Original Article

Future technology based HPLC Analytical Procedures and Pharmaceutical Description of Empagliflozin

Garima Kapoor[1], Rubina Bhutani[2*], Manni Dutta[2], Abhishek Sharma[2], Farhan Khan[2], Pankaj Bhatt[1]

[1]KIET School of Pharmacy, KIET group of Institutions, Ghaziabad, Uttar Pradesh

[2]Department of Pharmacy, School of Medical & Allied Sciences, G.D. Goenka University, Gurugram-Sohna Road, Sohna, Haryana

Email: rubinabhutani23@gmail.com

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Abstract

Empagliflozin known for its inhibition of SGLT-2 (sodium glucose co-transporter-2). SGLT-2 inhibitors are those category of prescription drugs that are given with diet and exercise helping in decreasing sugar level. SGLT-2 inhibitors lower blood sugar levels by inducing the excretion of sugar through urine via kidneys. With appropriate diet and exercise for management of type 2 diabetes mellitus in adults, therapy includes metformin or linagliptin in combination with empagliflozin. Genital mycotic infections are common and urinary tract infections are very rare. Empagliflozin's pharmacological and pharmaceutical features are discussed in this paper. The generic pharmaceutical industry may find empagliflozin to be an appealing target. In drug discovery, bioequivalence research, and pharmacokinetics evaluation, analytical method development strives to provide better estimation and cost-effectiveness. The growth of drug entities and their evaluation is a new topic of technique development study. Different approaches were created employing various type of combination of mobile phases and columns to produce run duration variation and efficacy in biological fluids or medication solution, according to a recent case study on empagliflozin. The case study showed that there is limited information on simultaneous estimate of fixed dose combinations of empagliflozin, making it a novel field to investigate.

Keywords: Bioequivalence; diabetes mellitus; Empagliflozin; Simultaneous estimate; Validation.

1. INTRODUCTION

Empagliflozin, also known as Jardiance, is an effective drug for diabetes mellitus which controls glucose level and decreases the death risk due to cardiovascular problems in adults leading to decrease in hospitalization and mortality because of heart failure. [1] This drug has benefits comparing to sulfonylureas and can be used instead of metformin. Empagliflozin raises the elimination of sugar in urine by working as SGLT-2 (sodium glucose co-transporter-2) inhibitor. [2][3] In 2014, countries like US and Europe Union approved empagliflozin for medicinal usage. World Health Organization already itemized it as an essential medicine. [4][5] With the increasing number of pharmacological moieties and fixed dose combinations, fresh analytical methods for quality evaluation and standardization are constantly being developed and validated. Chromatographic methods are expanding their scope by integrating them with techniques related to analysis for creation of new comprehensive procedures. The best method to be opted is HPLC useful in identifying chemical entities, evaluating their purity, and ensuring and controlling the quality of samples, intermediates, and finished compounds. Purpose of this analysis is to cultivate a complete and verified analytical method for isolating, identifying, and quantifying drug substance impurities.

The qualitative and quantitative analysis is done by investigation of identity, efficacy, purity, also known as the pharmaceutical analysis. The sample's nature is depicted by qualitative and sample amount is identified by quantitative test.

Chromatography is a separation-based technique for separating constituents based on solvent to polarity ratio in which a mixture is separated into its constituents based on their solvency/polarity while being influenced by a solvent or gas. According to IP 2016, liquid chromatography is a "technique of separating components between two phases which are not miscible and the liquid mobile phase elutes through a stationary phase in a column". Adsorption, partition and ion exchange are mostly used techniques of high-performance liquid chromatography. High Pressure Liquid Chromatography works on the basis that the pressure forces the mobile phase through the column at a considerably faster rate and is column chromatography which is used

in biochemistry, pharmaceutical and analytical chemistry and biomedical science for detection and separation of chemicals by the r polarities and interactions with the stationary phase of the column.

UV-Visible spectra region 190-180nm, is used whenever some organic compound or functional group is to be identified from the electromagnetic region. It is one of the most widely used tools in pharmaceutical research and is based on Lambert-Beer Law principle, which entails measuring the absorption of radiation by the sample (chromophore). When the HPLC and UV-visible spectroscopy are combined, the integrated method is best for quantitative analysis and is also precise, accurate, and cost-effective.

