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A Review Study on Reverse Phase HPLC & UV-Visible Methods for Active Pharmaceutical Ingredients [API] in Pure and Their Formulations Metformin-Canagliflozin

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Abstract – To develop and validate 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, Accuracy, Robustness and Ruggedness. Canagliflozin in phosphate buffer shows maximum absorbance at 240 nm. Beer's law was obeyed in the concentration range of 10-50 mcg ml-1.

Keywords: UV, HPLC, Canagliflozin, ICH Guidelines, Quantification, Validation etc.

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I. INTRODUCTION

Metformin (MET) has a chemical term which is N, Ndimethylimidodicarbonimidic diamide that can be seen in the Figure 1. Metformin is a first line oral pharmacotherapy for the type 2 diabetes. Energy-regulating enzyme AMPactivated protein kinase (AMPK) and its stimulation are mostly seen in the muscle and liver and it is regarded as a primary means of metformin action [1].

Canagliflozin (CANA) has a chemical term which is (2S, 3R, 4R, 5S, 6R)-2-[3-[[5-(4-fluorophenyl) thiophen-2-yl] methyl]-4-methylphenyl]-6-(hydroxymethyl) oxane- 3, 4, 5-triol (Refer to Figure 2). Canagliflozin is preferred in type 2 diabetes and it prevents Na+- reliant 14CAMG perception in a concentration-reliant means. It is the original C-glucoside with thiophene ring [2].

There are different UV & HPLC inspection techniques which are mentioned in literature to estimate the Metformin and Canagliflozin separately and along with rest of the drugs. Literature survey conducted states that no verified technique can be found to estimate Metformin and Canagliflozin at the same time by RP-HPLC in the integrated tablet dosage types. Due to this, the efforts were made for creating a new technique to estimate Metformin and Canagliflozin at the same time and verifying them in the tablet formulation in agreement with ICH guidelines [3].

Official method for the simultaneous estimation of Metformin and Canagliflozin by RP-HPLC in combined tablet dosage forms. Hence, an attempt has been made to develop a new method for simultaneous estimation and validation of Metformin and Canagliflozin in tablet formulation in accordance with the ICH guidelines.

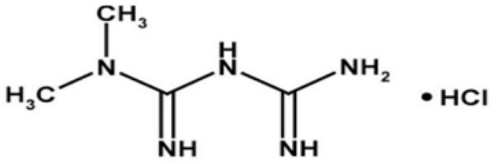

Figure 1.1: Chemical structure of Metformin Hydrochloride

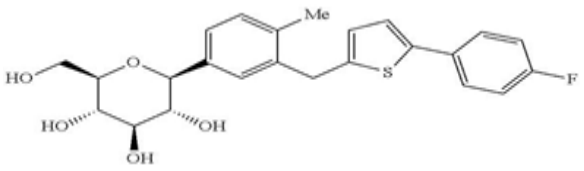

Figure 1.2: Chemical structure of Canagliflozin

Canagliflozin (C24H25FO5S) is a white to off white solid with melting range of 95- 105ºC[1] has a chemical term which is (2S,3R,4R,5S,6R)-2-[3-[[5-(4-fluorophenyl) thiophen-2-yl] methyl]-4- methylpheny]-tetrahydro-6-hydroxymethyl-2H-pyran-3,4,5-triol (Figure-1). It can be soluble in a lot of organic solvents such as methanol, Dimethyl sulfoxide but it is not soluble in aqueous forms. Canagliflozin is first Sodium-glucose co-transporter 2 (SGLT-2) inhibitor that was used in treating the patients who had type 2 diabetes. Canagliflozin helps in decreasing the reabsorption of purified glucose as it inhibits Sodium-glucose co-transporter 2 (SGLT2) and then lessens renal threshold for glucose (RTG) which leads to growth in emission of urinary glucose. Maddu et al In his study has described a sensitive and simple method of RP-HPLC to determine Canagliflozin in the form of pharmaceutical dosage. The separation of chromatographic has been achieved using the column of ODS (4.6 x150mm, 5µ particle size). Acetonitrile and water (45:55v/v) as the mobile phase, 1.0 ml/min was the flow rate. The eluent has been monitored using the detection of PDA at 214 nm. Resolution of Canagliflozin done at 2.8 minutes.

The parameters for validation have been assessed in lieu with the ICH guidelines. Further, the author suggests that the method would get induced for QC routine of the tablets of Canagliflozin in the pharmaceutical industry.

Studies of stability are done by exposure of drugs to basic, acidic, oxidative, photolytic stress and thermal conditions having Samples drawn at various time intervals. Samples were analyzed by the method. The method for estimation of Canagliozin and metformin hydrochloride in bulk form is accurate, easy, and precise and consumes less time.

