Research Article

Development and validation of novel stability indicating RP HPLC Method for quantitative Estimation of Empagliflozin in Tablets

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ABSTRACT

The current study was conducted to develop and validate a novel method for the qualitative and quantitative determination of Empagliflozin in bulk granules and tablets. This method was successively applied to the analysis of the approved dosage form of Empagliflozin available in the market with the brand name of Jardiance tablets. The developed method was found to be precise, economic, and rapid with a run time of 20 minutes. The Empagliflozin peak was eluted at 5.0 minutes. This elution was done on the C18 column (150 X 4.6mm) having a 5µm particle size using an isocratic mobile phase, a mixture of phosphate buffer pH 3.0 and methanol in the ratio of 70:30 at a flow rate of 1.0ml/min with PDA detector (maximum wavelength of 224nm) and column temperature 30°C. The method was found to be linear with LOD and LOQ, 0.068 µg and 0.35 µg, respectively. The correlation coefficient R2 calculated was 0.996. Precision and accuracy of the method evaluated by interday, intraday, and recovery study was found to be within limits. The forced degradation study proved the stability of the method. **Objectives:** This research article is aimed at developing a new method for developing and validating a novel method for the qualitative and quantitative determination of Empagliflozin in bulk granules and tablets. **Methods:** To conduct this research, all the validation parameters were employed according to International Council for Harmonization (ICH) guidelines and all the validation parameters e.g., Accuracy, specificity, linearity, LOD, LOQ, robustness, and degradation studies were performed. **Results:** Results of the proposed technique were found to be trustworthy, accurate, suitable, and robust for everyday application. **Conclusion:** Conclusively the results of the study indicated that the current method is simple, precise, accurate, stable, and economic, and thus can be used for routine quality control testing of Empagliflozin.

Keywords: Empagliflozin, RP-HPLC, Method Validation, Qualitative Determination

Chemically, Empagliflozin is (1-chloro-4[b d-glucopyranos-1-y1]-2-[4-([1]-tetrahydrofuran-3-yl-oxy) benzyl]-benzene [1-2]. Empagliflozin controls the excess glucose level in the blood. It is an inhibitor of sodium-glucose cotransporter-2(SGLT-2) and promotes the excretion of excess glucose in urine by reabsorbing it in the kidney, thus is used in the treatment of type 2 diabetes. [1,3-4]. The empirical formula of Empagliflozin is C23H27ClO7 and its molecular mass is 450.91 a.m.u [5-6]. Empagliflozin is available in 10 mg and 25mg oral tablet dosage forms alone or in combination with other drugs. Jardiance, Glyxambi, Synjardy, and Synjardy XR are examples of Empagliflozin tablets [7-10].

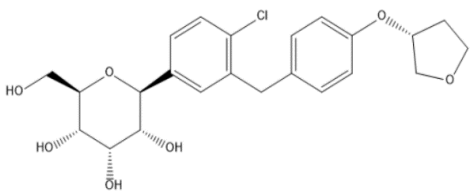

Figure 1 – Structure of Empagliflozin

The development of a quantitative method and its validation are fundamental and essential phases in pharmaceutical analysis to ensure that the results of the proposed technique are trustworthy, accurate, suitable, and robust for everyday application. The literature review revealed that Empagliflozin can be detected both qualitatively and quantitatively by using Liquid Chromatography (LC), Ultraviolet spectrophotometer (UV), High-Performance liquid chromatography (HPLC), and Ultra Performance Liquid Chromatography (UPLC) techniques [11-15]. However, few reports are available for the RP-HPLC measurement of empagliflozin in pharmaceutical preparations. In this article, we discussed the validation of a novel technique for precise Empagliflozin quantification in pharmaceutical dosage forms and bulk pharmaceuticals.

The validation is important as Drug regulatory governing bodies play a vital role and have placed emphasis on drug manufacturing companies to offer comprehensive information regarding the process for the validation of analytical methods

[15]. We performed the validation in accordance with ICH [16-18]. The current strategy, which is brand-new to the market, uses several mobile phase compositions to estimate Empagliflozin in a quality control laboratory. In accordance with the recommendations of the International Council for Harmonization, the method's performance was validated.