2. Reasons for developing HPLC & UV-Spectroscopic Method:

● Study and investigate profile of degradation products and impurities.

● Development of analytical method for formulated new pharmaceuticals and improvement of methods for accuracy, decreased time, and lower costs.

● Study stability, clinical release, and optimization of pharmaceutical formulations.

● Development of new combinations for simultaneous evaluation.

This study will act as one stop for development and validation of empagliflozin and also will be beneficial for future researchers to develop new methods spectroscopic for empagliflozin. It will help in the in assay and estimation of empagliflozin in future and present.

2.1. HISTORY

Eli Lilly and Company along with Boehringer Ingelheim collaborated in development. Empagliflozin/linagliptin, empagliflozin/metformin and empagliflozin/linagliptin/metformin are also available as combinations.

The Drugs Controller General of India has given Boehringer Ingelheim permission to market Jardiance (empagliflozin) in the country. The approval is based on an additional indication of lowering cardiovascular death risk and heart failure. Jardiance has approval from US Food and Drug Administration (USFDA). [6]

2.2. MEDICINAL BENEFITS

2.2.1. Diabetes mellitus/ Type-II diabetes

Empagliflozin is a drug that is used to relief people suffering from type 2 diabetes by lowering their sugar levels in blood when combined with a healthy diet and exercise. This drug can be combined with other drugs of this class like metformin, sulfonylureas, and insulin. [7]

2.2.2. Heart and kidney disease

For adults suffering from type 2 diabetes, empagliflozin can minimize chances of collapsing heart or the advancement of chronic kidney disease. Empagliflozin may lower the risk of mortality from cardiovascular causes in persons with type 2 diabetes who already have a heart condition. [8][9] This drug has approval in several countries to minimize mortality from cardiovascular disorders with type 2 diabetes. [10]

2.2.3. Weight and Blood Pressure

Body weight and blood pressure are both reduced moderately by empagliflozin can decrease blood pressure and body weight and sodium and glucose excretion from urine are seen. Patients on empagliflozin showed by clinical trials a loss of 2% of

baseline weight. Individuals receiving empagliflozin lost more than 5% of their body weight compared to their baseline control. This drug brought systolic blood pressure down by 3 to 5 millimetres of mmHg as many subjects having type 2 diabetes found to have overweight or being obese with high blood pressure, thus bending of body weight and blood pressure towards normal considered as positive for further exploration. [11]

2.2.4. Diabetes insipidus/ Type-I diabetes

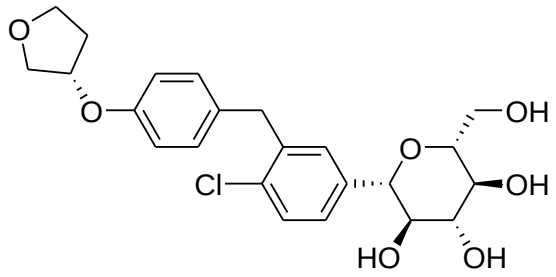
For type 1 diabetes, this drug is not being used. In one study, it was used alongwith insulin for adults in type 1 diabetes management. The drugs improved body weight slightly, but the same was associated to an exalted risk for diabetic ketoacidosis, a serious consequence of diabetes. The US Food and Drug Administration (FDA) has not approved empagliflozin for employing in management of type 1 diabetes. [12]

2.3. PHYSICOCHEMICAL PROPERTIES

Empagliflozin chemically is C-glycosyl compound comprising of a beta-glucosyl residue having a (4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy]benzyl}) phenyl group at the anomeric centre, with chemical formula C32H27ClO7. [13]

IUPAC name for empagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol.

Its molecular weight is 450.9 g/mol. Its solubility is 0.28 mg/ml. [14] It's physical form (at 20°C) stays white to yellowish as non-hygroscopic powder. Its melting point is 151-153°C. It is essentially insoluble in toluene and found soluble in water, methanol, ethanol, and acetonitrile; also soluble in 50 percent acetonitrile/water. Almost 86.2 percent of Empagliflozin is protein-bound in plasma. [15] Empagliflozin has six stereo-genic centres and is chiral. Appropriate analytical procedures are used to monitor enantiomeric purity on a regular basis. Empagliflozin comes in a single polymorphic form that is non-solvated and non-hydrated.