II. UV/VIS METHODS FOR DETERMINATION OF CANAGLIFLOZIN

Several researches done to determine Canagliflozin with the help of UV/VIS spectroscopy has been reported.

Ishpreet et al Validated and developed a sensitive, simple, rapid, precise, and a cost effective way to determine Canagliflozin in pharmaceutical and bulk formulations in temrs of guidelines of ICH. A double beam method of UV Spectrophotometric was developed and then validated having several parameters as Precision, Linearity, Repeatability, Limit of Detection (LOD), Accuracy, Limit of Quantification (LOQ), and Ruggedness and Robustness. Canagliflozin in the methanol reveals highest absorbance at the wavelength of 240 nm. The Beer's law has been obeyed in concentration range of around 10-50 mcg mL- 1, The LOD and LOQ are 0.15ug/ml and 0.46 ug/ml for Metformin Hydrochloride, 0.19ug/ml and 0.58ug/ml for Canagliflozin. Canagliflozin's recovery in formulation of tablet is seen in range of 80.00-120.00%. Canagliflozin's percentage assay we're greater than 99%.Beer's law was obeyed within 10-50µg/ml for the Canagliflozin. The Mean recovery for Canagliflozin of 100.23% suggests the methods accuracy. The above method has been used to estimate UV of canagliflozin in analytical labs and industries.

III. DETERMINATION OF CANAGLIFLOZIN IN HUMAN/RAT PLASMA

The first study to determine Canagliflozin in the human plasma relies on a sensitive and simple HPLC assay having florescence detector, such method has been developed for the Canagliflozin's accurate quantification in human plasma with the help of telmisartan as per internal standard (IS). Samples of Plasma have been extracted with the method of liquid–liquid extraction using diethyl ether being the extracting solvent. Canagliozin chromatographic separation has been done on the column of Nucleodur Isis C18 having the isocratic mobile phase with 20 M of potassium dihydrogen orthophosphate: acetonitrile in (40: 60, v/v) and flow rate, 1 mL min−1. IS and Canagliflozin has been eluted at 5.8 min and 2.8, and detected at wavelength of 280 and 325 nm for the excitation and also emission, The curve of plasma calibration displays linearity across the range of concentration of 16.13–6000 ng mL−1. Further, validation of assay has been done with selectivity & specificity, calibration curve's linearity, precision and accuracy, stabillty and recovery in lieu with several storage conditions.

Another 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) was established 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 i.d. × 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 + NH4] (+) → 191.0 for Canagliflozin and m/z = 451.2 [M + H](+) → 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 [4].

A sensitive assay UHPLC-MS/MS for quick Canagliozin determination in rat plasma has been validated and developed Muzzafar et al Canagliflozin chromatographic separation and Zafirlukast (IS) has been done on the column of Acquity BEH C18 (100×2.1mm, i.d. 1.7µm) inducing acetonitrile-water as (80:20, v/v) being the mobile phase and flow rate being 0.3mLmin(-1). IS and Canagliflozin has been expelled from plasma by the method of protein precipitation using acetonitrile. The detection of mass spectrometric has been using the electrospray source of ionization in a negative mode avoiding the formation of canagliflozin adduct ions. Several reaction monitored has been used to quantitate the precursor to the product ion at concentration's m/z 443.16 >364.96 for the drug canagliflozin and m/z for IS being 574.11>462.07. Validation of assay done with respect to Linearity, selectivity, precision, accuracy, stability, recovery, matrix effects. The method validated has been applied successfully to the oral pharmacokinetic characterization profiles of rata canagliflozin. The Canagliflozin's mean maximum concentration of plasma of 1616.79ngmL (-1) has been attained in 1.5h post oral administration in rats of 20mgkg (-1).

Another method of validated liquid mass spectrometry chromatography-tandem (LC- MS/MS) to analyze Canagliozin quantitatively in rat Plasma lower volume (0.1 mL) has been established and the applied to the rats pharmacokinetic study. Followed by the liquid-liquid extraction using tert-butyl methyl ether, Canagliozin chromatographic separation has been done on Quicksorb ODS (2.1 mm i.d. × 150 mm, 5 µm size) with acetonitrile-0.1% formic acid at (90:10, v/v) being mobile phase and flow rate 0.2 mL/min. Next, detection has been carried using the API 3200 mass spectrometer triple-quadrupole that operates in electrospray ionization mode. Selective transition of ion monitoring for Canagliozin of m/z = 462.0 [M + NH4] (+) → 191.0 and for Empagliflozin kf m/z = 451.2 [M + H] (+) → 71.0 obtained. The method validation investigated and found to be accurate, sufficiently specific, and precise. Concentration of Canaglifloz in the plasma of rat was found to be stable under analytical conditions. The above validated method has been applied successfully to assess the Canagliflozin's pharmacokinetics in rats using rat Plasma at 0.1 mL.

