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DEVELOPMENT AND VALIDATION OF THE RP- HPLC METHOD USING HYDROTROPIC SOLVENT TECHNOLOGY FOR THE ESTIMATION OF EMPAGLIFLOZIN IN BULK DRUG AND DOSAGE FORM

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ABSTRACT

By using hydrotropic solvents as the mobile phase, the bulk of the organic solvent in the analysis may be decreased. An RP-HPLC technique for estimating the amount of empagliflozin in tablets was developed. Excellent drug recovery percentage was attained, and the suggested approach was judged to be exact, reliable, innovative, and accurate. The ICH guidelines' constraints were met by the linearity, accuracy, range, and robustness. As a result, the process was discovered to be quick, innovative, straightforward, accurate, precise, economical, and repeatable.

KEYWORDS: Hydrotropy, Empagliflozin, Validation ICH guideline.

INTRODUCTION

Hydrotropy involves enhancing the solubility of a solute in water by introducing a substance known as hydrotropes. Hydrotropic solvents play a crucial role as solvents in spectrophotometry and chromatography, enabling accurate, rapid, and precise analysis. Hydrotropes, characterized by amphiphilic properties and a hydrophilic functional group, serve as a medium to enhance the solubilization of sparingly soluble substances in aqueous solutions. These organic salts, when present in aqueous solutions, significantly improve the solubility of hydrophobic organic substances in the aqueous phase. Commonly employed hydrotropes include hydroxy benzenes, hydroxy benzoates, benzene sulfonates, sodium benzoate, and sodium citrate.[1-4]

Empagliflozin, a recently introduced oral antidiabetic medication, functions as a selective inhibitor of sodium–glucose transport protein 2 (SGLT2). Administered in the form of a film-coated pill, it contains either 10 or 25 mg of empagliflozin as the active pharmaceutical ingredient. The United States Food and Drug Administration (USFDA) granted approval for this drug in 2014.[5]

Empagliflozin's chemical name is (1S)-1,5-anhydro-1-(4-chloro-3-{4-[(3S)-tetrahydrofuran-3 yloxy]benzyl}phenyl)-D-glucitol. It is also *refe*rred to as D-Glucitol, 1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3-furanyl]oxy]phenyl]methyl]phenyl]-(1S).

The molecular structure is depicted in Fig. 1. This substance is a white to yellowish, non-hygroscopic crystalline solid. It exhibits very slight solubility in water, slightly soluble in acetonitrile and ethanol, sparingly soluble in methanol, and practically insoluble in toluene.[6]

As a sodium–glucose co-transporter 2(SGLT2) inhibitor, empagliflozin represents one of the most recent advancements in the treatment of Type 2 Diabetes Mellitus (T2DM). SGLT2 inhibitors, functioning as glucose-lowering agents, operate through an insulin-independent mechanism, making them versatile for use in conjunction with other anti-diabetic agents for T2DM treatment. Beyond their primary role in glycemic control, they also contribute to diminished hyperglycemia, facilitate weight loss, and lower blood pressure.[7,8] The physico-chemical parameters[9] are shown in Table 1.

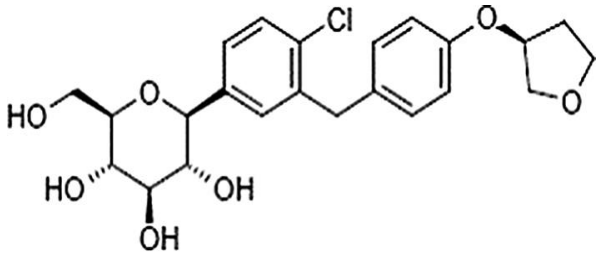

Fig. 1: Structure of Empagliflozin.

Table 1: Critical Physico-Chemical Parameters.