2.4. SYNTHESIS [16]

Composition

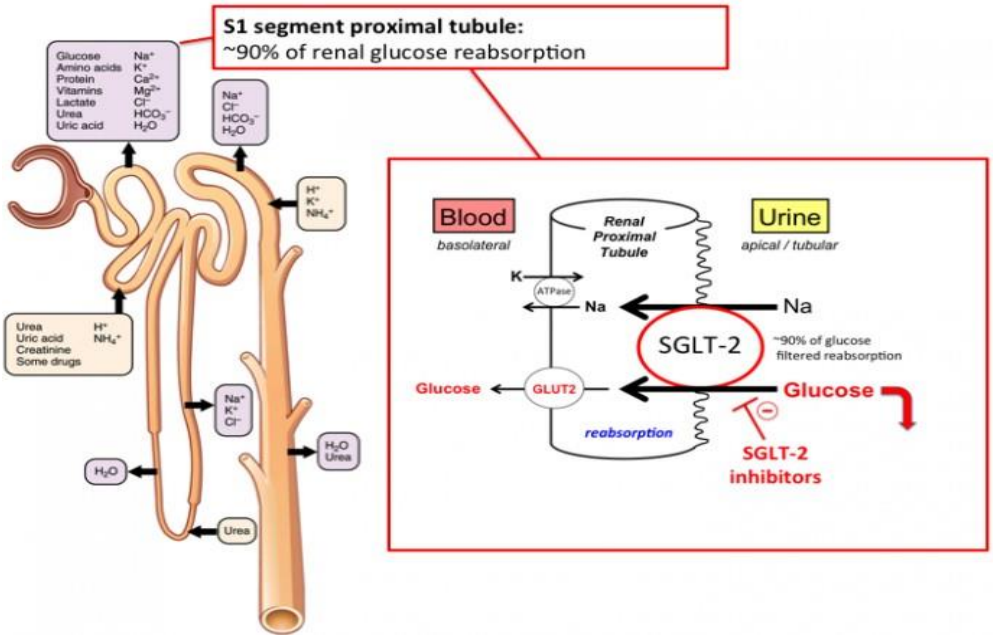
JARDIANCE (empagliflozin) film-coated tablets include 10 mg or 25 mg of empagliflozin as free base with other inactive constituents including microcrystalline cellulose, hydroxypropyl cellulose, lactose monohydrate, croscarmellose sodium, magnesium stearate and colloidal silicon dioxide, also film coating components named titanium dioxide, Hypromellose, talc,

yellow ferric oxide and polyethylene glycol. [17]

Mechanism

The drug is SGLT2 (Selective sodium-glucose transporter-2) inhibitor, which persists mainly in proximal renal tubules and is responsible for reabsorption of glucose via tubular lumen. This drug reduces glucose reabsorption and declines threshold of kidney meant for glucose causing further excretion of glucose in urine.

Reduces sodium reabsorption and increases salt supply to the distal tubule, which may have an impact on a variety of physiological activities, including decreasing cardiac pre- and afterload and downregulating sympathetic activity. [18]



2.5. PHARMACOKINETICS

2.5.1. Absorption

Empagliflozin peak plasma levels were reached 1.5 hours after oral administration. Plasma concentrations then decreased in a biphasic fashion, with a quick dispersion phase and a slow terminal phase. The steady state means plasma AUC and Cmax for 10 mg and 25 mg of empagliflozin once daily treatments, respectively, were 1870 nmol hr/L and 259 nmol/L, and they were 4740 nmol hr/L and 687 nmol/L, respectively. Empagliflozin peak plasma levels were reached 1.5 hours after oral administration. Plasma concentrations then decreased in a biphasic fashion, with a quick dispersion phase and a slow terminal phase. The steady state values for plasma AUC and Cmax with 10 mg of empagliflozin once daily therapy were 1870 nmol hr/L and 259 nmol/L, respectively.