This study was conducted to develop and validate the novel, accurate, precise, and stability-indicating RP-HPLC method for the analysis of Empagliflozin in bulk granules and tablets. In the development stage, the wavelength detection study was conducted by scanning the sample between 200nm and 400nm. A major peak was found at 224 nm (λ max). Then the method was validated for validation parameters I-e accuracy, precision (repeatability, intermediate precision), specificity, the limit of detection, the limit of quantification, linearity, range, and robustness as per ICH and United States Pharmacopeia (USP) guidelines using HPLC [11, 19]. Stress study was performed in acid, base, peroxide solutions, thermal, and photolytic stress conditions to check the stability of dosage form as well as the robustness of the method [20-21].

MATERIALS AND METHODS

All the chemicals, Methanol, Acetonitrile, Hydrochloric Acid, Hydrogen Peroxide, Sodium Hydroxide, and Monobasic hydrogen phosphate were purchased from Science Center Merk distributor Islamabad. All the chemicals were used without any further purification.

Instrumentation: Gradient HPLC model LC20 (Shimadzu Japan) (LC20AT) equipped with PDA detector (SPD M-20A), autosampler (SIL-20AHT), Column oven (CTO-20A) and degassing unit (DGU-20ASR) was used for the study. Computer-supported software-based Spectrophotometer model 1800 (Shimadzu Japan) for wavelength detection and FT-IR model Alpha (Germany) with ATR diamond ATR module for identification of Empagliflozin were employed. Calibrated analytical balance Metler Tolendo was used for weighing purposes. PH meter was served for the adjustment of the pH of the buffer for the mobile phase. A neutronic Photostability chamber was used for the photostability study, and thermal degradation was checked using a memart oven. An ultrasonic bath was used to dissolve the samples.

Chromatographic Conditions: In the isocratic mobile phase, the mixture of phosphate buffer pH 3.0 and methanol in the ratio of 70:30 with a flow rate of 1.0 ml/minute and column temperature of 30 °C was used. The flow rate was set at 1.0 ml/minute and the column temperature was kept at 30 °C. We kept the Injection volume at 20 µL. The mobile phase was also used as diluent and chromatograms were recorded at 224nm. The summary of chromatographic conditions is mentioned in Table 1.

Table 1 – Chromatographic conditions

Mobile phase	Phosphate buffer: Methanol (70:30%V/V) pH adjusted to 3.0
Column	ODS C18, 250×4.6 mm, 5µ
Wavelength	224 nm
Flow rate	1.0 mL/min
Injection volume	20 µL
Run time	10 min
Diluent	Phosphate buffer: Methanol (70:30%V/V) pH adjusted to 3.0

Preparation of solutions

Preparation of Mobile Phase: 6.8 grams of monobasic potassium phosphate was dissolved in 900 ml deionized water in a 1000ml volumetric flask, the pH was adjusted to 3.0 with orthophosphoric acid and the solution was filtered through a 0.5-micron filter and degassed. Then phosphate buffer and methanol were mixed in a ratio of 70:30 to make the isocratic mobile phase.

Preparation of standard solution: After weighing carefully, the Empagliflozin working standard equivalent to 25mg, was dissolved in a diluent in 50 ml volumetric flask. The solution was filtered and 2ml of the filtrate was taken to 50 ml volumetric flask, mixed well, and the volume was made with diluent to 50 ml and sonicated for 5.0 minutes.

Preparation of sample solution: We took 20 tablets, weighed, and powdered them. Then the powder equivalent to 25 mg Empagliflozin was dissolved in diluent and the volume was made up to 50 ml in the volumetric flask. After filtration, 2.0 ml of this solution was transferred to another 50 ml volumetric flask, mixed well with diluent, and sonicated for 5.0 minutes.

