm i.e stainless steel column 150 mm long, 4.6 mm internal diameter filled with amide groups chemically bonded to porous silica particles of 2.7 µm diameter maintained column oven temperature at 30°C. Ammonium acetate buffer is mobile phase A and acetonitrile is mobile phase B. Wavelength for UV detection: 290nm, flow rate: 0.7 ml/min and Injection volume: 10µl. The method suitability checked and validated according to the ICH guidelines for specificity, linearity, accuracy, precision, limit of quantification, limit of detection, robustness and ruggedness and also Canagliflozin was subjected to stress conditions of thermal, hydrolysis, humidity, peroxide and photolytic to observe the degradation products. Limit of detection of each RS is less than 0.008%w/w indicating that the developed method is highly sensitive.[9]

BIOANALYTICAL METHODS FOR DETERMINATION OF CANAGLIFLOZIN

Kulsum *et. al.*, has developed a simple, selective, rapid, precise and economical Reverse-Phase HPLC method has been developed and validated for quantitative determination of Metformin and Canagliflozin. Metformin Hydrochloride is an orally administered biguanide derivative used to lower blood glucose concentration in patients with non insulin dependent diabetes mellitus. Canagliflozin is an anti diabetic drug used to improve glycemic control in patients with type 2 diabetes. Pioglitazone is used as an internal standard. The method was carried out with Waters HPLC with auto sampler and PDA detector. Spursil (Dikma) ODS C_{18} column (4.6 x 250mm, 5 µm) is used at a flow rate of 1.0mL/min. Detection was carried out at 254 nm. The mobile phase used is Phosphate buffer (pH 3.0) with Acetonitrile in proportion 85: 15 v/v respectively. The retention times of Metformin and Cangliflozin were 4.738min and 8.352min respectively. The method was developed and tested for linearity range of 250 to 1250 ng/mL for Metformin HCl and 25 to 125 ng/mL for Canagliflozin. These bioanalytical validations play a significant role in evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic and toxicokinetic studies. In which different parameters like accuracy, precision, selectivity, sensitivity, reproducibility, and stability are performed.[10]

Muzaffar *et. al.*, developed a simple and sensitive HPLC assay with a florescence detector for accurate quantification of canagliflozin in human plasma using telmisartan as the internal standard (IS). Plasma samples were extracted by a liquid–liquid extraction method using diethyl ether as an extracting solvent. Chromatographic separation of canagliflozin and IS was performed on a Nucleodur Isis C_{18} column with an isocratic mobile phase of 20 mM potassium dihydrogen orthophosphate : acetonitrile (45 : 55, v/v) at a flow rate of 1 mL min^{-1}. Canagliflozin and IS were eluted at 2.8 and 5.8 min, respectively, and detected at 280 and 325 nm for excitation and emission, respectively. The plasma calibration curve displayed excellent linearity over the concentration range of 16.13–6000 ng mL^{-1}. The assay was fully validated in terms of selectivity & specificity, linearity of the calibration curve, accuracy & precision, recovery and stability under various storage conditions. This is the first validated HPLC-florescence detector assay for the quantification of canagliflozin in human plasma.[11]

Dudhe *et. al.*, developed a simple, specific, sensitive, precise, selective and accurate reverse phase high performance liquid chromatographic method was for the determination of canagliflozin in human plasma as per US-FDA guidelines. Plasma samples were extracted by protein precipitation method using methanol as extracting solvent. The chromatographic separation was performed with WATERS EA874 (250 × 4.6 mm, 5mm) column and mobile phase composed of 36.46 mM Acetate buffer: acetonitrile: methanol (30:50:20, v/v), pH 4.5 adjusted with acetic acid at a flow rate of 1.0 ml/min. Canagliflozin was detected at 290 nm with retention time of 5.1 min. Linearity was found to be 0.9929 over the range of 33.33 – 233.33 ng/ml and percentage recoveries were found to be 94.68 - 103.76 %.[12]