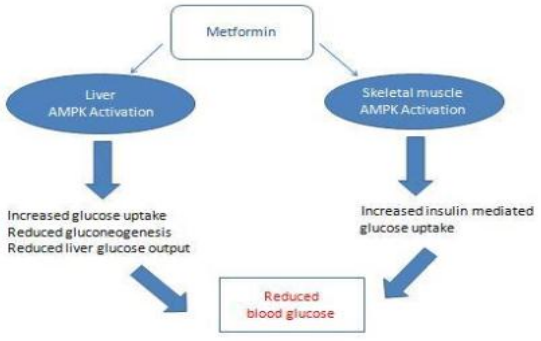

Figure 1.3: Mechanism of Metformin Action

The researches till now have documented alteration in fasting insulin as well as fasting glucose on the basis of fasting parameters to assess insulin sensitivity, instead of parameters correlating better with respect to the gold standard to estimate insulin sensitivity like the WBISI. Moreover, the metformin functional mechanism for reducing body weight as well as Body Mass Index is yet to be entirely explored [5].

A. Canagliflozin for Type 2 Diabetes

Sodium-glucose co-transporter 2 (SGLT2) inhibitors are the newly added ones for the therapeutic armamentarium that is made for curing Type 2 diabetes mellitus (T2DM). These orally consumed agents would comprise of huge levels of insulin that is not dependent on any of the mechanisms of action which would inhibit glucose and reabsorb it in the proximal convoluted tubule. Canagliflozin is a part of SGLT2, which is in the inhibitor class that has the authority to market in different countries like Europe, USA and Japan. This has to be taken in the dosage of 100mg every day and titrated to 300mg only when glycemic control is not sufficient. Canagliflozin efficacy along with tolerability is thoroughly assessed in different clinical trials. The main aim of conducting this review is to give a quick summary of the evidences that are available for canagliflozin that majorly focus on the safety profile and its impact on the population along with international information that is related to cost-effectiveness and reimbursement.

B. Safety of Canagliflozin

The Meta analysis that is carried out to show the hypoglycemia rate would be different for canagliflozin and placebo arms (risk ratio: 1.13; 95% CI: 0.40–3.20). The effect of hypoglycemia would be high on the patients who are receiving sulfonylurea or insulin that is used for treatment and background therapy. The patients who are suffering with stage 3 acute kidney problem would be given insulin or sulfonylurea and sometimes canagliflozin with the dosage of around 100 to 300 mg once every hypoglycemic episode of around 41.9 and 43.8%. There is placebo of 29.2% can be used as a substitute.

IV. CANAGLIFLOZIN IN THE TREATMENT OF TYPE 2 DIABETES

Bovine respiratory disease (BRD) can be treated with the help of the mixture of strong antimicrobial agent florfenicol and no steroidal anti-inflammatory flunixin meglumine and they are also helpful in dealing with the BRD-related pyrexia in the beef and non-lactating dairy cattle. Here in the research, advancement and authentication of HPLC-UV technique is defined to measuring the florfenicol and flunixin at the same time in the injectable formulation where the excipients are combined.

RP-HPLC technique that is put forth which was formed by reversed phase (RP) C18e (250 mm × 4.6 mm, 5 µm) column at room temperature involving the isocratic moveable stage of acetonitrile and water combination where pH was changed to 2.8 with the use of thinned phosphoric acid, flow rate being 1.0 mL/min along with the infrared discovery being 240 nm. Stability-indicating technique was the result when the drugs were vulnerable to pressure situations of acid and base hydrolysis, oxidation, photo degradation, and thermal deprivation. Damaged items that were acquired were put aside and away from APIs. The technique helped and it was authenticated with the agreement to FDA and ICH standards as there was exceptional linearity, preciseness, exactness, certainty, strongness, LOD, LOQ, and system appropriate outcomes falling under satisfactory standards acceptance criteria.

The mixture of Florfenicol and flunixin meglumine (Flr&Flx) can be efficient antimicrobial and nonsteroidal anti-inflammatory in the veterinary used directed for treating the BRD that is related to Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, along with the Mycoplasma bovis, and with the management of BRD-related pyrexia in beef and nonlactating dairy livestock. In the recent times, the general use of quality by design (QbD) methodology in the investigative technique development called analytical QbD (AQbD) is becoming well-known [6]. It is verified that it gives us the science-oriented and risk-oriented awareness of important investigative outlooks along with the primary aspects that have an impact on the investigative technique performance [7]. On the basis of these values of quality risk management and design of experiments, AQbD is also useful to completely understand the credible threats and related communication between technique variables [8]. It comprises of describing quality target method profile (QTMP) and CAAs, recognizing the important technique boundaries with the use of risk evaluation and broadcast, optimization of technique with the use of test designs, modelization and finest discovery with the help of response surface approach for undertaking the investigative design space and hypothesizing the control technique for the constant enhancement.

