Review Article

Analytical Method Development and Validation for Estimation of Empagliflozin in Drug and Dosage Form: A Review

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ABSTRACT

empagliflozin is (1S)-1,5-anhydro-1-(4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy]benzyl}phenyl)-D-glucitolFigure1, A compound called Empagliflozin is works by inhibiting the sodium-glucose co-transporter-2 (SGLT-2) found in the proximal tubules in the kidneys. Through SGLT2 inhibition, empagliflozin reduces renal reabsorption of glucose and increases urinary excretion of glucose. The vast majority of glucose filtered through the glomerulus is reabsorbed within the proximal tubule, primarily via SGLT2 (sodium-glucose linked co-transporter-2) which is responsible for ~90% of the total glucose reabsorption within the kidneys. Na+/K+-ATPase on the basolateral membrane of proximal tubular cells utilize ATP to actively pump Na+ ions into the interstitium surrounding the tubule, establishing a Na+ gradient within the tubular cell. SGLT2 on the apical membrane of these cells then utilize this gradient to facilitate secondary active co-transport of both Na+ and glucose out of the filtrate, thereby reabsorbing glucose back into the blood – inhibiting this co-transport, then, allows for a marked increase in glucosuria and decrease in blood glucose levels. Empagliflozin is a potent inhibitor of renal SGLT2 transporters located in the proximal tubules of the kidneys and works to lower blood glucose levels via an increase in glucosuria, whichis especially suitable for people with GERD who have recurring, chronic symptoms that can be disruptive and last a long time1. Literature survey reveals esomeprazole magnesium trihydrate that is determined by RP-HPLC, LC-MS/MS, UPLC, UVHPLC, UPLC—MS/MS and UV Spectrophotometry.

Keywords: Empagliflozin, HPLC, LC-MS/MS, UPLC, UVHPLC, UPLC—MS/MS, UV.

INTRODUCTION

Method development and validation are an iterative process. The influence of operating parameters on the performance of the method can be assessed at the validation stage which was not done during development/optimization stage of the method. The most significant point raised for validation is that the validity of a method can be demonstrated only through laboratory studies. Guidelines from the United States Pharmacopoeia (USP), International Conference on harmonization (ICH), Food and Drug Administration (FDA) etc., provide guidelines for performing such validations in pharmaceuticals. Results from method validation is used to judge the quality, reliability and consistency of analytical results[2]. Empagliflozin works by inhibiting the sodium-glucose co-transporter-2 (SGLT-2) found in the proximal tubules in the kidneys. Through SGLT2 inhibition, empagliflozin reduces renal reabsorption of glucose and increases urinary excretion of glucose

A variety of methods have been used to estimate and validate Empagliflozin both alone and in combination[3]. The most frequent separation technique in HPLC is reversed-phase chromatography. The reversed-phase method's versatility and ability to handle compounds with varying polarities and molecular masses contribute to its popularity. Reversed phase chromatography has analytical and preparative uses for biological separation and purification. Reversed phase chromatography is highly effective at separating hydrophobic molecules like proteins, peptides, and nucleic acids. This review discusses the significance of RP-HPLC in analytical method development, including methodologies and essential chromatographic parameters that must be tuned for efficiency. In high-performance liquid chromatography, a compound with lower affinity for the stationary phase travels faster and covers a longer distance, while a compound with higher affinity moves slower and covers a shorter distance. This differential migration facilitates effective separation and analysis of sample components. High-performance liquid chromatography (HPLC) proves invaluable in pharmaceutical analysis, efficiently isolating and quantifying major medications, reaction impurities, synthesis intermediates, and degradants. As a preeminent analytical tool, HPLC excels in identifying, measuring, and separating diverse sample components soluble in liquid. Its precision is paramount for both quantitative and qualitative drug product analysis, playing a pivotal role in determining drug product stability. By offering a meticulous approach to characterizing pharmaceutical samples, HPLC stands as an indispensable technique in ensuring the quality and safety of medicinal formulations in the field of analytical chemistry[4]. Liquid chromatography—tandem mass spectrometry (LC—MS/MS) h is becoming a popular technology for analyzing prescription medicines. LC-MS/MS is the only practical measuring approach for substances without natural

chromophores or fluorophores, providing enhanced specificity and sensitivity compared to immunoassay. LC-MS/MS is considered a superior technology to immunoassay because to its higher specificity and sensitivity; however, the main disadvantages of LC-MS/MS are the high instrument costs, increased technical complexity, and analytical speed and turnaround[5].Ultra-high performance liquid chromatography (UPLC) has revolutionized analytical separation procedures, allowing for faster findings without losing quality. UPLC, a derivation of HPLC, works on the idea that decreasing column packing particle size leads to increased efficiency and resolution.