Iqbal et. al., developed a sensitive UHPLC–MS/MS assay for rapid determination of canagliflozin in rat plasma was and validated for the first time. Chromatographic separation of canagliflozin and zafirlukast (IS) was carried out on Acquity BEH C18 column (100×2.1 mm, internal diameter 1.7 µm) using acetonitrile–water (80:20, v/v) as mobile phase at a flow rate of 0.3 mL min−1. Canagliflozin and IS were extracted from plasma by protein precipitation method using acetonitrile. The mass spectrometric detection was performed using electrospray ionization source in negative mode to avoid canagliflozin adduct ions formation. Multiple reaction monitoring were used for quantitation of precursor to product ion at *m/z* 443.16 >364.96 for canagliflozin and *m/z* 574.11>462.07 for IS, respectively. The assay was fully validated in terms of selectivity, linearity, accuracy, precision, recovery, matrix effects and stability. The validated method was successfully applied to the characterization of oral pharmacokinetic profiles of canagliflozin in rats.[13]

Kobuchi *et. al.*, validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitative analysis of canagliflozin in a lower volume of rat plasma (0.1 mL) and applied to a pharmacokinetic study in rats. Following liquid-liquid extraction by tert-

butyl methyl ether, chromatographic separation of canagliflozin was performed on a Quicksorb ODS (2.1 mm internal diameter × 150 mm, 5 µm size) using acetonitrile-0.1% formic acid (90:10, v/v) as the mobile phase at a flow rate of 0.2 mL/min. The detection was carried out using an API 3200 triple-quadrupole mass spectrometer operating in the positive electrospray ionization mode. Selected ion monitoring transitions of m/z = 462.0 $[M + NH^4]^{(+)} \rightarrow 191.0$ for canagliflozin and m/z = 451.2 $[M + H]^{(+)} \rightarrow 71.0$ for empagliflozin (internal standard) were obtained. The validation of the method was investigated, and it was found to be of sufficient specificity, accuracy and precision. Canagliflozin in rat plasma was stable under the analytical conditions used. This validated method was successfully applied to assess the pharmacokinetics of canagliflozin in rats using 0.1 mL rat plasma.[14]

DIFFERENT METHODS USED IN DETERMINATION OF CANAGLIFLOZIN AND METFORMIN COMBINATION IN DOSAGE FORMS

Deepak *et al.* has developed a new stability indicating RP-HPLC method for estimation Metformin and Canagliflozin in bulk and pharmaceutical dosage form. To choose the mobile phase various combinations of organic solvents were used on Kromosil C18 250 column, Then the mobile phase containing a mixture of phosphate buffer and acetonitrile in the ratio of 65:35% v/v was selected at a flow rate of 1.0ml/min and a peak with a good shape and resolution was found resulting in short retention time. The retention time of metformin and canagliflozin were 2.413 and 3.548 min respectively, quantitative linearity was obeyed in the concentration range of 50-300µg/ml and 5-30 µg/ml of Metformin and Canagliflozin respectively. LOD and LOQ were found to be 0.30µg/ml and 0.91µg/ml (Metformin), 0.361µg/ml and 1.094µg/ml (Canagliflozin) respectively, which indicated the sensitivity of the method. The high percentage recovery indicated that the proposed method was accurate, no interfering peaks in the chromatogram which indicate that the excipients used in tablet formulation didn't interfere with the estimation of the drug by the proposed method.[15]

Uttam *et al.* reported a novel approach to develop and validate a rapid isocratic RP-HPLC method for simultaneous estimation of Metformin and Canagliflozin in bulk and pharmaceutical dosage form with forced degradation studies. The separation was performed by Kromasil C18 column (250mm×4.6 mm, 5mm particle size), Waters Alliance e2695 HPLC system with 2998 PDA detector and mobile phase consisted of a mixture of 0.01M Ammonium acetate (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (65:35, v/v). The flow rate was 1ml/min and the detection achieved at 254nm. The retention time of Metformin Hydrochloride and Canagliflozin was 2.440min and 3.713min respectively. Linearity was established in the range of 50-300µg/ml for Metformin Hydrochloride and 530µg/ml for Canagliflozin with correlation coefficients (r^2=0.999). The percentage recoveries were between (99.45%-100.65%) and (99.95%-100.74%) for Metformin Hydrochloride and Canagliflozin respectively. Validation parameters were evaluated according to the (ICH) Q2 R1 guidelines. The forced degradation studies were performed by using HCl, NaOH, H_2O_2, thermal, UV radiation and water. Metformin Hydrochloride and Canagliflozin were sensitive towards oxidative degradation condition. The developed method was successfully applied for the quantification and hyphenated instrumental analysis.[16]