Administration of 25 mg empagliflozin after a high-fat, high-calorie meal resulted in slightly lower exposure compared to fasting circumstances; AUC fell by nearly 16% and Cmax by roughly 37%. It was determined that meals have no clinically meaningful impact on the pharmacokinetics of empagliflozin, therefore it can be given with or without food. [19]

2.5.2. Distribution

The apparent steady-state volume of distribution was estimated to be 73.8 L based on a population pharmacokinetic analysis. After giving healthy participants an oral empagliflozin solution, the red blood cell partitioning was approximately 36.8% and the plasma protein binding was 86.2 percent.

2.5.3. Metabolism

Three glucuronide conjugates were the most prevalent metabolites in human plasma, and no significant empagliflozin metabolites were discovered there (2-O-, 3-O-, and 6-O-glucuronide). Less than 10% of the total drug-related material was exposed systemically to each metabolite. Studies conducted in vitro indicate that glucuronidation by the uridine

The main pathway for empagliflozin metabolism in humans is through the 5'-diphospho-glucuronosyltransferases UGT2B7, UGT1A3, UGT1A8, and UGT1A9. [19]

2.5.4. Excretion

The apparent terminal elimination half-life and apparent oral clearance of empagliflozin were 12.4 hours and 10.6 L/h, respectively, according to the population pharmacokinetic analysis. When an oral empagliflozin solution was administered to healthy volunteers, about 95.6% of the drug-related radioactivity was eliminated in faeces (41.2%) or urine (54.4 percent). Nearly half of drug-related radioactivity released in urine and the bulk of drug-related radioactivity recovered in faeces were both unaltered parent drugs. [20]

2.6. PHARMACODYNAMICS

Empagliflozin works by reducing glucose reabsorption in the kidneys and so increasing the quantity of glucose discharged in the urine. It has a long duration of action and only requires a once-daily dose. Empagliflozin may be paused in cases of acute kidney injury and/or discontinued in individuals who develop chronic renal illness because its mechanism of action is dependent on renal glucose excretion. [21]

2.7. DOSAGE & ADMINISTRATION

2.7.1. Patients with Type 2 diabetes

Empagliflozin is prescribed at a dose of 10 mg once a day in the morning, with or without food. The dose of empagliflozin can be increased to 25 mg in those who tolerate it well. [22]

2.7.2. Patients with Cardiovascular risk

The recommended starting dose is 10 mg orally once a day in the morning. Patients who tolerate therapy well may be increased to 25 mg orally once a day. [22]

2.7.3. Patients with Renal impairment

Renal function should be checked before starting empagliflozin and then every few months after that. Patients with an eGFR of less than 45 mL/min/1.73 m2 should not start it. Patients with an eGFR greater than or equal to 45 mL/min/1.73 m2 do not require dose adjustments. If your eGFR is less than 45 mL/min/1.73 m2, you should stop using empagliflozin. [23]

2.8. RESEARCH

According to a study, half a pill of empagliflozin 25mg has equivalent glycaemic efficacy as a full tablet, with the added benefit of lower monthly medication costs.

Side effects

Urinary tract infection is one of the most reported side effects of empagliflozin. Vulvovaginal candidiasis, cervicitis, genital candidiasis, genitourinary infection, vaginal infection, vulvitis, and vulvovaginitis are some of the other adverse effects. [24][25]

Common

●Empagliflozin raises your chances of getting a vaginal fungal infection. People having a history of genital fungal infections are at the greatest risk.

●Urinary tract infections (UTIs) may be more frequent with empagliflozin. Even though some specific clinical trials have indicated an increase in risk, overall data from several trials reveals no such increase. [26]

●There are slight increases in LDL cholesterol that range from 2% to 4% from baseline. [27]

●Empagliflozin and other SGLT-2 inhibitors have the potential to increase the frequency of diabetic ketoacidosis (DKA), an uncommon but potentially lethal condition. While euglycemic ketoacidosis, a disease brought on by SGLT-2 inhibitors, can happen with abnormally normal blood glucose levels, DKA is frequently associated with higher blood glucose levels. The absence of elevated blood glucose levels in SGLT-2 inhibitor users may make detecting DKA more challenging. The risk of empagliflozin-associated DKA may rise in the presence of illness, dehydration, surgery, and/or alcohol consumption. Changes to the prescribing instructions for SGLT2 inhibitor diabetic medications have been authorised by the FDA, and now it is suggested that these medications be temporarily discontinued prior to scheduled surgery to lower the chances of evolving ketoacidosis (condition in which the body yields high levels of blood acids called ketones). Empagliflozin withdrawal is advised at least three days before to surgery. [28]