Sr.No.	Parameters	Description
1	Drug Name	Empagliflozin
2	CAS Number	864077-44-0
3	Molecular formula	C23H27ClO7
4	Molecular weight	450.9
5	Creation	Empagliflozin was discovered and developed by Boehringer-Ingelheim. It was approved by the FDA in 2014 and is marketed in four formulations: Jardiance (Empagliflozin), Glyxambi (Empagliflozin and Linagliptin), Synjardy (Empagliflozin and Metformin) and Synjardy XR (Empagliflozin and Metformin extended-release).
6	Category	Antidiabetic
7	Specification	white to yellowish powder
8	Appearance	Crystalline Solid
9	Melting point	151.0–153.0 ° C
10	PKa	12.57
11	Solubility	Methanol, Ethanol,
12	Drug type	Approved

Mechanism of Action

Empagliflozin works by blocking the sodium-glucose co-transporter-2 (SGLT-2) located in the proximal tubules of the kidneys. By inhibiting SGLT2, empagliflozin decreases the reabsorption of glucose in the kidneys and enhances the excretion of glucose through urine. The glucose-lowering impact of the drug is not reliant on insulin. Empagliflozin also lessens the levels of sodium and fluid, leading to a reduction in intravascular volume through its diuretic and natriuretic properties.[10]

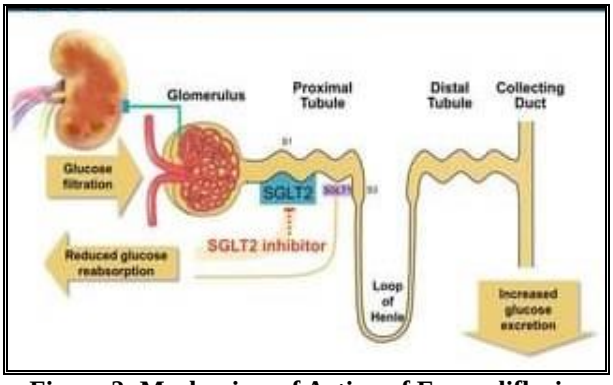

Figure 2: Mechanism of Action of Empagliflozin.

The prevalence of type 2 diabetes mellitus has a tremendous impact on obesity, which is a growing problem. Because of the rise in obesity, there is an increased demand for the researchers to develop drug therapies that not only effectively treat hyperglycemia but also promotes weight loss. Metformin, a biguanide2, is the preferred oral hypoglycaemic agent for initial therapy in patients with type-2 diabetes and later combination therapy in which metformin along with other medication is given to the patient and in other scenarios where the condition worsens the patient, they are advised to take insulin with metformin. But long-term use of Metformin is associated with lactic acidosis which is serious side effect.[3]

There are many other options for further oral therapy. Agents that can be used along with metformin include: sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 receptor agonists, sodium-glucose cotransporter 2 (SGLT2) inhibitors and insulin.[4,5]

Empagliflozin (Jardiance, Boehringer Ingelheim), a sodium-glucose cotransporter 2 (SGLT2) inhibitor, belongs to a new class of oral hypoglycemic agents, which includes canagliflozin and dapagliflozin. In August 2014, empagliflozin became the most recent medication in its class to be approved by the Food and Drug Administration.[6] Empagliflozin has less side-effects when used in combination with other anti-diabetic medications. Empagliflozin had the highest selectivity for SGLT2 over SGLT1 compared to dapagliflozin and canagliflozin. It is a sodium glucose co-transporter-2 inhibitor (SGLT2). SGLT2 co-transporters reabsorbs glucose from the glomerular filtrate in the kidney. By inhibiting the SGLT2 co-transporter, it reduces renal absorption of glucose and it increases glucose excretion through urine.[7]

There is minimum or no risk of hypoglycemia with empagliflozin because the mechanism of action is not dependent on beta-cell function and insulin pathway. Empagliflozin is preferred over metformin for early diabetes and in combination it can prevent lactic acidosis caused by Metformin. Empagliflozin is suggested for the improvement of glycemic control along with diet and exercise in adults with type-2 diabetes mellitus.[8] It has additional cardioprotective effects.

Long-term complications of type 1 diabetes due to exposure to chronic hyperglycaemia, blindness, cardiovascular disease and kidney failure, remain a fear for many patients living with type1 diabetes.[9] Intensive insulin therapy reduces the risk of complications, but the majority of people with type 1 diabetes fail to achieve

the acceptable glycaemic targets. It is also seen that long-term intensive insulin therapy is associated with weight gain which is a relevant side-effect as a significant proportion of patients with type 1 diabetes are now overweight. Empagliflozin can be used even for type-1 diabetes patients for greater glycemic control.