Empagliflozin Fig.1 is used along with diet and exercise, and sometimes with other medications, to lower blood sugar levels in adults and children 10 years of age and older with type 2 diabetes (condition in which blood sugar is too high because the body does not produce or use insulin normally). Empagliflozin is also used to reduce the risk of stroke, heart attack, or death in people who have type 2 diabetes along with heart and blood vessel disease. Empagliflozin is in a class of medications called sodium-glucose co-transporter 2 (SGLT2) inhibitors.

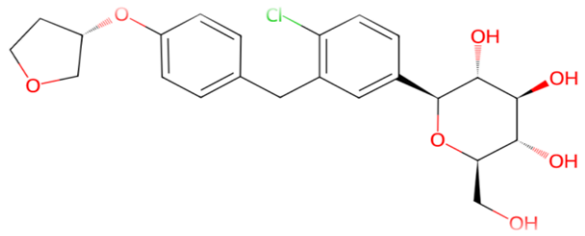

Figure 1: Scheme of Empagliflozin

This study aims to develop a simple, precise, authentic, cost effective and validated analytical method for the estimation of empagliflozin in drug and dosage form. Analytical method development is very important in development and production of new drugs and formulations. The validation of analytical method is a regulatory requirement for submission of New Drug Application (NDA) and Abbreviated New drug Application (ANDA) as a part of U.S generic drug approval. Analytical method development and validation play a vital role in bio-availability, bioequivalence studies and the risk assessment/management[6].

ANALYTICAL METHODOLOGIES

❖ **Shilpa Rathwa:** Authors reported UV Spectroscopy method is simple and sensitive zero order derivative method was developed for the simultaneous estimation of Finerenone and Empagliflozin in their synthetic mixture. Developed methods were successfully applied to synthetic mixture and assay were found to be 98.28% to 101.28% of Empagliflozin and 98.28% to 101.98% of Finerenone. Simple and sensitive. Use the Shimpack ODS C18 column (250 m x 4.6 mm, 5µm) was chosen for the method development process. The proposed analytical method was validated according to ICH guidelines, yielded good results concerning range, linearity, precision, accuracy, robustness, and ruggedness[7]

❖ **Venkatesh Prasath N et al:** To expand an easy to use, available, reliable, and retable RP-HPLC method for determining the formulation and pure form of empagliflozin. Using an isocratic C18 reverse column (250 x 4.6 mm, particle length: 5 µm) with an ammonium acetate and acetonitrile (62:38 v/v) cell section, the method operates at a drift fee of 1ml/minute at a detection wavelength of 265 nm. Empagliflozin within the gift of deterioration merchandise as fast, economic, and dependable products. Forced degradation examine for acid, alkali, oxidation, picture stability, dry warmth, and impartial degradation. Empagliflozin and had measured retention times of 5.3 minutes each. The percentage recoveries of Empagliflozin have been 98.24% respectively. It became discovered that the assay's relative general deviation for empagliflozin was much less than 2%. The correlation coefficient for empagliflozin became determined to be 0.998. The limits of detection and quantification for empagliflozin had been 4.14 ng/ml and 12.55 ng/ml, respectively. For the motive of estimating empagliflozin, a completely unique, quick, sensitive, and solid RP HPLC method changed into evolved and validation changed into performed according with ICH guidelines[8].

❖ **Ashim kumar Sen et al:** Trijardy XR® consisting of empagliflozin, linagliptin, and metformin hydrochloride, a fixed-dose combination (tablets) that improves glycemic control in individuals with diabetes mellitus (type 2). The present work presents four spectrophotometric methods that are quick, effortless, accurate and reproducible for the concurrent assessment of the ternary mixture. Materials and Methods: The 1st approach works on the principle of solving established equations (simultaneous) by measuring absorbance at 224.6, 226 and 237.2 nm for empagliflozin, linagliptin and metformin hydrochloride, respectively. The second method namely ratio difference spectroscopy works by measuring the difference in amplitude at two different wavelengths in the ratio spectra. Whereas, the derivative ratio spectrum zero-crossing approach (third approach) relied on the utilization of the derivative ratio signals at zero-crossing locations. The fourth approach is the double divisor-ratio spectra derivative approach in which the first derivative of ratio spectrum was acquired and the concentrations of all 3 drugs in their combination were quantified. Results and Discussion: All the three drugs exhibited excellent linear correlation in the concentration series of 2-10 µg/ml for simultaneous equation method and 0.5-10 µg/ml for all the other methods with an exceptional correlation coefficient value. Furthermore, the projected approaches were validated as per ICH strategies and which displayed good precision, accuracy and sensitivity. Conclusion: The developed spectrophotometric approaches when compared to other analytical procedures are regarded to be more cost-effective because they do not require expensive solvents or sophisticated instruments.