●When used with a sulfonylurea or insulin, empagliflozin can increase the risk of low blood sugar. When administered in addition to metformin it does not appear to increase the risk of hypoglycaemia.[28]

●Empagliflozin and other SGLT-2 inhibitors are more likely to cause Fournier's gangrene, an uncommon but deadly groin infection. Fever, a general feeling of malaise, and soreness or swelling around the genitals or in the skin behind them are all symptoms. The illness spreads swiftly, necessitating immediate medical intervention. [29]

2.9. CONTRAINDICATIONS

Empagliflozin is contraindicated in patients with severe renal impairment, defined as a GFR of less than 30 mL/min/1.73m2. Empagliflozin should not be used if GFR is found to be lower than 45 mL/min or if you are pregnant with second or third trimesters. End-stage renal illness, dialysis, and a severe allergic reaction to empagliflozin are some of the other contraindications. In situations of hepatic impairment, empagliflozin may be utilized. Patients with Type 1 diabetes and diabetic ketoacidosis should not take empagliflozin. [27]

Safety profile in special subjects

2.9.1. Pregnancy/Nursing

In pregnancy, empagliflozin is classified as a category C drug. There are no controlled human studies of the medication in pregnant women currently available. Empagliflozin has been shown in animal experiments to pass the placenta, impairing kidney growth and maturation. In rats and rabbits, however, no evidence of teratogenicity was discovered at dosages 48 and 128 times the maximum human therapeutic dose, respectively. The medicine should only be used during pregnancy if the possible benefit outweighs the potential danger to the foetus and there are no other options. Similarly, empagliflozin is thought to be potentially harmful during lactation, while it is unknown whether the medicine is excreted in human milk. Empagliflozin should be stopped when nursing because of the risk of major adverse reactions in nursing infants due to the drug's glucose lowering and volume contraction effects. [30]

2.9.2. Elderly

The geriatric population is a special population that is of relevance in terms of empagliflozin treatment. The negative effects of empagliflozin treatment may be amplified in this population since older patients usually have impaired autonomic function, may be using diuretics, and are commonly administered several medications with a significant risk of drug-drug interactions. Furthermore, empagliflozin is probably less effective than it once was since elderly people frequently experience concurrent renal impairment. Although only 6% of participants in randomised clinical trials were over 75, this age group was more susceptible to adverse events linked to volume depletion and UTIs than younger participants. As a result, caution is suggested while giving empagliflozin to the elderly, and careful monitoring for side effects is needed. [31]

2.10. TOXICITY

Dehydration, hypotension, hypovolemia, and syncope have all been reported as a result of its diuretic effects associated with volume depletion. Fournier gangrene is a kind of necrotizing fasciitis of the perineum for which the FDA has issued a warning.

There were twelve cases documented, and all of them required hospitalization and surgical debridement. Stop the drug if you feel it's the cause, and have the patient go to the emergency room right away for a surgical assessment. [32]

2.11. DRUG INTERACTIONS [33]

Drug drug interactions can result in increased or compromised efficacy, or engender unexpected side effects in the body. Drugs that have a clinical impact are given below;