Pharmacokinetics

Absorption: After oral administration, the highest levels of the drug in the bloodstream are achieved around 1.5 hours (Tmax). When the drug is administered regularly (steady-state), the plasma area under the curve (AUC) and maximum concentration (Cmax) were 1870 nmol·h/L and 259 nmol/L, respectively, for empagliflozin 10mg daily. For empagliflozin 25mg daily, these values were 4740 nmol·h/L and 687 nmol/L, respectively. It's worth noting that taking the medication with food does not significantly impact the absorption of empagliflozin.[11]

Distribution: The calculated apparent steady-state volume of distribution is 73.8 liters.[11]

Metabolism: Empagliflozin experiences minimal metabolism, with its primary metabolic pathway involving glucuronidation catalyzed by 5'-diphospho-glucuronosyltransferases 2B7, 1A3, 1A8, and 1A9. This process leads to the formation of three glucuronide metabolites: 2-O-glucuronide, 3-O-glucuronide, and 6-O-glucuronide. It's important to note that none of these metabolites individually constitutes more than 10% of the total drug-related material.[11]

Elimination: Following oral administration of radiolabeled empagliflozin, about 41.2% of the administered dose was eliminated in feces, and approximately 54.4% was eliminated in urine. The primary component of radioactivity in the feces was the unchanged parent drug. In contrast, around half of the radioactivity in urine was attributed to the unchanged parent drug.[11]

Special Populations

Half-life: The apparent terminal elimination half-life of empagliflozin was determined to be 12.4 hours, as determined through population pharmacokinetic analysis.[11]

Clearance: The apparent oral clearance of empagliflozin was determined to be 10.6 L/h based on a population pharmacokinetic analysis..[11]

Toxicity: Experience with empagliflozin overdose is limited. In case of overdose, it is recommended to employ standard symptomatic and supportive measures. Gastric decontamination may be considered when appropriate. However, the use of hemodialysis in cases of empagliflozin overdose has not been studied. Moreover, it is considered unlikely to be beneficial due to the drug's relatively high protein-binding, which may limit its removal through dialysis and contribute to altered plasma concentrations.[11]

RP-HPLC Technique

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) is a robust analytical technique extensively employed in chromatography. It falls under the category of liquid chromatography, where molecules are separated based on their hydrophobicity, or more precisely, their affinity for a hydrophobic stationary phase.

RP-HPLC is known for its versatility and finds widespread applications across various scientific disciplines, including pharmaceuticals, biochemistry, environmental analysis, and food and beverage testing. This technique is particularly valuable for separating and quantifying complex mixtures of organic compounds. By utilizing a stationary phase with hydrophobic properties and a mobile phase that is more polar, RP-HPLC allows for efficient separation of different components within a sample.

The ability of RP-HPLC to handle a diverse range of compounds and its sensitivity make it an indispensable tool in laboratories for qualitative and quantitative analyses in a multitude of fields.[12]

Indeed, the fundamental principle of Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) is centered on the interaction between the analyte and the stationary phase. In RP-HPLC, the stationary phase is nonpolar, while the mobile phase is polar. This contrasts with normal phase chromatography, where the stationary phase is polar, and the mobile phase is nonpolar.

The nonpolar stationary phase in RP-HPLC is typically composed of hydrophobic materials, such as hydrocarbons or bonded alkyl groups. This setup encourages interactions between the hydrophobic analytes and the nonpolar stationary phase. The more hydrophobic a molecule is, the longer it will be retained on the column, resulting in a separation of different compounds based on their hydrophobic properties.

This reversed phase arrangement allows RP-HPLC to be highly effective in separating nonpolar and moderately polar compounds, making it a widely used technique in various analytical and preparative applications.[13]

In RP-HPLC, the stationary phase is often composed of a hydrophobic material, and one of the commonly used materials is C18-bonded silica, where C18 denotes an 18-carbon alkyl chain. This hydrophobic stationary phase provides a surface with a strong affinity for hydrophobic analytes.

