RESEARCH ARTICLE

RP-HPLC Method Development and Validation for an Estimation of Empagliflozin from Bulk and Pharmaceutical Dosage Form

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ABSTRACT

The objective of a recent investigation was to develop an RP-HPLC technique for assessing empagliflozin in both bulks and pharmaceutical dosage forms. An Agilent Eclipse XDE C-18 (4.6 mm x 250 mm, 5.0 m particle size) column for separation, a mobile phase of 0.1 M TEA pH 5.5 corrected by methanol and OPA in a proportion of 4:96 percent v/v at a flow rate of 0.7 mL/minwas utilized towards achieving the desired peak resolution. Diagonal array detectors (DADs) are utilized to measure the eluent at a wavelength of 270 nm. The regression equations for empagliflozin in bulk were y = 58.924x-5.693 and the regression equations for empagliflozin in tablet dosage form were y=57.927x+5.027. The calibration curve shows that the peak size was proportionate toward the concentration. In the precision study, the % of the amount was found in the 100 to 101% range. In %accuracy, %RSD was found to be 99.18 to 99.84 for empagliflozin in bulk and 99.29 to 99.53 for empagliflozin in the tablet dosage form. The limit of detection (LoD) for empagliflozin was determined to be 0.309143 µg/mL, while the limit of quantitation (LoQ) was determined in the direction of 0.936798 µg/mL. Based on the findings of the robustness experiments, it was determined that the method's accuracy and specificity remain within acceptable ranges when subjected to minor adjustments to flow rate, wavelength, and mobile phase. The established technique was suitable for use in quality control labs to determine the quantity of empagliflozin present in bulk and tablet formulation.

Keywords: Empagliflozin, Method development, RP-HPLC, Methanol, Bulk, Tablet

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INTRODUCTION

The development of analytical methods and their subsequent validation are essential aspects of the development of any pharmaceutical product. The development and validation activities relevant to drug products will be the primary emphasis of this technical brief. Undervalued is the advantage that well-developed analytical procedures may bring to the overall efficiency of a program's development in terms of both time and cost, even though this benefit is often considered normal.[1,2] The development of analytical methods and their validation are two separate but interrelated tasks that are carried out continuously throughout the drug development process. The term "validation" refers to the process by which the capabilities of a measuring technique are determined and the accuracy with which a given method assesses a parameter is verified.[3-5]

People who have T2D often use empagliflozin in conjunction with dietary changes, physical activity, and even additional drugs to get their blood sugar amounts down. Cardiovascular disease, as well as T2D in many other individuals or blood vessel disease, may benefit from taking empagliflozin since it lowers their chance of having a stroke, heart attack, or passing away altogether.[6-8] Empagliflozin is also administered to people with heart failure to lessen the likelihood that they will need hospitalization and will pass away due to heart and blood vessel illness. Empagliflozin belongs to the type of drugs recognized as SGLT2 potent inhibitors. SGLT2 refers to sodium-glucose co-transporter 2.[9-11] It does this by making the kidneys excrete more glucose via urine, which in turn decreases blood sugar levels. Type 1 diabetes

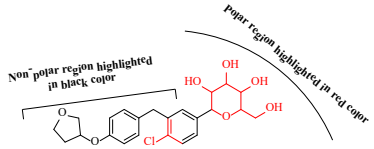

Figure 1: The structure of Empagliflozin with the polar and non-polar areas

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or diabetic ketoacidosis is not treated with Empagliflozin by prescription.[12-14] In people with type 1 diabetes, the body stops producing insulin and, as a result, is unable to manage the quantity of sugar that is present in the blood. It was observed that empagliflozin possesses polar and non-polar regions that are highlighted in different colors in Figure 1. The IUPAC name of empagliflozin is 2-(4-chloro-3-(4-((tetrahydrofuran-3-yl) oxy)benzyl)phenyl)-6hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol.[15]

Empagliflozin can be estimated using limited high-performance liquid chromatography (HPLC), reverse-phase high-performance liquid chromatography (RP-HPLC), and ultraviolet (UV) methods, according to a survey of the relevant published research.[16-28] This study was undertaken to determine the best RP-HPLC technique aimed at determining the quantity of empagliflozin in both bulks and pharmaceutical dosage forms. The recommendations laid out by ICH were used to perform the technique validation.