Therefore, the projected methods could be effectively employed for the concurrent assessment of empagliflozin, linagliptin and metformin hydrochloride in ternary mixture[9]

❖ **Wael Abu Dayyih et al:** Empagliflozin is a sodium-glucose co-transporter-2 (SGLT2) inhibitor. SGLT2 transporters are primarily responsible for glucose reabsorption in the kidney. In 2014, empagliflozin was approved for medical use in the United States and the European Union. With over 4 million prescriptions in 2019, it was the 146th most commonly prescribed medication in the United States in 2019. The spectrophotometric determination of empagliflozin is described using coupling agents such as 3-chloro-4- nitroaniline or sulfanilamide. These methods are straightforward and are based on the reaction of empagliflozin with diazotized products of 3-chloro-4-nitroaniline or sulfanilamide to produce colored azo dyes with absorption maxima at 470 and 480 nm. Empagliflozin was linear from 1.2 to 26.6 µgml−1 or 0.8 to 20.4 µgml−1 when combined with diazotized 3-chloro-4-nitroaniline or sulfanilamide, respectively. Empagliflozin's molar absorptivity and Sandell's sensitivity to 3-chloro-4-nitroaniline or sulfanilamide azo dyes were 3.179 × 104 l mol−1cm−1 or 4.367 × 104 l mol−1cm−1 and 1.149 × 10−2 µgcm−2 or 8.368 × 10−3 µgcm−2, respectively. The formed colored azo dyes are stable for more than 12 hours. The optimal reaction conditions and other analytical parameters are assessed. Foreign organic compound interference has been studied. The method has been successfully used to determine empagliflozin in pharmaceutical sample [10] .

❖ **A. Raja Reddy et al**: The present study was to develop a simple, precise, accurate method was established for simultaneous estimation of Metformin hydrochloride and Empagliflozin in bulk and pharmaceutical dosage form. The separation was done effectively by injecting 10 µl and standard solution were injected to C18 150 mm I.D × 4.6 mm,5 µm column using 0.1N Potassium dihydrogen phosphate as mobile phase A and Acetonitrile as mobile phase B in the ratio of 50:50. The gradient flow rate was optimized as 1 mL/min. Wavelength selected for detection was 260nm. Results: Only as per ICH guidelines the complete validation has been carried out. The retrieval study was carried in between 25% to 150% level of concentration and the results obtained were in the range of 99.3-99.6%for both the Analytes. The linearity was attained in between the range of 25-125 mg/ml and 1.25-25 mg/ml for Metformin hydrochloride and Empagliflozin respectively with a linear regression curve(R=0.999). Conclusion: The developed method was robust for different parameters like column temperature flow rate, mobile phase, PH, composition and gradient. The HPLC method which has been developed for estimation of Metformin hydrochloride and Empagliflozin can be successfully used as release test in manufacturing units of quality control department[11]

❖ **Shilpi Pathak A et al:** Stability-indicating RP-HPLC method was developed and validated for the estimation of empaglifozin drug and its tablet dosage form using a DAD detector. The mobile phase consisted of methanol/ acetonitrile/0.1%OPA (75:20:5). The peak was observed at 2.54 min using 222.0 nm absorption maxima. Results: Calibration curve plot was found within the range of 10–50 µg/mL. The coefcient of determination (R2) was found to be 0.9990. Forced degradation studies were performed for the empaglifozin in various conditions, and the results were calculated as %RSD values and were found to be within the limits. Conclusion: The method was validated as per ICH guidelines with respect to all validation parameters[12].

❖ **T Anas M. Hanifhe et al:** current investigation is based on efficient method development for the quantification of empagliflozin in raw and pharmaceutical dosage forms, as no pharmacopoeial method for the drug is available so far. The developed analytical method was validated as per ICH guidelines. C18 column with mobile phase (pH 4.8) consisted of 0.1% trifluoroacetic acid solution and acetonitrile (70:30 v/v) was used for drug analysis. The calibration plot showed good linear regression (r 2>0.999) over the concentration of 0.025-30 µg mL-1. The LOD and LOQ were found to be 0.020 µg mL-1 and 0.061 µg mL-1, respectively. The percentage recovery was estimated between 98.0 to 100.13%. Accuracy and precision data were found to be less than 2%, indicating the suitability of method for routine analysis in pharmaceutical industries. Moreover, the drug solution was found to be stable in refrigerator and ambient room temperature with mean % accuracy of >98%. Empagliflozin contents were also tested in both the raw API and marketed tablet brands using this newly developed method. The mean assay of raw empagliflozin and tablet brands were ranged from 99.29%±1.12 to 100.95%±1.69 and 97.18%±1.59 to 98.92%±1.00 respectively. Based on these findings, the present investigated approach is suitable for quantification of empagliflozin in raw and pharmaceutical dosage form[13]

❖ **Geetha Susmita**: The objective of this study was to develop and validate a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of the metformin and empagliflozin in tablet dosage forms. The chromatographic conditions were optimized and it was run through Std. BDS (250 mm × 4.6 mm, 5 m) column with mobile phase consisting of 0.1% orthophosphoric acid buffer: acetonitrile in the ratio of 50:50. The flow rate was 1 ml/min and optimized wavelength was 210 nm.Temperature was maintained at 30°C. A sensitive, rapid, and specific method has been developed for the simultaneous estimation of metformin and empagliflozin using RP-HPLC [14.]

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