On the other hand, the mobile phase in RP-HPLC is typically a mixture of water and an organic solvent, such as acetonitrile or methanol. The composition of these

solvents in the mobile phase can be adjusted to control the elution strength. By altering the ratio of water to the organic solvent, chromatographers can manipulate the polarity of the mobile phase, affecting the retention and separation of analytes on the hydrophobic stationary phase.

This flexibility in adjusting the mobile phase composition is a key advantage of RP-HPLC, allowing for optimization of separations based on the specific characteristics of the analytes being analyzed.[14,15]

Mechanism of RP-HPLC

The separation mechanism in Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) relies on the differential affinity of analytes for the stationary phase. In this chromatographic technique, hydrophobic analytes have a stronger interaction with the nonpolar stationary phase (such as C18-bonded silica). This interaction results in a longer retention time for hydrophobic analytes on the column.

On the other hand, hydrophilic analytes, having weaker interactions with the nonpolar stationary phase, move more quickly through the column and are eluted earlier. By manipulating the composition of the mobile phase, chromatographers can control the degree of hydrophobicity or hydrophilicity of the mobile phase, thereby influencing the retention times and separation of different analytes.

This differential affinity of analytes for the stationary phase based on their hydrophobic or hydrophilic nature is the key principle that enables the effective separation of compounds in RP-HPLC.[16]

Equipment

RP-HPLC systems typically include several key components, and their arrangement is crucial for the effective separation of compounds. Here's a breakdown of the components mentioned:

High-Pressure Pump: The high-pressure pump is a critical component that is essential for maintaining a consistent and controlled flow of the mobile phase through the chromatographic column. The high pressure is required to ensure efficient and rapid separation of analytes on the column.

Sample Injector: The sample injector is responsible for introducing the sample into the chromatographic system. It allows for precise and controlled injection of the sample onto the column. Different injection techniques, such as manual injection or automated auto samplers, can be employed.

Column with Stationary Phase: The column contains the stationary phase, which, in the case of RP-HPLC, is typically a hydrophobic material like C18-bonded silica. This is where the separation of analytes based on their differential affinity for the stationary phase occurs.

Detector: The detector monitors the eluent from the column and detects the separated compounds based on their properties (e.g., UV absorbance). Common detectors include UV-Vis detectors, diode array detectors, fluorescence detectors, and more.

Data Analysis System: The data analysis system processes and analyzes the signals from the detector, generating chromatograms that represent the separation of compounds over time. This system aids in quantifying and identifying the components of the sample.

The high-pressure pump is particularly crucial in maintaining the desired flow rate, which is essential for reproducible and accurate chromatographic separations. The overall system, as you described, is depicted in Figure 3.[17]

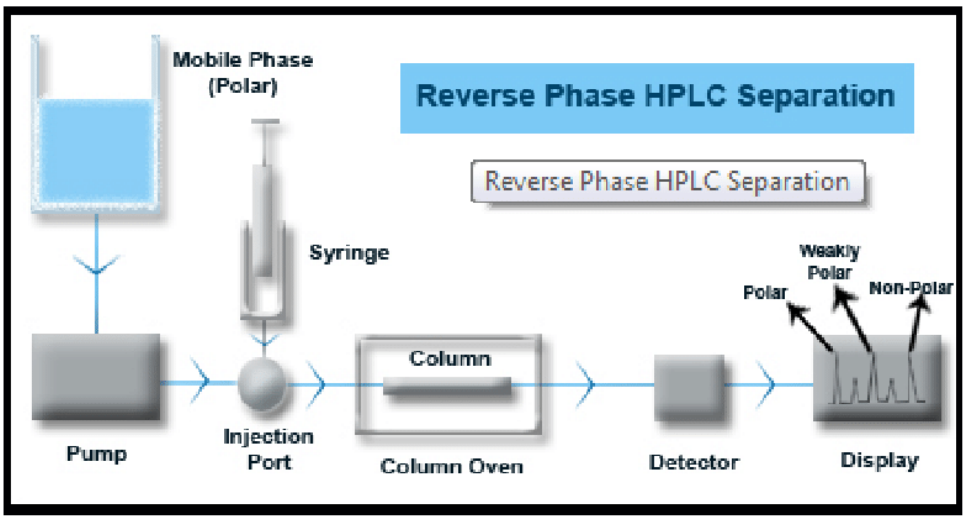

Figure 3: RP-HPLC Technique.

