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Original Research Article

AQbD-Inspired Design and Development of a Reverse Phase HPLC Assay to Estimate Quantification of Empagliflozin

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Abstract: To determine empagliflozin in bulk and tablet preparations under quality-by-design principles, we designed and optimized a special, simple, sensitive, appropriate, specific and solid (RP-HPLC) technique. A Central Composite Design was applied to the optimization of the chromatographic conditions. Separating was done using HPLC, Chromeleon 7.3 software and Zorbax C18 column (250 mm x 4.6 mm; 5 mm). Detection occurred at 224 nm. The developed RP-HPLC used phosphate buffer and acetonitrile (60:40 v/v) as the mobile phase with fixed flow rate of 1.0 mL/min and the retention period was 4.5 minutes. The validity of the analytical findings was considered in terms of Method development. The given method proved to be linear with the correlation coefficient (r) of 0.9939. The nominal error of the method did not exceed 2.0 percent standard deviation. Empagliflozin recovery was set to 99.85 percent. The proposed solution had a high level of predictability and robustness. The proposed solution is very predictable and strong.

Keywords: RP-HPLC, QbD, Empagliflozin, Method Development, Validation.

INTRODUCTION

The population of individuals having diabetes in the world today is 463 million and it is likely to grow to 700 million in 2045. [1]. Diabetes mellitus (DM) is a long-term and complex disease that must be treated medically on a continuous basis, along with the multifactorial approach to the minimization of risks and glucose regulation. Empagliflozin is a new medicine with antihyperglycemic properties, and it has been licensed to be used clinically in 2014[.4]Empagliflozin is a relative drug that is capable of inhibiting the sodium-glucose cotransporter 2 (SGLT2), which elevates the glucose secretion in the urine and thus, improves the glycemic regulation, glucose metabolism, reduces glucotoxicity, and insulin resistance.

Empagliflozin was found to positively improve the actions of the beta-cell by lowering the burden and glucotoxicity experienced by the pancreatic beta cells.

Central composite design (CCD) is a good design used in optimization of important characteristics. CCD proposes the optimal value of a variable which results in the best and meaningful response, and conditions a process which is resilient to any deliberate alterations in the factors. It can also provide a mathematical model which can correlate the response to the underlying variables so that a prediction of the response can be made with a minimum error[10-

14].

Numerous researches have led to the RP-HPLC procedures of empagliftozin discovery under both bulk and in a pharmaceutical dosage type by C18 column with a moving phase of methanol or acetone. Most of the techniques use a constant retention period of approximately 7.0-7.8 minutes under different chromatography conditions. Other than the conventional assays, other researchers have been in a position to establish stability-indicating and QbD/AQbD based RP-HPLC procedures that enable the estimation in the presence of other antidiabetic agents like metformin, linagliptin and dapagliflozin, which have shown to be robust, accurate and controlled. It has been established in the literature review that determination of medicine has been achieved using HPLC. [15-21].

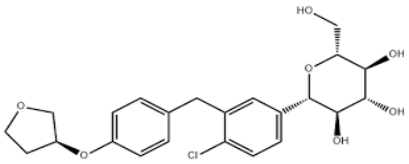
Experimental Method :

Materials:

Empagliflozin was obtained from the Arni Analytical Laboratory, Nashik. HPLC-grade solvents such as Acetonitrile, Methanol, and HPLC system were obtained from Thermo Ultimate 3000.

Fig 1 : Chemical structure of Empagliflozin

Instrumentation



Method development and validation was done using the HPLC Thermo Ultimate 3000 with software Chromeleon 7.3 Agilent Zorbax C18 column (150 mm × 4.6 mm 5 µm). Design -The CCD model was designed using the Design- Expert version 11.0 software.

Mobile Phase Preparation:

Preparation of Buffer:

Dissolve 0.01M orthophosphate of potassium in water.

Mobile Phase Preparation:

Prepare (60:40) v/v ratio of buffer and Acetonitrile.

Standard solutions Preparation:

Weigh 25mg of empagliflozin in a 50 ml volumetric flask, add 30 ml of the mobile phase and sonicate the mixture (5 min). The above solution was then diluted to 50ml mobile phase.