MATERIALS AND METHODS

Chemicals, Reagents, and Instruments

Agilent's LC 1100 series autosampler system with reciprocating plunger pump, auto-injector, and diode array detectors (DAD) was used for chromatography. The wavelength was determined using an analytical technology UV spectrophotometer. All calibrated volumetric glassware (Borosil) was utilized in this validation process. Swapnroop medicine and pharmaceuticals, Aurangabad, Maharashtra, India, provided effective pharmaceutical grade specified empagliflozin as a gifting sample. A neighborhood pharmacy sold a combo product, including the three medications. Merck Ltd., India, sent TEA, OPA, sodium hydroxide, hydrochloric acid, hydrogen peroxide, HPLC-grade acetonitrile, and water, in addition to methanol.

Method Development

Chromatographic Condition

We employed an Agilent LC 1100 series autosampler system for this chromatography experiment equipped with a reciprocating plunger pump, auto-injector, and DAD. The data gathered during and after chromatographic analysis was recorded and evaluated using a Data Ace chromatography data system. The chromatographic parting was performed on an Agilent Eclipse XDE C-18 with a mobile phase of 0.1 M TEA pH 5.5 modified with methanol and OPA in a proportion of 5:95 v/v at a flowing rate of 0.7 mL/min (4.6 mm x 250 mm length, 5.0 m particle size). DAD detection was utilized to evaluate the eluent at a wavelength of 238 nm. The mobile phase was filtered through a 0.45 µm nylon filter and then sonicated in an ultrasonic bath to prepare it for use (an ultrasonic electronic instrument). The stress-degraded investigation used a highly precise pan of water, including an MV controller to carry out the required reactions in solution (i-therm, Biomedical, India). Thermal stability was evaluated using a dry-air oven. In addition, micropipettes and an analytical balance (a WENSARTM High-Resolution Balance) were employed.[29-30] Table 1 provides a summary of the chromatographic parameters.

Table 1: The chromatographic conditions applied in the current investigation

Sr. no	Parameters	Conditions
1	HPLC	AGILENT (1100)
2	Software	CHEMSTATION
3	Column	Id 4.6 × 250 mm length
4	Particle size packing	5.0 µm
5	Stationary phase	C18 (AGILENT)
6	Mobile phase	Methanol: 0.1 % OPA Water pH 2.8 (4:96)
7	Detection wavelength	270 nm
8	Flow rate	0.7 mL/min
9	Temperature	33ºC
10	Sample size	20 µL

Preparation of Standard and Stock Solutions

Empagliflozin 10 mg was measured in an A-grade volumetric flask to form a stock solution (1000 g/mL), and the remaining space was filled with HPLC-grade mobile phase. Aluminum foil was used to shield the stock solution from light. Using an A-grade bulb pipette, aliquots of the standard stock solution of the commercially available formulation were pipetted into 10 mL volumetric flasks, as well as then the volume was then filled up with mobile phase towards getting final concentrations of 5, 10, 15, 20, and 25 µg/mL of empagliflozin. For the calculation of the assay, the standard solution and the sample solution are introduced into the chromatographic system.[31]

Selection of Wavelength for Empagliflozin

After adjusting for background, a 10 µg/mL working standard solution was examined. in a UV spectrophotometer amongst 200–400 nm against methanol. At 272 nm, the maximum lambda value was detected, making this wavelength the one chosen for further investigation.

System Suitability

System suitability testing was initially thought to be used throughout the industry of pharmaceuticals towards deciding in any pharmaceuticals workplaces when the accuracy of the data is crucial and a particular analysis is appropriate. Chromatographic systems are being employed regularly. However, recent research has shown that system suitability testing is not used for this purpose. Following the optimization of the chromatographic parameters, an identical standard solution of empagliflozin at a concentration of 10 µg/mL was injected into the RP-HPLC column and the resulting chromatogram was then recorded. We performed an analysis on the chromatogram to determine the retention duration, peak area, amount of theoretical plates, and tailing factor, in addition to other variables. These findings were compared to the limitations specified in the ICH recommendations Q2R1.[31] The same technique was carried out an additional five times, and the results of each instance of the chromatogram were

seen recorded. A calculation was made to determine the mean retention time as well as the mean area.

Preparation of Sample for Calibration Curve

The calibration curve was taken from the sample solution that was prepared by pipetting out the stock solution and making a series of samples in diluent with concentrations between 10 and 50 µg/mL. The linear equation indicating the empagliflozin calibration curve can be shown by graphing the absorbance versus concentration data. A wavelength of 272 nm was used to measure the concentration's absorbance.