Advantages

- Wide Applicability: RP-HPLC is indeed versatile and can be applied to a broad spectrum of compounds, ranging from small organic molecules to large biomolecules. This flexibility makes it a widely used technique in various scientific fields, including pharmaceuticals, environmental analysis, biochemistry, and more.
- High Sensitivity: RP-HPLC is known for its high sensitivity, allowing for the detection of trace amounts of analytes. This is especially crucial when dealing with samples where the target compounds are present in low concentrations, as is often the case in pharmaceutical and environmental analyses.
- Reproducibility: RP-HPLC provides reproducible results, making it a reliable method for routine analysis. Consistent and repeatable separations are essential for obtaining accurate and reliable data, and RP-HPLC's reproducibility contributes to the robustness of the technique.
- Resolution: RP-HPLC offers high-resolution separations, enabling the identification and quantification of individual components in a mixture. The ability to separate closely related compounds is critical in analytical chemistry, and the high resolution of RP-HPLC facilitates the precise analysis of complex samples.
- Overall, these characteristics contribute to the widespread use and effectiveness of RP-HPLC in analytical laboratories for various applications.[18]

Method Development

Empagliflozin being a hydrophobic drug indicates that it has a stronger affinity for nonpolar environments. Therefore, a reverse phase column with a nonpolar stationary phase, such as a C18 column, is indeed suitable for the analytical method development and validation of empagliflozin.

Hydrophobic Nature of Empagliflozin: The fact that empagliflozin is hydrophobic implies that it interacts well with nonpolar surfaces. In reverse phase chromatography, the nonpolar stationary phase (like C18) promotes the retention of hydrophobic compounds.

Choice of C18 Column: C18 columns, which consist of an 18-carbon alkyl chain, provide an appropriate nonpolar environment for hydrophobic compounds like empagliflozin. These columns are commonly used in reversed-phase HPLC for separating and analyzing such substances.

Mobile Phase Selection: Knowledge of empagliflozin's pKa (acid dissociation constant), solubility, and chemical stability is crucial for selecting an appropriate mobile phase. This information helps in optimizing conditions that enhance the separation and detection of empagliflozin on the C18 column.

In summary, the choice of a C18 column in reverse phase chromatography, coupled with a well-informed selection of the mobile phase based on the compound's properties, is key for the successful analytical method development and validation of empagliflozin.[19,20]

Method Development and Optimization

The appropriateness of the column and the mobile phase in the optimized method has been determined based on selectivity, sensitivity, and acceptable chromatographic parameters of the generated peaks. We utilized the mobile phase as a solvent for all samples to minimize noise and eliminate any undesired solvent peaks.

Selection of UV wavelength

Empagliflozin exhibits a maximum absorption (λmax) at 225 nm in methanol.[6] A satisfactory response was achieved by detecting both brands of the drug at 225 nm (Fig. 2). The optimized HPLC conditions are detailed in Table 1.

Method validation

The method has been validated following the guidelines outlined in the International Conference of Harmonisation (ICH) Q2 (R1).[7] This validation encompasses the assessment of system suitability, precision, accuracy, linearity, the limit of detection (LOD), the limit of quantitation (LOQ), and forced degradation studies.

1. System suitability

System suitability parameters, including tailing factor, number of theoretical plates, and retention time for the Empagliflozin peak, were evaluated by injecting a blank mobile phase followed by six replicates of Empagliflozin (50 µg/ml).

2. Linearity

The linear regression data covering the range of 2 to 12 µg/mL for Empagliflozin, with a correlation coefficient of 0.999, indicates a strong linear relationship between the area and concentration in the calibration curve.

3. Precision, repeatability (intra-day precision) and intermediate (inter-day precision)

System and method precision were evaluated by injecting five independent samples of Empagliflozin (50 µg/ml each) on the same day under identical operating conditions. Intermediate or inter-day precision was assessed by comparing the results of five independent determinations conducted on three different days.

4. Accuracy study and recovery

The accuracy of the method was determined using the standard addition method, involving the addition of pure Active Pharmaceutical Ingredient (API) at three different concentration levels (70%, 100%, and 130%), each performed in triplicate. The accuracy of the method is calculated in terms of the percentage recovery of the API.