Another study by Nareddy *et al.* who has developed a new HPLC method for simultaneous estimation of Metformin and Canagliflozin in pharmaceutical dosage forms. Chromatography was carried out on an ODS 250mm x 4.6 mm, 5µ particle size with an isocratic mobile phase composed of Buffer, Acetonitrile and methanol at a flow rate of 1mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 212 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention times for Metformin and Canagliflozin were 2.783 min and 3.781 min respectively. The percentage recoveries of Metformin and Canagliflozin were 100.1% and 100.2% respectively. The relative standard deviation for assay of tablets found to be less than 2%. The method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and pharmaceutical industries.[17]

Lotfy *et. al.* performed several spectrophotometric methods for the quantification of canagliflozin (CGZ) and metformin hydrochloride (MTF) simultaneously in their binary mixture. Two of these methods; response correlation (RC) and advanced balance point-spectrum subtraction (ABP-SS) were developed and introduced for the first time in this work, where the latter method (ABP-SS) was performed on both the zero order and the first derivative spectra of the drugs. Besides, two recently established methods; advanced amplitude modulation (AAM) and advanced absorbance subtraction (AAS) were also accomplished. All the proposed methods were validated in accordance to the ICH guidelines, where all methods were proved to be accurate and precise. Additionally, the linearity range, limit of detection and limit of quantification were determined and the selectivity was examined through the analysis of laboratory prepared mixtures and the combined dosage form of the drugs. The proposed methods were capable of determining the two drugs in the ratio present in the pharmaceutical formulation CGZ:MTF (1:17) without the requirement of any preliminary separation, further

dilution or standard spiking. The results obtained by the proposed methods were in compliance with the reported chromatographic method when compared statistically, proving the absence of any significant difference in accuracy and precision between the proposed and reported methods.[18]

Zaghary et. al. developed a new HPLC-UV method (method A), for simultaneous determination of metformin (MET) and canagliflozin (CANA), and compared to another novel UPLC-UV method (method B) in their tablet combination. In terms of accuracy and precision, method Accuracy was 99.81±0.73 and 99.37±0.54, while method B gave accuracy of 99.47±1.03 and 99.73±0.89 for CANA and MET, respectively. For precision, the %RSD was found to be less than 2% for three concentrations analyzed three times. The two methods are convenient for quality laboratories, yet the UPLC method offered the advantage of shorter run times and higher sensitivity.[19]

Trivedi *et. al* modified quantification through high-performance liquid chromatography analysis for canagliflozin and metformin hydrochloride in bulk and tablets using ecofriendly green solvents. The developed method follows all the validation parameters like accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method was found to provide faster retention time with sharp resolution with linearity at a lowest concentration as compared to previous methods and this method is validated as per International Conference on Harmonization guidelines and successfully applied to the simultaneous estimation of Canagliflozin and metformin in bulk and dosage forms. There was no such novel method for simultaneous estimation of Canagliflozin and metformin. Hence the developed method is suitable for industrial analysis of Canagliflozin and metformin with ecofriendly, green and less expensive solvents.[20]

Kommineni *et. al.* developed a new stability indicating RP HPLC method and validated for simultaneous estimation of Metformin Hydrochloride and Canagliflozin in bulk and dosage forms. The method involves separation on Kromasil C18 column (250mm x 4.6mm x5µm particle size). The optimized mobile phase consists of 0.1% OPA (pH 2.8) and Acetonitrile (45:55v/v) with a flow rate of 1ml/min and UV detection at 254nm. Retention time was 2.112 min for Metformin Hydrochloride, 2.671 min for Canagliflozin. RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Canagliflozin in their combine dosage form was developed and validated as per the ICH guidelines. Forced degradation studies indicated the suitability of the method for stability studies. When the validation parameters of the method developed are compared with those of the earlier reported methods the developed method was found superior in certain respects such as RT, LOD and the method was more economical when compared to others. Accuracy and precision, ruggedness and robustness were similar to earlier reported methods.[21]