Validation of the Developed Method

Linearity and Range

The degree to which a calibration plot of response vs. concentration maintains a linear relationship indicates the linearity of a process. Linearity is an evaluation of how accurate a method is. It is possible to evaluate linearity by carrying out a single set of measurements at several analyte concentrations. After that, a linear regression using the least-squares method is used to analyze the data. Slope, interception, and correlation coefficient can be calculated from the resulting plot to provide the necessary information on linearity. The concentrations of empagliflozin in the various solutions that were produced ranged from 5 to 25 µg/mL. The mean peak areas were calculated by injecting all of these standard working solutions of empagliflozin as a combination in triplicate to the improved chromatographic conditions. The calibration curve for empagliflozin was produced using various concentrations of standard solutions. Following each chromatographic measurement, the mean peak area was calculated as a consequence estimate. Estimates of the correlation coefficient and intercept were derived from the calibration curve's corresponding equation of the line.[32-34] The equation that describes a straight line in its most generic form is shown below.

$$Y = mX+c$$

Where,

Y = Peak region; m = slope; X = measured concentration; c = intercept.

Precision

To evaluate the accuracy of the procedure, the concentration of empagliflozin was varied from 10 to 20 µg/mL and then measured using three different standard solutions. The reproducibility of the measurements was established by carrying out several measurements of the identical standard solutions on the same day. However, the intermediate precision also called the system precision, was calculated on two consecutive days. After injecting the three standards at 10, 15, and 20 µg/mL, the chromatograms were used to estimate the average peak area using the integration method. In each instance, the percent RSD was computed, and then conformity with the stipulated requirements was determined by examining the results.[32-34]

%Accuracy

According to the precision study, the accuracy of the procedure was decided by utilizing three different standard solutions of the empagliflozin that were included in the combination (API mixture). After injecting the solutions three times, the concentration of each solution was calculated by extrapolating from the calibration curve. After that, an estimate of the accuracy percentage was calculated using the subsequent formula.[32-34]

$$\%\ Accuracy = \frac{Mean\ measured\ concentration}{Nominal\ concentration}\ X\ 100$$

Limit of Detection and Limit of Quantitation

A sample's LoD is the lowermost amount of analyte that can be recognized through a given assay, whereas a sample's LoQ is the lowermost amount of analyte that can be determined. The following formula was used to obtain these values for the parameters.[32-34]

$$LOD = \frac{3.3\ X\ s}{S}$$

$$LOQ = \frac{10\ X\ s}{S}$$

Where,

s = Standard deviation and S = Slope of the calibration curve.

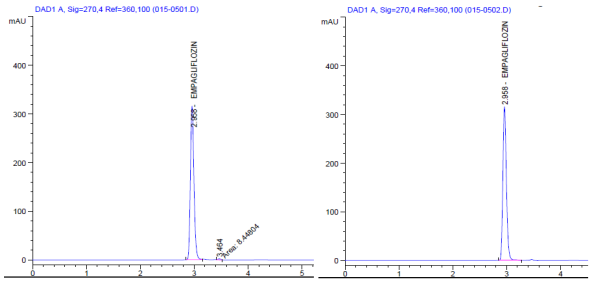
Robustness

The ability of an analytical technique to retain its precision despite minor and purposeful changes in the method's parameters is referred to as the method's resilience. To test the robustness of the approach, it was subjected to a wide range of situations, including those in which variables were purposefully altered, factors, for example, mobile phase composition, flow velocity, in addition to wavelength.[32-34]

RESULTS AND DISCUSSION

Method Development and System Suitability

A technique based on fast RP-HPLC has been established to measure the concentration of empagliflozin in bulk as well as in tablet dosage form. This technique employs a wide range of chromatographic settings, flow rates, and mobile phase ratios. The optimal conditions for the mobile phase (methanol: 0.1% OPA) were found to be a ratio of 05:95, a column size of 250 mm 4.6 mm, a particle size packing of 5 m, and a flow rate of 0.7 mL/min. We have successfully estimated empagliflozin in both bulks as well as tablet dosage forms with the chromatographic situations described in Table 1, and the resulting chromatograms are shown in Figure 2. The peak was seen at a residence time of 2.96 for bulk and at 2.95 for tablet dosage form, which is acceptable and indicates the applicability of the developed method. In system suitability, the obtained chromatogram is illustrated in Figure 3 and then the outcomes with acceptable criteria are tabulated in Table 2. The retention time was observed at 3.43, which is within the



(a) **(b)**

Figure 2: The obtained chromatograms of empagliflozin, a) in bulk drug; b) in the tablet dosage form.