5. LOD and LOQ

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Empagliflozin were calculated from the linear regression equation using the standard deviation of the intercept (Q) and the slope (S) with the formulas:

LOD = 3.3 * Q / S

LOQ = 10 * Q / S

Where Q is the standard deviation of the intercept, and S is the slope of the calibration curve.

6. Forced degradation studies

To evaluate the stability-indicating property of the developed HPLC method, stress studies were conducted under conditions recommended by the International Conference of Harmonisation (ICH). Forced degradation of Empagliflozin involved exposing the bulk sample to acidic, alkaline, oxidative, photolytic, and neutral conditions. The objective was to investigate the method's capability to measure the analyte response in the presence of its degradation products.

Acid and alkali hydrolysis: A 1 ml aliquot of Empagliflozin solution (1 mg/ml) was transferred to a small round bottom flask. The solution was then mixed with 9 ml of 0.1N hydrochloric acid or 0.1N sodium hydroxide. The prepared solutions underwent reflux for 2 hours in a boiling water bath. Following reflux, the samples were cooled to room temperature (25ºC) and neutralized with an amount of acid or base equivalent to that which was added previously. From the resulting neutral solution, 20 µl of each was injected into the HPLC system.

Oxidation: A round bottom flask received one milliliter of Empagliflozin solution (1 mg/ml). The contents were mixed with 9 ml of a 30% hydrogen peroxide solution, and the reaction mixture was allowed to proceed at room temperature (25ºC) for 2 hours with intermittent shaking. Subsequently, a volume of 20 µl was injected into the HPLC system.

Irradiation with ultraviolet light: A sample powder of Empagliflozin (10 mg) underwent exposure to UV light (254 nm) for 48 hours. The material was then dissolved in 5 ml of water, and the resulting solution was filtered using a syringe filtration disk, yielding a claimed concentration of 1 mg/ml. This solution was appropriately diluted, and a volume of 20 µl was injected into the HPLC system.

In addition, an aqueous solution of Empagliflozin (1 mg/ml) was also exposed to UV light (254 nm) for 48 hours. After dilution, 20 µl of this solution was injected into the HPLC system.

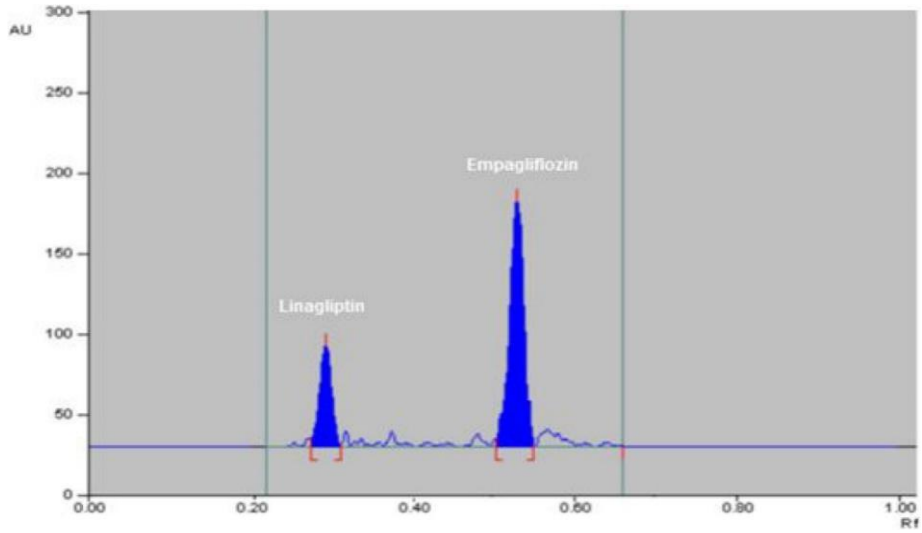

Fig. 2: Empagliflozin λ max at 225 nm in methanol.

Table 1: HPLC conditions.