Method Development: We performed some preliminary tests for the development of a stable and effective method for qualitative and quantitative estimation of Empagliflozin. First, the wavelength was detected using a UV-Visible spectrophotometer by scanning the sample and standard solution between 200nm to 400nm. A major peak of Empagliflozin was observed at 224nm (Figure 2).

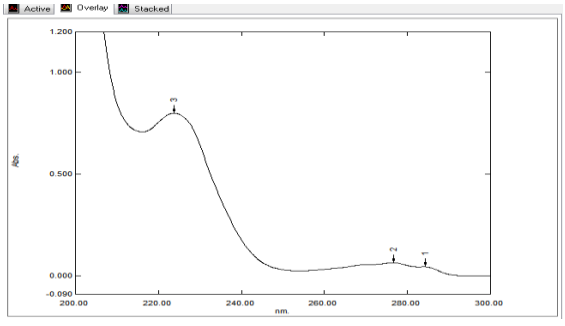

Figure 2 – UV spectrum of Empagliflozin

Then many injections of the sample were applied on HPLC using water, methanol and acetonitrile, and phosphate buffer in different compositions (to determine the optimal mobile phase combination) as mobile phase. The mobile phases tested included methanol and Acetonitrile in the ratios of 60:40 and 70:30, phosphate buffer, and methanol with 65:35, 60:40, and 70:30 ratios to observe the separation and characteristics of the peak. It was observed that the best symmetry of peak with less retention time and low pressure of HPLC pump was achieved using phosphate buffer and methanol in the ratio of 70:30 as mobile phase. The optimum flow rate selected after hit and trial basis was 1.0 ml per minute.

System Suitability: A suitability study of the system was conducted to confirm its performance.

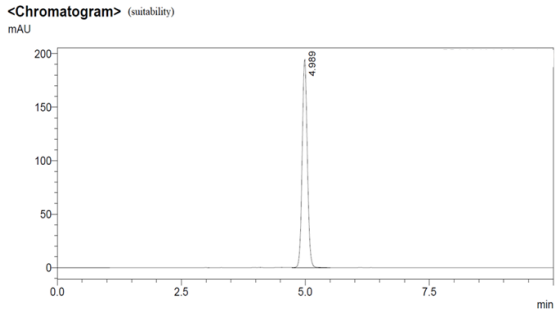

Figure 3 – Suitability chromatogram

All critical parameters including percentage Relative standard deviation (RSD), tailing factor, USP plate count, and USP resolution were verified by injecting six injections of standard solution. The results of system suitability are depicted in Table 2.

Table 2 – System suitability

S.NO.	RT (Min)	Peak Area	No. of Theoretical plates (USP)	Tailing (%)
1	4.992	1404259	9283	1.068
2	4.989	1404395	9300	1.069
3	4.990	1404188	9245	1.069
4	4.992	1404778	9308	1.065
5	4.991	1404595	9327	1.066
6	4.996	1403433	9329	1.067
Mean Area		1404280.67		
SD		426.27		
%RSD		0.03		

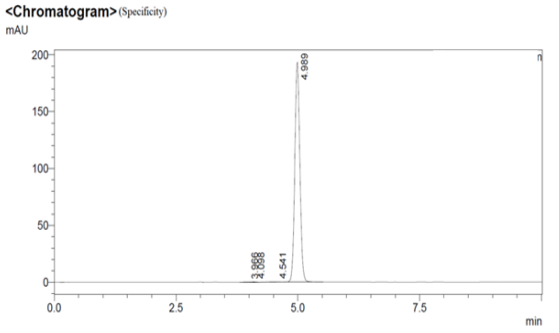

Figure 4 – Specificity chromatogram

Specificity: The analytical method should have the ability to resolve all its process impurities, known and unknown degradation impurities during stress study. A placebo should not interfere with the retention times of active drugs and Impurities. The specificity of the method was confirmed by injecting the blank, placebo, and standard solution.

Linearity: The linearity of the method was checked by injecting 5 standard solutions of concentration ranging from 25 µg/ml to 125 µg/ml. Then the graph was plotted between concentration and peak areas. A straight line was obtained which confirmed the linear response of the method (Fig. 5).