Sample solutions Preparation:

Properly weigh 10 tablets and the mean weight was determined. The pills were then ground to a fine powder. The accurate weight of the powder was equal to the average weight (tablet weight) in a 100 ml volumetric flask. Make up the volume by adding 60 ml mobile phase and sonicating it 10 min. The solution above was filtered using Whatman filter paper no. 41. Then 5 ml of the diluted filtrate, 50 ml to 5 ml using a Mobile Phase.

Experimental Design: Scouting Step:

This was achieved through a series of trials and error whereby the mobile phase that attained a satisfactory separation was found. It was determined that at the first technique preparation stage, the mixture of the both buffer and acetonititrile was giving fair peak; therefore, mixture of buffer and acetonititrile in the proportions of 60:40%v/v was selected as a mobile phase. Finally, the 2-factor selected percent acetonitrile and flow rate that could potentially affect the selected responses was selected.

Optimization design :

Another design that is most commonly used is the central composite design (CCD) since it is rather effective and can lead to the reduction of the number of the runs. The k factors used in a CCD must require 2k factorial runs, 2k axial experiments, which are evenly spaced along each axis of variable at + 8 q and at least at a center point. The three significant ones were so designed to form a rotatable CCD (= 1.68) to form the most desirable amount of requisite replies in 5 levels of each variable (= - +) and 13 random runs which is a combination of 5 point center runs. The major aim of the HPLC mode of analysis is to get a fast analysis of the analysis and sufficient separation of the peaks. When these aspects are defined by the system of HPLC when the chromatographic or other

conditions separate, and the effect of such responses are identified, then these aspects are optimized using a central composite design regimen. CCD suggests the two-factorial designs as the expansion or the star point to obtain the rotatability or orthogonality to erroneously approximate the second order equation, as Box and Wilson put it. Thus, it allows obtaining the coefficient of the quadratic regression model that will result in the forecasting of the nonlinear influences of the variables. Design expert (version 11, Stat-Ease Inc., Minneapolis, Minnesota, USA) was used to model the CCD model[20-21].

The CCD matrix has been performed on the thirteen trial runs and the results have been tabulated in Table 1 where the independent variable forms the independent variable and the values of the response are provided in Table 1 and the levels in Table 2.

Table 1: Central composite design that included 13 randomized trail runs.

Run	% ACN	Flow rate	Rt empagliflozin
1	40	1.2	4.392
2	40	0.7	4.792
3	40	1	4.534
4	50	0.8	4.581
5	54	1	4.128
6	30	1.2	4.392
7	25	1	5.128
8	40	1	4.551
9	50	1.2	3.892
10	40	1	4.558
11	30	0.8	4.928
12	40	1	4.546
13	40	1	4.581

Table 2: Factors and levels used in the central composite design were experimental.

Name	Low (-1)	Medium (0)	High (+1)
Independent factor			
A : Acetonitrile %	30	40	50
C :Flow rate	0.8	1	1.2
Dependent factor			
R1	Retention time of empagliflozin		

Table 3: Optimized results and conditions of the experiment.

Parameters	Description
Program	Isocratic
Mobile phase	Phosphate buffer: ACN (40:60 v/v)
Wavelength	224 nm
Injection volume	20 µL
Flow rate	1 mL/min

Method validation:

System compatibility, linearity, DL, QL, accuracy, precision and robustness had been considered in the validation of the method against the standards of the International Conference on Harmonization (ICH) (Q2R2). [22]

Specificity:

Specificity is a factor that considers the capability of introducing the analyte in circumstances where desired elements are needed The samples produced with the advertised dosage forms and the HPLC peak of the pure active

compounds of the samples were compared to the blank.and placebo to determine specificity of the method used in the analysis (method).

Precision:
It applied in the establishment of the reproducibility of an analytical procedure, which is described in the percentage form of the relative standard deviation of the area and retention time of the formed solution.

Intermediate precision:
Precision when the test is conducted under the conditions of analytical repeatability i.e. conducting tests on the same sample in the same laboratory using the same operator and with the same equipment in the short run.

Accuracy
This is because the accuracy of the analysis method shows the proximity of results of the test which the method provides with real values. The accuracy was expressed as a percentage of recovery and was done at three levels namely: 80 percent, 100 percent and 120 percent.