System	Agilent 1260 Series
Mobile Phase	water: acetonitrile (55:45, v/v)
Flow rate	rate 1 ml/min
Column	C-18, 100 x 4.6 mm, 3.5 µm (Symmetry, Waters)
Oven Temperature	40 degree Celsius
Wavelength of detection	225 nm
Back Pressure generated	134-136 bars
Total Run time	6 min

RESULTS AND DISCUSSION

Linearity Studies

Table 2: Linearity of Empagliflozin.

Parameter	Result
Linearity range	2-12 mcg/ml
Slope	1.14412 x 10-5
Intercept	0.0463436
Coefficient of correlation	0.9994

Table 3: System suitability parameters.

Parameter	Results
Retention time	3.00 min
Tailing factor	1.22
Theoretical plates	7834
% RSD	1.02918

Precision Studies

Table 4: Intra-day precision studies of Empagliflozin.

Conc.	3 mg/ml	5mg/ml	11mg/ml
Mean Conc.	2.986267	4.9276	11.1774
SD	0.042023	0.028758	0.109132
SE	0.010859	0.007431	0.028199
CV	0.014072	0.005836	0.009764
% CV	1.407207	0.583617	0.976359

Table 5: Inter-day precision studies of Empagliflozin.

Conc	3 mg/ml	5mg/ml	11mg/ml
Mean Conc.	2.995111	4.962111	11.25838
SD	0.071386	0.056114	0.113857
SE	0.010655	0.008375	0.016994
CV	0.023834	0.011308	0.010113
% CV	2.383408	1.130847	1.011312

Accuracy and Recovery Studies

Table 6: Accuracy Studies of Empagliflozin.

Amount of sample taken (µg/ml)	5	5	5
Amount of standard added (µg/ml)	2.5	5	7.5
Percentage of Standard added	50	100	150
% Recovery	99.5	99.1	99.8
Relative Standard Deviation	1.13	1.01	1.45

LOD and LOQ

The calculated LOD and LOQ were 0.002669 ng and 0.008007 mg for Empagliflozin.

DISCUSSION

Various mobile phases of different compositions were tested to develop an optimum mobile phase to achieve a satisfactory separation and good peak symmetry for Empagliflozin. A mobile phase consisting of was developed. The analysis was carried out based on peak area with UV detection at 225 nm (Fig. 2). The retention time obtained for Empagliflozin was at 3.00 min. The detector response was linear in the concentration range of 1-12 µg/ml.

Validation of the Proposed Method

A. System suitability

The obtained results of 6 replicate injections showed that the parameters tested were within the acceptable range. Empagliflozin was repeatedly retained at 3.00 min with RSD% of the recorded retention 2.02918 to indicate good repeatability of replicate injections on the integral HPLC system used, the tailing factor never exceeded 1.24 in all peaks indicating good peak symmetry (acceptance limit is < 2) and the number of theoretical plates was always >2000 in all chromatographic runs to ensure good column efficacy throughout the developed separation process.

B. Linearity

A linear correlation was attained between peak area used absorbance vs concentration of Empagliflozin in the range of 2-12 mcg/ml. The linearity of the calibration curve was validated by the high value of the correlation coefficient of regression as shown in Fig. 8 and the results are shown in Table 2.

C. Precision

The %RSD values of intra-day and inter-day for Empagliflozin are less than 2% which reveal that the proposed method is precise and is shown in Tables 4 and 5.

D. Accuracy

The accuracy experiments were carried out by the standard addition method. The high value of recoveries obtained for Empagliflozin indicates that method is accurate as shown in Table 6.

E. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of Empagliflozin were found 0.002669 ng and 0.008007 ng, respectively.

The developed procedure was applied to two marketed formulations of these two compositions of empagliflozin 10 mg and excipients to q.s. The analysis obtained was in uniformity in the claimed amount in the marketed sample. Validation performed according to the ICH guidelines where the results are fast, accurate, robust, specific and linear.

CONCLUSION

The current research represents a report focused on developing a High-Performance Liquid Chromatography (HPLC) method for determining Empagliflozin. The accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ) values were found to be within acceptable limits. Empagliflozin is highly sensitive, making it unstable in alkaline, oxidative, and thermal conditions. However, it remains stable in UV light or acidic conditions. The method is recommended for routine use in quality control laboratories within the industry.

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