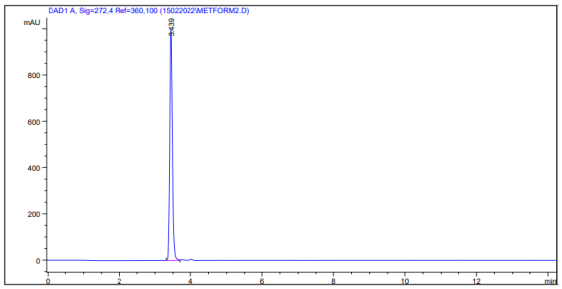


Figure 3: The obtained chromatogram from standard empagliflozin solution using the developed method to check the system suitability

Table 2: The observations noted during system suitability testing of empagliflozin with acceptance criteria as per ICH guidelines.

Parameter	Empagliflozin	Acceptance criteria
asymmetric factor	0.68	< 2.00
Number of theoretical plates	11287	> 2000
Retention Time	3.439	>1

Table 3: Empagliflozin linearity in bulk and tablet dosage form

Concentration (µg/mL)	*Area in bulk drug*	*Area in tablet dosage form*
5	295.46	296.8
10	572.67	579.94
15	875.45	874.89
20	1184.74	1166.13
25	1462.53	1451.87

*mean n = 5

acceptable limit range per the ICH guidelines. The entire system's attributes, including the plate count in addition to the tailing factor, come under the permitted limits, and the resolution between the analyte peaks was outstanding. Since the analyte peaks are unaffected by the excipients and mobile phase, we can conclude that the method is sensitive.

Validation of the Developed Method

Linearity and Range

The approach demonstrated linearity amongst the 05–25 µg/mL concentration range aimed at empagliflozin in both its bulk and tablet dosage forms. The regression equations for empagliflozin in bulk were y = 58.924x-5.693, while the regression equations

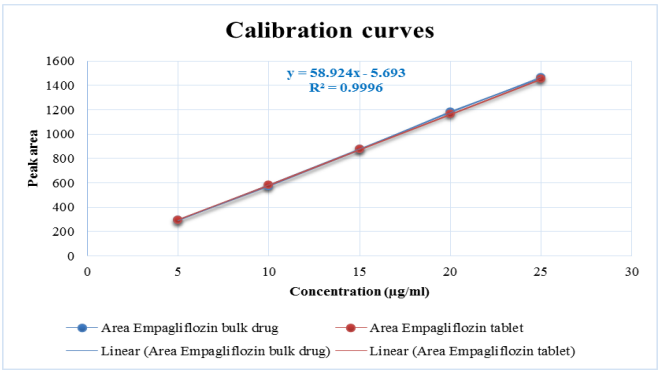


Figure 4: The calibration curves of empagliflozin in bulk (blue) and tablet dosage form (red)

for empagliflozin in tablet dosage were. As the calibration figure shows, a linear relationship exists between the peak size and the concentration over a comprehensive assortment of concentrations. The calibration curves derived for the bulk and tablet dosage forms, respectively are given in Figure 4. The outcomes are described in Table 3, which is available.

Precision

The experiment on precision was performed in two distinct ways: 1) Repeatability: precision achieved under varying working circumstances, by an individual with the same level of expertise over a short period; 2) Intermediate precision: the system is evaluated on a variety of days. To meet the requirements of the ICH standards, documentation of repeatability must include either nine determinations across the method's standard range (for instance, three concentrations with three replicates each) or six repeated measurements at 100% of the concentration being examined. In this instance, it was completed using the procedure from before. Empagliflozin was injected at three different concentrations spanning the range of the technique, and chromatograms were recorded after each injection. The three standard solutions were utilized. The peak area was integrated before being analyzed statistically to establish the mean peak area, and standard deviation, besides the percentage of a quantity. The outputs may be shown to fall within the parameters that were set. The tabulated findings from the precision research may be seen in Tables 4 and 5. In the precision study, the% of the amount was found in the 100 to 101% range.

%Accuracy

By the ICH recommendation Q2R1, the accuracy of the technique should be determined at three different levels, with three repeated measurements made at each level over the method's full range. Three standard concentrations of empagliflozin were used at three different values across the range to determine the accuracy. Table 6 contains a tabulation of the observed findings about accuracy. The %RSD was found to be in the range of 99.18 to 99.84 for empagliflozin in bulk and 99.29 to 99.53 for empagliflozin in the tablet dosage form. All of the findings that were generated remained within the permitted range.