Trivedi *et. al.* developed an accurate, precise, specific modified HPLC method for the simultaneous quantification of canagliflozine and metformin in bulk and dosage forms. A C18 column (250 x 4.6mm; 5 µm Phenomenex) with mobile phase containing 0.05% v/v triethylamine (pH 6.5): acetonitrile (45:55% v/v) 20^0C was used and isocratic pump is used for elution and eluents were monitored at 215 nm. The developed method follows all the validation parameters like accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method was validated as per ICH guidelines and successfully applied to the simultaneous estimation of canagliflozine and metformin in bulk and dosage form.[22]

Jyothi *et.al.* developed a simple, precise, economical, accurate, reproducible, and sensitive method for the estimation of of Metformin and Canagliflozin drug product by RP-HPLC method. The chromatographic separation was achieved on C18 column (Inertsil ODS 3V C18 250*4.6mm) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v TFA in water: Acetonitrile (20:80). All validation parameters were within the acceptable range.[23]

Kommineni *et. al.* developed a new stability indicating RP HPLC method and validated for simultaneous estimation of Metformin Hydrochloride and Canagliflozin in bulk and dosage forms. The method involves separation on Kromasil C18 column (250mm x 4.6mm x5µm particle size). The optimized mobile phase consists of 0.1% OPA (pH 2.8) and Acetonitrile (45:55v/v) with a flow rate of 1ml/min and UV detection at 254nm. The method developed is more sensitive, accurate and precise than the methods reported earlier. Retention time and run time were also less and hence the method is economical. When applied for tablet assay, drug content was within 98.55-101.4% of labeled content. Forced degradation studies indicated the suitability of the method for stability studies.[24]

UV/VIS METHODS FOR DETERMINATION OF CANAGLIFLOZIN

Few researches for Determination of Canagliflozin using UV/VIS spectroscopy were reported. Ishpreet et al. has developed and validated a simple, sensitive, precise, rapid and cost effective method for determination of Canagliflozin in bulk and pharmaceutical formulations as per ICH Guidelines. A simple double beam UV Spectrophotometric method has been developed and validated with different parameters such as Linearity, Precision, Repeatability, Limit of Detection (LOD), Limit of Quantification (LOQ), Accuracy, Robustness and Ruggedness. Canagliflozin in methanol shows maximum absorbance at 290 nm. Beer's law was obeyed in the concentration range of 5-10 mcg mL^{-1}, The LOD

and LOQ were found to be 0.084 mcg/ml and 0.255 mcg/ml respectively. A recovery of Canagliflozin in tablet formulation was observed in the range of 80.00-120.00%. Percentage assay of Canagliflozin tablets (INVOKANA®) was found to be more than 99%.[25]

Another method developed by Patel *et. al.*, depending on UV spectroscopy for the estimation of Canagliflozin in tablet formulation. Canagliflozin was estimated by using the mode at 290 nm in their solution in methanol. The Beer's law obeyed the concentration range of 5-25µg/ml for Canagliflozin. Mean recovery of 100.47% for Canagliflozin signifies the accuracy of the method. This method can be used for the routine UV estimation of Canagliflozin in industries and other analytical laboratories.[26]

Vani *et.al.*, describes the development, validation and stable studies of a new, simple and reliable visible spectroscopy procedure for the analysis of Canagliflozin in pharmaceutical formulations. Studies were carried out to investigate the reaction between Canagliflozin with 5 different chromogenic dyes. All these dyes shown specific colors on reaction with Canagliflozin and prominent wavelength maxima was observed. All the developed methods were validated as per the ICH guidelines and results shows that the methods were valid. Formulation analysis shows good argument with the true values. Hence the proposed methods for the estimation of Canagliflozin are simple, rapid, accurate, economical and are useful for the estimation of Canagliflozin in pharmaceutical formulation.[27]

Vani *et.al.*, developed Simple, fast and reliable UV and derivative Spectrophotometric methods for determination of Canagliflozin in pharmaceutical formulation. Absorption for NH was measured at maximum wavelength 227nm. Analytical Calibration curves were linear within a concentration range from 2-12µg/ml. The developed method was applied to directly and easily to the analysis of the pharmaceutical tablet preparations. The percentage recovery of Canagliflozin in pharmaceutical dosage form was found to be 98.57-100.75% for UV, 98.50-99.880% for first derivative and 98.87-99.90% for second derivative methods respectively. The method was completely validated and proven to be rugged. The results obtained were statistically evaluated and were found to be accurate and reproducible. The excipients did not interfere in the analysis. The results showed that this method can be used for rapid determination of Canagliflozin in pharmaceutical tablet.[28]