Diuretics	
Clinical impact	When empagliflozin was combined with diuretics, the volume and frequency of voids increased, thereby increasing the risk of volume depletion.
Intervention	Assess your volume status and renal function before starting empagliflozin. Correct volume depletion before starting empagliflozin in those who have it. After starting therapy, keep an eye out for signs and symptoms of volume depletion, as well as renal function.
Insulin or Insulin Secretagogues	
Clinical impact	When empagliflozin is used with insulin secretagogues (e.g.,sulfonylurea) or insulin, the risk of hypoglycemia is enhanced.
Intervention	To lessen the risk of hypoglycemia when empagliflozin is used with an insulin secretagogue (e.g., sulfonylurea) or insulin, smaller dosages of the insulin secretagogue or insulin may be required.
Positive Urine Glucose Test	
Clinical impact	SGLT2 inhibitors cause increased glucose excretion in the urine,resulting in positive urine glucose tests.
Intervention	In patients on SGLT2 inhibitors, urine glucose testing is not advised for glycemic control monitoring. Use different strategies to keep track of your glycemic control.
Interference with 1,5-anhydroglucitol (1,5-AG) Assay	
Clinical impact	In patients on SGLT2 inhibitors, measurements of 1,5-AG are inaccuratefor determining glycemic control.
Intervention	It is not suggested to use the 1,5-AG test to monitor glycemic control. Usedifferent strategies to keep track of your glycemic control.

2.12. CLINICAL TRIALS

2.12.1. Empagliflozin as monotherapy [34]

Double-blind, placebo-controlled research for 986 patients having T2DM was conducted to evaluate the efficacy and safety of empagliflozin monotherapy. Patients with poorly managed T2DM who were treatment-naive entered a two-week open-label placebo run-in period. Patients with a HbA1c between 7% and 10% were assigned to placebo, empagliflozin 10 mg, empagliflozin 25 mg, or a reference comparator if they remained poorly managed at the conclusion of the run-in phase. Compared to placebo, individuals receiving a daily dose of empagliflozin 10 mg or 25 mg had significantly lower HbA1c, body weight, and systolic blood pressure at week 24.

The primary outcome revealed placebo-adjusted decreases in HbA1c of 0.74 percentage points (P 0.001) and 0.85 percentage points (P 0.001) for the empagliflozin 10-mg and 25-mg dosages, respectively, from baseline to week 24. Empagliflozin 10 mg and 25 mg patients had significant placebo-adjusted weight loss of 1.93 kg (P 0.001) and 2.15 kg (P 0.001), respectively. Empagliflozin 10 mg reduced systolic blood pressure by 2.6 mm Hg (P = 0.023) and empagliflozin 25 mg reduced it by 3.4 mm Hg (P = 0.003) as compared to placebo. Patients on empagliflozin had a greater rate of vaginal infections than those taking placebo: 4.2 percent with empagliflozin 10 mg and 3.6 percent with empagliflozin 25 mg, compared to 0.7 percent with placebo.

2.12.2. Empagliflozin with Insulin [35][36]

During a 78-week, double-blind, randomized, placebo-controlled trial, empagliflozin was studied as an auxiliary medication in individuals with T2DM on basal insulin. Patients were given either a placebo (n = 170), empagliflozin 10 mg (n = 169), or empagliflozin 25 mg (n = 155) as a treatment. The study included an initial 18-week period of set insulin doses, following which the dose was modified at the discretion of the investigators. The primary objective at week 18 was a change in HbA1c from baseline. At week 78, secondary objectives with empagliflozin included a statistically significant decrease in HbA1c, insulin sparing, and weight loss relative to placebo. Empagliflozin 10 mg and 25 mg reduced HbA1c by 0.6 and 0.7 percentage points, respectively, at week 18 when corrected for placebo (P 0.001). HbA1c reductions of 0.4 and 0.6 percentage points (P 0.001) were observed in the empagliflozin 10-mg and 25-mg groups at week 78. In addition to lower HbA1c levels at week 78, the placebo-adjusted required daily insulin dose for empagliflozin 10 mg (P = 0.002) and 25 mg (P = 0.009) was reduced by 6.7 IU and 6.0 IU, respectively. The placebo arm had a 5.5 IU rise from baseline. The results also demonstrated that both empagliflozin 10 mg and 25 mg reduced body weight by 2.4 kg, compared to 0.7 kg for placebo. FPG and systolic blood pressure were found to be lower after further investigation. Hypoglycemia was reported in 36.1 percent of empagliflozin 10 mg and 25 mg individuals and 35.3 percent of placebo participants. Adverse events, such as urinary tract infections, were reported in 14.8%, 11.6%, and 8.8% of patients receiving empagliflozin 10 mg, empagliflozin 25 mg, and placebo, respectively.