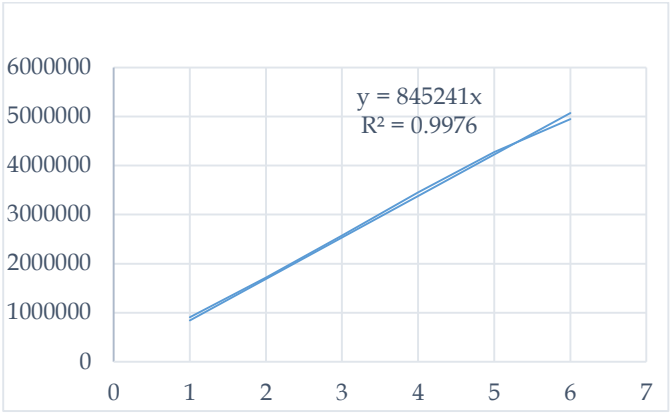

Figure 5 – Linearity curve

The intercept, slope, correlation coefficient of regression, and sum of squares were calculated. The linearity results are presented in Table 3.

Precision

a) **Repeatability:** For repeatability testing, we applied six replicate injections of the same concentration of sample solution on the same day. All the parameters including percentage RSD, tailing factor and USP plate were checked and are mentioned in Table 4.

Accuracy: The accuracy of a method describes how close the results are to a true value. The accuracy study was conducted by calculating the percentage recovery of Empagliflozin. It was processed by injecting three replicates of three different concentrations of the analyte. In the end, the percentage recovery of the analyte was determined to confirm the accuracy of the system and depicted in Table 6.

Table 3 – Linearity

Concentration	Peak Area
25 µg/ml	910323
50 µg/ml	1715682
75 µg/ml	2568999
100 µg/ml	3452698
125 µg/ml	4274632
150 µg/ml	4947380

Table 4 - Repeatability

Injection/Replicates No.	Retention time(min)	Peak Area
1	4.990	1376056
2	4.984	1384229
3	4.993	1379343
4	4.994	1381435
5	4.989	1381614
6	4.990	1381372
Mean	4.990	1380674
SD	2745.37	
%RSD	0.20	

Table 5 – Intermediate precision

Conc.	Peak Area (Day1)		Peak Area (Day 2)	
	666757	Mean 667245.67	668277	Mean 668547
50% (0.25 mg/ml)	667094	SD 579.8	668184	SD 550.73
	667886	%RSD 0.09	669181	%RSD 0.08
	1382257	Mean 3301765	1370368	Mean 1371163
100%(0.5 mg/ml)	1383726	SD 1976.45	1371396	SD 707.86
	1384232	%RSD 0.06	1371725	%RSD 0.05
	2049014	Mean 2050650	1961242	Mean 1962663
150% (0.75 mg/ml)	2050820	SD 1558.91	1962357	SD 1596.15
	2052118	%RSD 0.08	1964390	%RSD 0.08

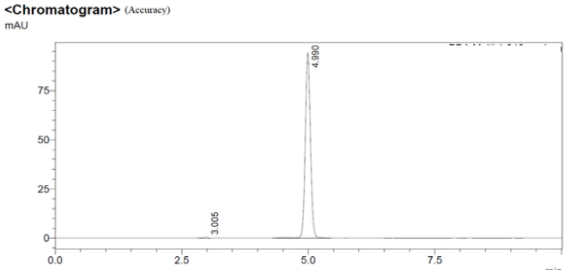

Figure 6 – Accuracy chromatogram

Limit of Detection (LOD) & Limit of Quantitation (LOQ): Qualitative analysis of an analyte in a tablet at the lowest possible concentration is called the limit of detection. It can be calculated by the formula LOD = 3.3σ/S, where σ is the standard deviation of response and S is the slope of the calibration curve. Whereas the lowest possible concentration of an active ingredient in a tablet that can be detected quantitatively is called the limit of quantitation. In the recent study, the limit of quantitation was calculated in the sample with acceptable precision and accuracy limits by the formula LOQ=10σ/S. The results of the limit of detection of Empagliflozin calculated in XR tablets were found to be 0.068µg/ml while the limit of quantitation came out to be 0.35 µg/ml.