HPTLC METHODS FOR DETERMINATION OF CANAGLIFLOZIN

Determination of Canagliflozin has been reported by Ishpreet et al. who developed and validated a simple, authentic and stability indicating high performance thin layer chromatographic method for determination of Canagliflozin in bulk and pharmaceutical formulations as per ICHQ2 R1 Guidelines. HPTLC aluminium plates Precoated with Silica Gel 60F254 using Toluene: Ethyl acetate: Methanol (2:2:1, v/v/v) as mobile phase were used for the chromatographic separation and it was validated with different parameters. Also, Forced degradation study was carried out in different media. The densitometric analysis of the spots was performed at 290 nm. The Linearity was achieved over the range of 10500ng/spot with a good correlation coefficient of 0.9988. The LOD and LOQ were found to be 0.39 and 1.19 respectively. A recovery of Canagliflozin in tablet formulation was observed in the range of 99.04-99.82%. Percentage assay of Canagliflozin tablets (INVOKANA®) was found to be 99.8%. Forced degradation studies of canagliflozin showed the degradation in acidic, alkaline, photolytic and oxidation but were most stable in thermal degradation.[29]

Bhole *et. al.*, Developed and validate HPTLC method for simultaneous estimation of canagliflozin and metformin hydrochloride in bulk and tablet dosage form. This work describes the development and validation of an HPTLC method for the simultaneous determination of MET and CANAG in a combined dosage formulation. The chromatography was performed on pre-coated silica gel 60 F 254 plates using methanol: toluene: ethyl acetate: ammonia (2: 4: 4: 0.1) as mobile phase. A thin layer chromatographic (TLC) scanner set at 254.0 nm was used for direct evaluation of the chromatograms in reflectance/absorbance mode. The drugs were satisfactorily resolved with Rf 0.15 for MET and 0.50 for CANAG. The method was validated according to The International Council on Harmonization (ICH) guidelines. The calibration plot was linear between 0.5–3.0 µg/band for MET and 50–300 ng/band for CANAG respectively. Accuracy and precision of the proposed method were evaluated by recovery studies and intra-day and inter-day precision studies respectively. In stability testing, MET and CANAG were found to be susceptible to acid hydrolysis and alkaline degradation. Because the method could effectively separate the drugs from their degradation products, it may be used as a stability-indicating method.[30]

Emam *et. al.*, developed and validated a green high-performance thin-layer chromatographic densitometric determination for the accurate quantification of canagliflozin and its main oxidative degradation product. Separation was performed on high-performance aluminum plates precoated with silica gel using acetone/ethanol (80:20, v/v) as a developing system and scanning at 290 nm. Retardation factor values were 0.64 and 0.81 and linearity ranges were 0.4-3.6 and 0.2-3.2 µg/band for the drug and the degradation product, respectively. It was a matter of interest to use green solvents with no harmful effects on the environment. The comparison between the proposed and the reported high-performance liquid chromatography method regarding greenness profile showed that the proposed method was greener and so could be used as an alternative method to

the reported one with no environmental harm. Method validity was tested as per international conference on harmonization and method utility was verified by application to Invokana® tablets.[31]

CONCLUSION

Many methods for determination of Canagliflozin have been reported. Some HPLC assay methods were used to monitor canagliflozin. Methods for the analysis of active and inactive metabolites of canagliflozin in plasma have also been reported. Some articles related to the determination of canagliflozin alone or in combination with metformin in pharmaceutical dosage forms have been mentioned.

Literature survey suggested that various UV-VIS Spectroscopic, bioanalytical, RP-HPLC, HPTLC methods were developed and reported. There are many methods used in determination of canagliflozin and metformin combination in dosage forms. The published methods were validated for various parameters as per ICH guidelines.