Table 4: System interday precision of the established technique for the assessment of empagliflozin

Conc (µg/mL)	Empagliflozin bulk drug		Empagliflozin tablet	
	Mean ± SD	% Amt found	Mean ± SD	%Amt found
10	585.4 ± 1.97	100.92	582.61 ± 1.97	100.92
15	874.84 ± 3.61	100.99	879.95 ± 3.61	100.99
20	1171.26 ± 4.56	101.34	1177.71 ± 4.56	101.34

All value presented as *mean ± SD, n = 3

Table 5: System interday precision of the established technique for the assessment of empagliflozin

Conc (µg/mL)	Empagliflozin bulk drug		Empagliflozin tablet	
	Mean ± SD	%Amt Found	Mean ± SD	%Amt Found
10	579.84 ± 0.83	100.10	578.67 ± 0.83	100.10
15	871.28 ± 3.97	100.61	876.89 ± 3.97	100.61
20	1163.59 ± 2.16	100.27	1160.54 ± 2.16	100.27

All value presented as *mean ± SD, n = 3

LoD and LoQ

The LoD and LoQ were defined utilizing this methodology by calculating the standard deviation of the responses acquired for each of the standard concentrations of empagliflozin during the linearity research. The tabulated findings of the investigation are shown in Table 7. The LoD for empagliflozin was determined near be 0.309143 µg/mL, although the LoQ was determined to be 0.936798 µg/mL, as indicated in Table 7. The low values for the LoD and the lower limit of LoQ supplementary indicate the method's sensitivity towards detection and the quantification of empagliflozin.

Robustness

The stability of the current method was investigated by making minute but calculated adjustments to the parameters of the method, such as the detector wavelength, which was measured in nm, the flow rate of the mobile phase, which was measured in mL/min, and the organic concentration of the mobile phase, which was measured in percent volume/volume. In the experimental portion that was referenced above, Table 8 shows the detailed experimental setup for the robustness experiment. The results obtained from the robustness experiment it was observed that small changes in flow rate, wavelength, and mobile phase do not affect the accuracy and specificity of the method, which is acceptable as per the standard guidelines.

CONCLUSION

Throughout this investigation, we established a method aimed at detecting the amount of empagliflozin in tablet and bulk dose forms that is simple, sensitive, specific, precise, exact, and cost-effective. This technique employs a wide range of chromatographic settings, flow rates, and mobile phase ratios. The optimal conditions for the mobile phase (methanol: 0.1% OPA) were found to be a ratio of 05:95, a column size of 250 mm 4.6 mm, a particle size packing of 5 m, and a flow rate of 0.7 mL/min. Empagliflozin was effectively quantified from several commercially available tablet dosage forms on the market, ensuring the method's continued usefulness in a variety of settings. In addition to this, the approach was sensitive enough to measure empagliflozin when the sample matrix was present. As a result of the aforementioned research, the conclusion was reached that the technique developed is suitable for use in quality control labs to determine the quantity of empagliflozin present in bulk and tablet formulation.

Table 6: Accuracy levels developed a method for empagliflozin

Level of Recovery (%)	Empagliflozin bulk drug			Empagliflozin tablet		
	Mean% recovery	± SD	%RSD	Mean% recovery	± SD	%RSD
80	99.18	0.25	99.18 ± 0.25	99.53	0.25	99.53 ± 0.25
100	99.84	0.39	99.84 ± 0.39	99.29	0.39	99.29 ± 0.39
120	99.79	0.29	99.79 ± 0.29	99.38	0.29	99.38 ± 0.29

*mean ± SD, n = 3

Table 7: LoD and LoQ of empagliflozin for the developed method

Drug name	LoD (µg/mL)	LoQ (µg/mL)
Empagliflozin	0.309143	0.936798

Table 8: The findings obtained from the robustness experiment, which involved varying the detector wavelength, flow rate, and mobile phase

Parameter	Modification	Level	± SD	%RSD
Flow rate	± 0.2 mL/min	0.7	3.17	0.19
		0.9	0.09	0.01
Wavelength	± 2 nm	1484.9	1.62	0.11
		1405.30	3.34	0.24
Mobile phase (Methanol: OPA)	4:96	97:3	0.21	0.01
		95:5	0.11	0.01

*mean ± SD, n = 6

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