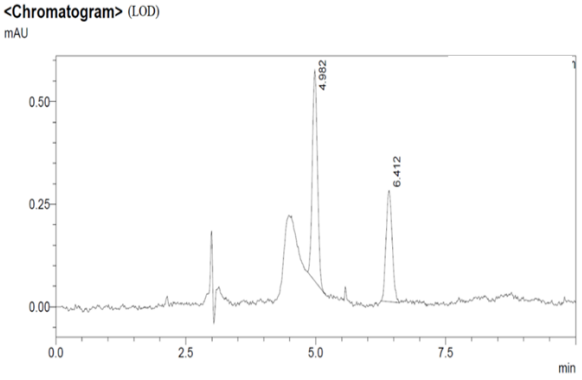

Figure 7 – Limit of detection

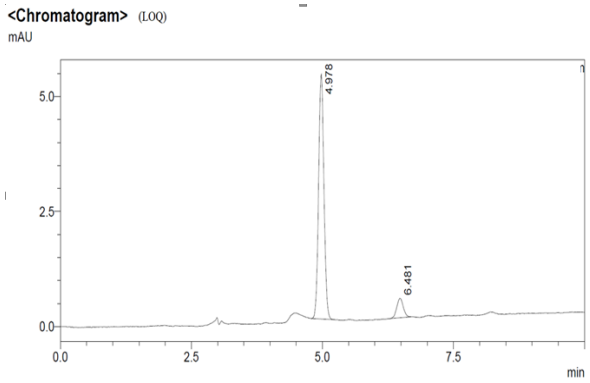

Figure 8 – Limit of Quantitation

Robustness: The robustness of the analytical method is the capacity to remain unchanged by a small change in conditions. High system robustness ensures a positive indication in normal use. The robustness of the method was checked by making minor changes in the composition of the mobile phase, flow rate, temperature of the column, and the pH of the buffer. It was confirmed that no significant changes occur after deliberately changing the system conditions. The robustness results are depicted in Table 7.

Table 6 – Accuracy of the system

Concentration, µg/mL	%Age Recovery	Statistical analysis
40 µg/mL	99.5	Average = 99.26%
	99.4	S.D. = 3.21
	98.9	%R.S.D. = 0.32%
	99.2	Average = 99.46%
60 µg/mL	99.5	S.D. = 2.52
	99.7	%R.S.D. = 0.25
	98.6	Average = 98.90%
80 µg/mL	99.3	S.D. = 5.57
	98.8	%R.S.D. = 0.56%
Avg. % Recovery	99.2	Overall Avg.SD = 3.11
		Overall Avg. %RSD = 0.313

Table 7 – Robustness of the method

Conc.	Flow Rate ± 5%		Temperature ± 5° C		Mobile Phase	
5mcg/ml	1.05ml/min	0.95ml/min	25° C	35 ° C	65:35	75:25
Avg. Peak Area.	12153 19	14788 16	133 897 8	134 867 6	132 166 3	134 671 5
Retention Time	4.96	5.0	4.98	4.97	4.97	4.99
SD	677.44	490.64	359 3.96	634 9.12	256 7.10	626.78
%RSD	0.06	0.03	0.27	0.47	0.19	0.05

Table 8 – Stress stability study

Type of Degradation	Avg. Peak area	% Recovery	% Degraded
Acid	1374560	97.6%	2.4%
Alkali	1361089	97.2%	2.8%
Dry Heat	1296005	96.8%	3.4%
UV	1308775	27.5%	2.5%

Stress stability study: This study was conducted to assess the stability of the method under stressed conditions. The forced degradation parameters like acid, alkali, oxidative, photolytic, thermal, and hydrolytic analyses were carried out under the scope of this study.