REFERENCES

1. www.en.wikipedia.org/wiki/Canagliflozin.
2. Suneetha A.; & Sharmila D.; A Validated Stability Indicating RP-HPLC Method for Estimation of Canagliflozin in Dosage Form. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2015; 6(5): 1186-1194.
3. Suma M.; Manasa K.; Rajakumari C.; & Lakshmaiah B.; RP-HPLC Method Development and Validation For The Estimation Of Canagliflozin In Tablet Dosage Form. International Journal of Pharmacy, 2014; 5(4): 1288-1292.
4. Ishpreet K, Sharad W, Harsharan PS, Satish M. Development and Validation of a Stability-Indicating Reverse Phase HPLC-PDA Method for Determination of Canagliflozin in Bulk and Pharmaceutical Dosage Form. Pharm Methods, 2016; 7(1): 54-62.
5. D'souza S.; Krishna M.; Sushmitha G. S.; & Vasantharaju S. G.; Stability Indicating Assay Method Development and Validation to Simultaneously Estimate Metformin Hydrochloride and Canagliflozin by RP-HPLC. Current Trends in Biotechnology and Pharmacy, 2016; 10(4): 334-342.
6. Marella V. L.; Syed A.; Prasanna L. M.; & Nalluri B. N.; A novel validated RP-HPLC method for the estimation of canagliflozin in bulk and pharmaceutical dosage forms. International Journal of Advances in Pharmaceutical Analysis, 2017; 07(3): 24-27.
7. Bossunia M. T. I.; Urmi k. F.; & Shaha C. K; Quality-By-Design Approach to Stability Indicating RP-HPLC Analytical Method Development for Estimation of Canagliflozin API and Its Validation. Pharm Methods, 2017; 8(2): 92-101.
8. Iadva B. J.; Dobariya P. V.; Pancholi H. D.; Nayak B. S.; & Jain S.; Development and Validation of Chromatographic Method For Estimation of Canagliflozin In API And Tablet Dosage Form. International Journal of Recent Scientific Research, 2016; 7(5): 10976-10979.
9. Sen G, Babu KR, Annapurna N, Vekariya NA, Kumar VJ, Kumar KSRP, Sharma HK: Validation of Stability-Indicating Reverse Phase HPLC Method For The Determination of Related Substances in Canagliflozin Drug Substance. Journal of Pharmacy and Biological Sciences, 2017; 12(6): 86-94.
10. Kulsum S, Vidya Sagar G, Senthilkumar R, Kazim SM: Bio-Analytical Method Development and Validation for Metformin and Canagliflozin by RP-HPLC. World Journal of Pharmacy and Pharmaceutical Sciences, 2017; 6(12): 371-381.
11. Muzaffar I, Nasr YK, Amer MA, Khalid A. A simple and sensitive high performance liquid chromatography assay with a fluorescence detector for determination of canagliflozin in human plasma. Anal. Methods, 2015; 7: 3028-3035.
12. Dudhe P.B.; & Kamble M.C.; RP-HPLC Method Development and Validation forthe Determination of Canagliflozin in Human Plasma. International Journal of PharmTech Research, 2016; 9(8): 174-181.
13. Iqbal M, Ezzeldin E, Al-Rashood KA, Asiri YA, Rezk NL. Rapid determination of canagliflozin in rat plasma by UHPLC–MS/MS using negative ionization mode to avoid adduct-ions formation. Talanta, 2015; 132: 29-36.
14. Kobuchi S, Yano K, Ito Y, Sakaeda T. A validated LC-MS/MS method for the determination of canagliflozin, a sodium-glucose co-transporter 2 (SGLT-2) inhibitor, in a lower volume of rat plasma: application to pharmacokinetic studies in rats. Biomed Chromatography, 2016; 30(10): 1549-1555.
15. Deepak G, Patil RN, Mangesh H. A Validated Stability Indicating RP-HPLC Method for Simultanious Determination Of Metformin And Canagliflozin In Pharmaceutical Formulation. World J Pharm Pharm Sci, 2015; 4(12): 631-640.
16. Panigrahy U. P.; & Reddy A. S. K.; A Novel Validated RP-HPLC-DAD Method for the Simultaneous Estimation of Metformin Hydrochloride and Canagliflozin in Bulk and Pharmaceutical Tablet Dosage form with Forced Degradation Studies. Oriental Journal of Chemistry, 2015; 31(3): 1489-1507.
17. Nareddy PR, Naga TC. RP-HPLC Method development and validation for the Simultaneous Estimation of Metformin and Canagliflozin in Tablet Dosage Form. Int J Pharma Sci, 2015; 5(4): 1155-1159.
18. Lotfy H. M. Dalia M.; & Elshahed M. S.; Different mathematical processing of absorption, ratio and derivative spectra for quantification of mixtures containing minor component: An application to the analysis of the recently co-formulated antidiabetic