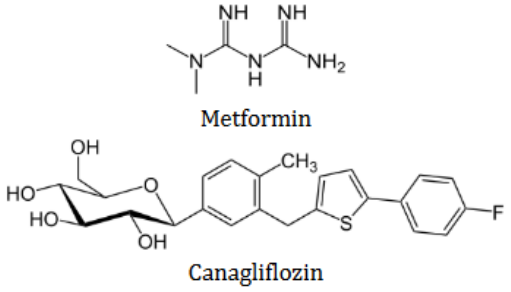

Figure 1.3: Chemical structures of drugs

Therefore, there were efforts made to come up with the fresh technique to determine and authenticate Metformin and Canagliflozin in the tablet formulation at the same time in the agreement with ICH standards. Kidney has an important part to play in the glucose homeostasis and pathophysiology of the type 2 diabetes mellitus (T2DM). Sodium glucose co-transporter 2 (SGLT2) inhibitors are fresh group of antihyperglycemic mediators in T2DM therapy where autonomous insulin instrument of the steps targets the kidney. SGLT2 inhibitors lead to reduction in renal glucose reabsorption which leads to the growth in urinary glucose emission along with the reduction in plasma glucose levels for the patients who have hyperglycemia.

V. GENITAL MYCOTIC INFECTIONS WITH CANAGLIFLOZIN

The occurrence of GMIs was enhanced acrss both the genders treated having canagliflozin in relation with placebo or other dynamic comparators (sitagliptin or glimepiride), but it was displayed to be proportion connected [9][10][11]. On the whole, almost 10% of females who went under the treatment of canagliflozin advanced GMIs of mild-to-moderate strength in relation with 3% of those went under placebo in the medical experiments. Female who had a GMIs having canagliflozin directed to tale them in the initial days having deterioration in danger along with the time period. No life- intimidating GMIs or hospitalization happened in female in these experiments, and more than that of 99% of GMIs were taken into consideration from the range of mild to moderate in concentration [11]. The whole GMIs reacted to general antifungal therapy, and in most of the strategies were their own treated by the patient; only some of the GMIs move towards the treatment cessation (0.5–0.9%) [34, 38]. Most of the females (78.5%) who progressed a GMIs having canagliflozin shows only one episode; and 18.3% shows two episodes while only 3.2% has shown either three or more episodes [34, 38].

People who are with unrestrained hyperglycemia must be administered to reach out to glycemic targets, which might decrease their GMI danger. GMI that evolve at the time of canagliflozin treatment is same as to those detected in noncanagliflozin-treated females, and female people who are with T2DM go with similar danger influences for VVC. These hazard aspects consist the utilization of extended-estrogen oral contraceptives, intrauterine instruments, diaphragms having spermicide, and antibiotics. Antiquity and medical performance are moderate for many of the treatements. Antiquity must consist of beleaguered questions regarding health performance, modern medication utilization, vaginal symptoms, preceding VVC incidents, cleanliness and utilization of more-the-counter self-governed medicines. Diabetes-associated queries

need to determine the T2DM drugs utilized and the scope of glycemic regulator accomplished utilizing present treatments. Indications are similar to that of females having no T2DM, consisting of vulvar longing, erythema, annoyance and vaginal evolution that might be curdy (VVC) or yellowish. In medical preliminaries, ladies who create VVC during the treatment with the canagliflozin was having comparable reaction to run of the mill antifungal specialists, for example, OTC azoles, intra-vaginal operators as well as fluconazole [5]. In an investigation of ladies with the T2DM (n is equal to 198) treated with canagliflozin, two among the nine contaminations that have outcomes of culture have been distinguished as the Candida glabrata; not a single lady experienced repeat after one quality path of the anti-fungal treatment.

A. Irradiation with Ultraviolet Light

A sample powder of Canagliflozin (10 mg) was exposed to UV light (254 nm) for 48 h. The material was dissolved in 5 ml water. The solution was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was suitably diluted and a volume of 20 µl was injected into the HPLC system. As well, an aqueous solution of Canagliflozin (1 mg/ml) was exposed to UV light (254 nm) for 48 h, and after diluting 20 µl was injected into the HPLC system.

VI. 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. Canagliflozin is an antidiabetic drug used to improve glycemic control in patients with type 2 diabetes. A sensitive UV spectrophotometric method was developed for the estimation of canagliflozin in bulk and pharmaceutical dosage form. Validation of the developed method was done as per the ICH guidelines Q2 (R1).

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