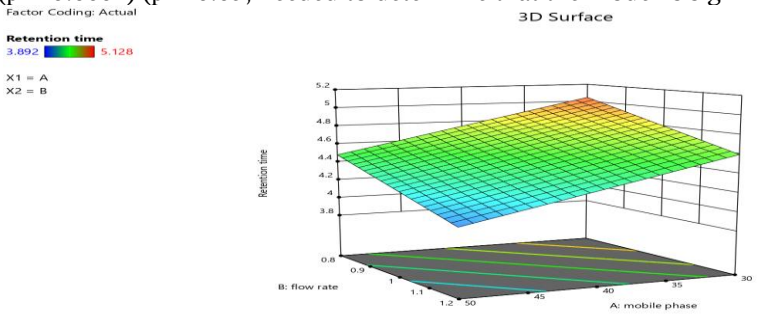
Linearity
This evaluation technique was found to be linear to the 80-120 concentrations. Build calibration curves. The concentrations are equated to the peaks.

QL and DL
DL is the lowest limit of concentration observable and impossible to quantify and QL is the lowest limit of concentration measurable.

Solution Stability:
The empagliflozin stability in the solution of the assay was measured by merely placing the working standard in a rigorously closed volumetric flasks at room temperature after 24 h. The test sample was also made and stored as long as 24 h.

Result and Discussion :

Method optimization and design of experiment:
Lastly the 2-factor chosen the acetonitrile percent and flow rate which can definitely influence the chosen responses were chosen. A spectral examination of drug in the 200-400 nm range revealed that empagliflozin has a 224 nm 2max. Chromatographic detection was done at 224 nm. It was done in an Agilent Zorbax C18 column (250 mm x 4.6 mm; 5µm). The best mobile phase was determined as phosphate buffer: acetonitrile (60: 40 v/v) below a flow rate of 1.0 mL/min. Each of the two variables was obtained a sweet spot (also called the brilliant yellow area) and the rest of the parameters were kept at a constant value. CCD using two variables (concentration of acetonitrile and, flow rate) on the 5 level and 13 trials. Table 3 contains the equation of the quadratic regression model to be used, which is developed out of the interaction of factors and answers using the design expert software. The efficacy of the model and the effect of variables were evaluated by analyzing the variance (ANOVA) which confirmed the P- and F-values shown in Table 4. An independent effect was also high on the response as evidenced by the probability ($p < 0.0001$) ($p < 0.05$, needed to determine that the model is significant).



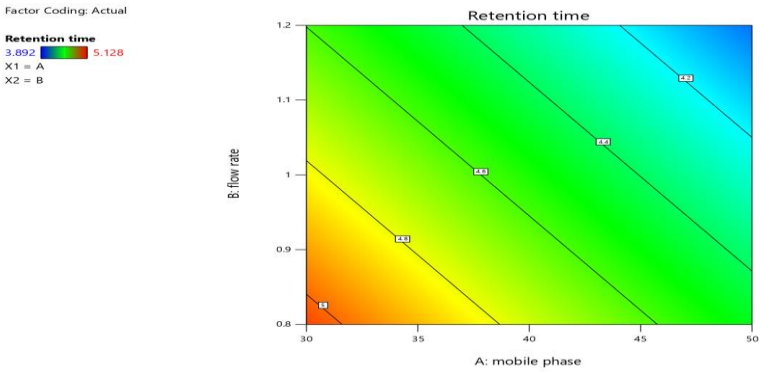


Fig 2: 2D and 3D plot of the interaction effect of critical factors on the retention time of empagliftoin.

In ***Fig 2*** the 2D and 3D graphs show the links between each of the dependent variables and two independent variables. The figures clearly show that when the flow rate and mobile phase acetonitrile concentration were increased, the retention time decreased.

Table 4 : Statistical parameter obtained from ANOVA

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.04	2	0.5200	33.09	< 0.0001	significant
A-mobile phase	0.6391	1	0.6391	40.67	< 0.0001	
B-flow rate	0.4008	1	0.4008	25.51	0.0005	
Residual	0.1571	10	0.0157			
Lack of Fit	0.1559	6	0.0260	85.35	0.0004	significant
Pure Error	0.0012	4	0.0003			
Cor Total	1.20	12				

As it is indicated in ***Table No.4,*** the F-value is 33.09, and this indicates that the model is significant. There is a probability of 0.04 percentage of this large F-value arising as a result of noise.P-values of less than 0.0500 indicate significant model terms.