drugs; canagliflozin and Metformin. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2018; 189: 100-109.
19. Zaghary W. A.; Mowaka S.; & Hendy M. S.; Comparative Liquid Chromatographic Study for Concurrent Determination of Canagliflozin and Metformin in Combined Tablets. Journal of Analytical Methods in Chemistry, 2017, Article ID 9197230:1-9.
20. Trivedi A.; Dixit N.; & Jhade D. N.; Modified Quantification Through High-Performance Liquid Chromatography Analysis For Canagliflozin And Metformin Hydrochloride In Bulk And Tablets Using Ecofriendly Green Solvents. International Journal of Applied Pharmaceutics, 2017; 9(5): 97-101.
21. Kommineni V.; Chowdary K.P.R.; & Prasad S.V.U.M.; Development And Validation of A New RP-HPLC Method For Simultaneous Estimation of Metformin Hydrochloride And Canagliflozin And Its Comparison With The Reported Methods. World Journal of Pharmacy and Pharmaceutical Sciences, 2017; 6(3): 696-713.
22. Trivedi A.; Dixit N.; & Jhade D. N.; Modified HPLC Quantification Analytical Technique for Canagliflozin and Metformin Hydrochloride in Bulk and Tablets. EIJO: Einstein International Journal Organization Journal of Medical, Health Science and Innovative Research (EIJO– JMHSIR), 2017; 1(1): 36–44.
23. Jyothi U.; & Umadevi P.; Analytical Method Development and Validation for the Simultaneous Estimation of Canagliflozin and Metformin in Drug Product by RP-HPLC. European Journal of Biomedical and Pharmaceutical sciences, 2017; 4(11): 298-303.
24. Kommineni V.; Chowdary K. P. R.; & Prasad S. V. U. M.; Development of A New Stability Indicating RP-HPLC Method For Simultaneous Estimation of Metformin Hydrochloride and Canagliflozin and Its Validation As Per ICH Guidelines. International Journal of Pharmaceutical Sciences and Research, 2017; 8(8): 3427-3435.
25. Kaur I.; Wakode S.; & Singh H. P.; Development and Validation of UV Spectroscopic Method for Determination of Canagliflozin in Bulk and Pharmaceutical Dosage Form. Pharm Methods, 2015; 6(2): 82-86.
26. Patel N. D.; Shah D. B.; & Maheshwari D. G.; Development And Validation of UV-Spectrophotometric Estimation Of Canagliflozin In Its Pharmaceutical Dosage Form. International Journal of Pharmacy & Technology, 2015; 7 (3): 9779-9784.
27. Vani B. K. K.; UV and Derivative Spectrophotometry Method for the estimation of Canagliflozin in pharmaceutical formulations. International Journal of Applied Pharmaceutical Sciences and Biological Sciences, 2017; 6(1): 22-29.
28. Vani B. K. K.; Extractive Visible Spectrophotometer Method development and validation for the estimation of Canagliflozin in pharmaceutical formulations. International Journal of Science and Technology, 2017; 40(3): 2376-2381.
29. Kaur I.; Wakode S.; & Singh H. P.; Development and Validation of a Stability-Indicating High Performance Thin Layer Chromatography (HPTLC) Method for estimation of Canagliflozin in bulk and Pharmaceutical Dosage Form. Journal of Applied Pharmaceutical Science, 2016; 6(5): 51-57.
30. Bhole R. P.; Gandhi J. S.; & Wankhede S. B.; Development and validation of HPTLC method for simultaneous estimation of canagliflozin and metformin hydrochloride in bulk and tablet dosage form. African Pharmaceutical Journal, 2017; 84(6): 65-71.
31. Emam AA: Canagliflozin stability study and ecofriendly chromatographic determination of its degradation product: A comparative study. Journal of separation science, 2017.