2.12.3. Empagliflozin with Metformin [37][38]

A 12-week randomized, double-blind, placebo-controlled trial comprising 495 people aged 18 to 80 of 16 countries who were poorly managed on metformin alone or metformin in conjunction with another oral antidiabetic (OAD) drug was done. These patients needed a daily maintenance dose of 1,500 mg or more of metformin, not to exceed the maximum tolerated dose. HbA1c requirements were as follows: 6.5 percent to 9 percent for patients on metformin monotherapy; 7 percent to less than 10% for patients who were on metformin plus another OAD medication prior to the study and for all other patients at the start of the placebo phase. Patients who were taking another antidiabetic medicine with their metformin had to wait four weeks before taking it again. During the washout phase of the second OAD drug, metformin was continued. Patients were dispensed with a placebo, empagliflozin 1, 5, 10, 25, or 50 mg, or sitagliptin 100 mg once a day, with or without food, for the trial. Sitagliptin was included to give a clinical comparison to other OAD medications and to test the trial's sensitivity. A change in HbA1c from baseline to week 12 was the primary outcome assessed. A change in HbA1c over time, a change in FPG and body weight from baseline to week 12, and the proportion of patients who attained a HbA1c of 7% or less or whose HbA1c fell by at least 0.5 percentage points at week 12 were the secondary endpoints assessed.

When compared to the placebo, empagliflozin administered once a day, as an add-on therapy with metformin showcased clinically substantial reductions in HbA1c, FPG, and body weight, with the 10-, 25-, and 50-mg formulations showing the greatest reductions. The 10-, 25-, and 50-mg doses of empagliflozin reduced HbA1c levels by 0.56, 0.55, and 0.9 percent (P 0.0001), respectively; this was better than or comparable to 100 mg of sitagliptin, which only reduced HbA1c by 0.45 percent (P 0.0001). Overall, empagliflozin was well tolerated, with few adverse events including urinary tract infections, which occurred in 14 people (4 percent of the trial group), mostly women. Other genital infections, which occurred in 14 people, were self-reported and were classified as mild to moderate. Although this is a common adverse effect with other SGLT2 inhibitors, the genital infections in this trial were less common than with other SGLT2 inhibitors and were comparable in male and female patients.

3. LITERATURE REVIEW

S. No.	Year	Column	Solvent	Absorbance	Flow Rate	Temperature	Retention Time	Reference No.
1	2022	DiscoveryC18	Acetonitrile:Phosphate buffer	222 nm	1 ml/min	30°C	3.9 mins	39
2	2022	Phenyl column	0.1% Perchloric Acid:Acetonitrile	226 nm	1.2 ml/min	30˚C	15.7 mins	40
3	2021	ACE C18	Orthophosphoric acid buffer:Acetonitrile	230 nm	0.5 ml/min	25°C	3.2 mins	41
4	2021	Zorbax eclipse C18 plus	Phosphate buffer:Acetonitrile	220 nm	1.5 ml/min	28°C	1.4 mins	42
5	2021	C-18	0.1% orthophosphoric acid:Acetonitrile	230 nm	1 ml/min	25°C	2.05 mins	43
6	2021	Phenomenex C18	1% Orthophosphoricacid	223 nm	1 ml/min	30°C	14.5 mins	44
7	2021	C18 column	Acetonitrile:Phosphate buffer	222 nm	1 ml/min	30˚C	3.9 mins	45
8	2021	C18 column	Methanol:Water	224 nm	1 ml/min	25°C	4.8 mins	46
9	2021	C18 column	Methanol:Phosphate buffer	240 nm	1 ml/min	30˚C	3.9 mins	47
10	2021	ZORBAxC18	Acetate buffer:Acetonitrile	232 nm	1 ml/min	28°C	2.4 mins	48
11	2021	BEH C18	Acetonitrile:Phosphate buffer	220 nm	0.3 ml/min	28°C	1.2 mins	49
12	2021	HypersilODS 3V	Water:Acetonitrile	225 nm	1 ml/min	25°C	8.3 mins	50
13	2021	Kromasil C18	0.1% OPA buffer:Acetonitrile	226 nm	1.1 ml/min	28°C	2.9 mins	51