Acid Degradation study: In the acid degradation study, 1ml of 2N HCl was added to 1ml of stock solution of Empagliflozin and the mixture was refluxed for 30 minutes at 60°C. The resultant solution was diluted with diluent to 5 µg/ml. Then three replicate injections of 10 µl solution were injected into the chromatograph and chromatograms were observed to check the stability of the solution. The percentage degradation was checked by comparing the results with the standard.

Alkali Degradation Study: This study was conducted by taking 1ml of stock solution and 1ml of 2N NaOH solution and the mixture was refluxed for 30 minutes at 60°C. Then the solution was diluted to 5 µg/ml. Three replicate injections of 10 µl solution were then injected into the chromatograph and the behavior of the peak area was observed. This peak area was then compared with that of the standard.

Dry Heat degradation study: Thermal degradation was carried out by placing powder of Empagliflozin in a petri dish at 105°C for 1 hour. Then 5 µg/ml solution of Empagliflozin was made and triplicate injections were applied to the chromatograph and the degradation was observed by comparing the peak areas with the standard solution.

Photo stability: For photolytic degradation, a standard stock solution of Empagliflozin was placed in a photostability chamber for 5 days. Then after 5 days, the powder was diluted to make the concentration of about 5 µg/ml and then triplicate injections were applied to the chromatograph and the results for possible degradation were observed. The standard solution kept in normal condition was studied versus the photolytic standard solution and then the percentage degradation was calculated. The results of degradation studies were depicted in Table 8.

RESULTS AND DISCUSSION

With the advancements in technology and instrumentation, there comes the requirement to design an efficient, dependable, and selective quantification approach for active pharmaceutical components. Though numerous analytical techniques have been employed to estimate drugs, researchers continue to develop alternative and more efficient HPLC methods to quantify a wide range of compounds.

We also employed a novel method for the pharmaceutical assay of empagliflozin tablets. We developed an accurate, simple, and stable reverse phase HPLC method for both qualitative and quantitative estimation of Empagliflozin using different ratios of mobile phase, different chromatographic conditions, diluents, and flow rates for analysis. In optimal conditions of isocratic mobile phase with the combination of phosphate buffer pH 3.0 and methanol (70:30), C18 column (150X4.6mm) with particle size 5µm and flow rate of 1.0 ml per minute, accurate and precise results were obtained. The results of system suitability, specificity, linearity, and robustness were all within good acceptable limits. There was no interaction of solvents like mobile phase, diluents, and excipients with the peak of Empagliflozin. Additionally, statistical analysis revealed negligible differences between the peak areas during precision, suitability, and repeatability studies. A fit value of 0.9996 was obtained for the regression equation y = 845241x, indicating a strong correlation. It was discovered that the minimal detection and quantification limits were 0.068 µg/ml and 0.35 µg/ml, respectively. Due to the proper selection and composition of the mobile phase, the reduced detection values in our investigation were noted. Lower standard deviations and coefficients of variation were found in the drug assay results, indicating good procedure accuracy.

The peak purity of a sample depends on the selectivity and specificity of an analyte. For confirmation of peak purity, the drug solution was also tested against a placebo mixture (with excipients used in tablet formulations).A lack of co-elution peaks during the drug sample's retention time indicated that the current approach was suggestive of both selectivity and specificity. Since there was no peak of any additive during the analytical run of empagliflozin, the devised method can be effectively used to quantify the medicine in the marketed tablets. The underlying methodology was confirmed to be reliable because no concentration change was seen when the flow rate, column temperature, or mobile phase composition

was changed. Previous studies also confirmed the reliability of analytical methods, frequently by altering the solvent composition, flow rate, and column temperature to a limited extent [22]. Based on the results, it can be concluded that the present method can be employed in pharmaceutical labs for routine quality control testing and can serve as a basis for further modifications in the method in the future.

CONCLUSION

Validation parameters mentioned in the recent study were checked according to ICH guidelines and satisfactory results were obtained. A stress stability study confirmed that the method is stability-indicating. Therefore, based on the study conducted it can be ensured that the method can be applied to the pharmaceutical lab for routine analysis of tablet dosage form.

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