14	2021	Kromasil	Acetonitrile	233 nm	1 ml/min	25°C	2.7 mins	52
15	2021	Inertsil C8	0.1% OPA:Acetonitrile	230 nm	1.2 ml/min	24°C	11.5 mins	53
16	2021	C18 column	0.1% Perchloric acid:Acetonitrile	230 nm	1 ml/min	30˚C	2.0 mins	54
17	2021	Hypersil BDS	0.1% OPA:Acetonitrile	233 nm	1 ml/min	28°C	3.2 mins	55
18	2021	BDS C18	Methanol:0.1% orthophosphoric acid	254 nm	1 ml/min	25°C	2.6 mins	56
19	2021	Inertsil ODS	Water:Acetonitrile: Methanol	265 nm	0.8 ml/min	30˚C	3.8 mins	57
20	2021	RSLC 120 C18	Potassium dihydrogen phosphate buffer:Methanol	225 nm	0.4 ml/min	30˚C	1.5 mins	58
21	2021	Inertsil	1.01M Acetate buffer:Methanol	260 nm	2 ml/min	25°C	1.2 mins	59
22	2021	Phenome nex C18	Methanol:Water	224 nm	1 ml/min	28°C	4.8 mins	60
23	2020	Ascentis C18	0.1% orthophosphoric acid:Acetonitrile	260 nm	1 ml/min	25°C	3.1 mins	61
24	2020	Zorbax SB Phenyl	Phosphate buffer:ACN:Methan ol	220 nm	1 ml/min	28°C	5.5 mins	62
25	2019	Enable C18G	Methanol:Water	233 nm	1 ml/min	30˚C	6.2 mins	63
26	2019	X-Select-HS	Phosphate	255	1	30˚C	6.4 mins	64
		S C18 SV	buffer:Acetonitrile	nm	ml/min			
27	2019	Kromasil	0.1% o-phosphoric acid buffer:acetonitrile	230 nm	1 ml/min	28°C	2.1 mins	65

28	2019	Cosmosil C18	Methanol:Potassiu m dihydrogen phosphate buffer	227 nm	0.8 ml/min	25°C	6.5 mins	66
29	2019	Xselect-HSS C18 SB	Acetonitrile	255 nm	1 ml/min	28°C	6.4 mins	67
31	2018	Kromasil C18	Buffer:Acetonitrile	226 nm	1.1 ml/min	30˚C	2.9 mins	68
32	2018	Discovery C18	0.1% orthophosphoric acid:Acetonitrile	218 nm	1 ml/min	25°C	4.6 mins	69
33	2017	Dikma C18	Phosphate buffer:Methanol	240 nm	1 ml/min	30˚C	1.7 mins	70
34	2017	Kromasil C18	Buffer:Acetonitrile	226 nm	1.1 ml/min	30˚C	2.9 mins	71
35	2016	Phenomene x C18	Methanol:Water	224 nm	1 ml/min	25°C	3.6 mins	72
36	2015	ODS	Buffer:Acetonitrile	245 nm	1 ml/min	25°C	3.6 mins	73

4. CONCLUSION

Empagliflozin comes under the class of drugs known as SGLT2 (sodium-glucose co-transporter 2) inhibitors. It decreases blood sugar via kidneys by excreting more glucose in the urine. Empagliflozin abridged the risk and total number of inpatient and outpatient worsening heart failure episodes in patients of heart failure, with benefits observed soon after medication initiation and lasting throughout the double-blind trial. When empagliflozin was added to standard therapy, subjects with type 2 diabetes having elevated risk for cardiovascular events found to have a lower rate of the primary composite cardiovascular events and mortality from the same when compared to those subjects who were administered placebo. As a result, the medication is a viable therapeutic choice for patients with type 2 diabetes along with high risk of cardiovascular disease. Thus, it can be a desirable target for the generic drug industries.

Rationale of this analysis was to compare different methods for estimating and quantifying empagliflozin in pharmacological samples, fixed dose combinations and biological samples. This research will contribute to the creation of a novel approach with a shorter run time, higher efficacy, and lower cost. With the rising complexity of treatment regimens, it is necessary to simplify analytical techniques and look for more environmentally friendly methods of analysis.

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