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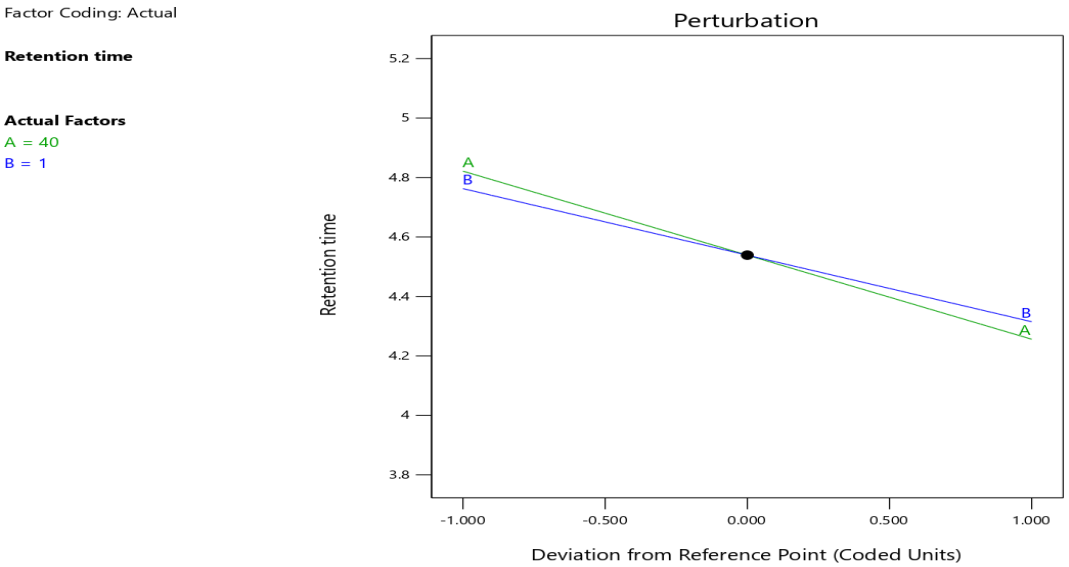

Fig 3 : Perturbation plot show effect of factor on responses

As shown in Fig 3, the percent acetonitrile in the mobile phase and flow rate had the most adverse effect on the retention time of empagliflozin because the two parameters increased the retention time with an increase in the percentage of acetonitrile in the mobile phase and flow rate.

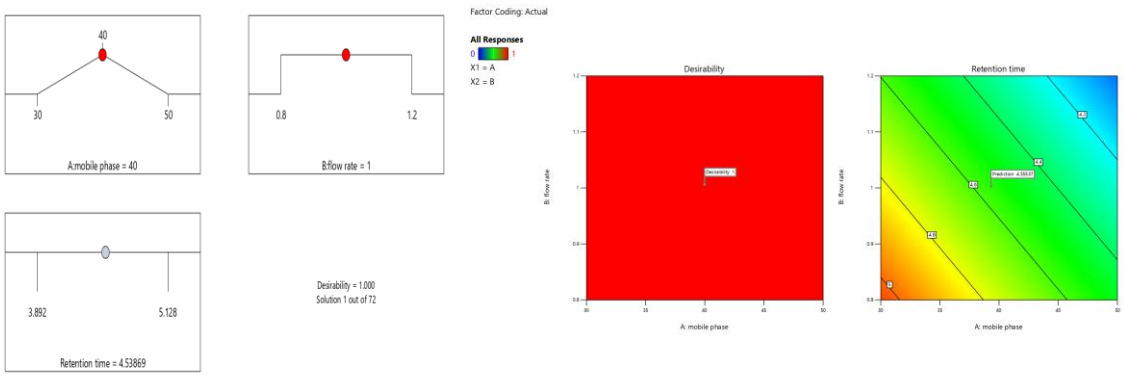

Fig 4.Graphical analysis of constraints that can be determined as accepted to determine global desirability and obtained optimal state of affairs.

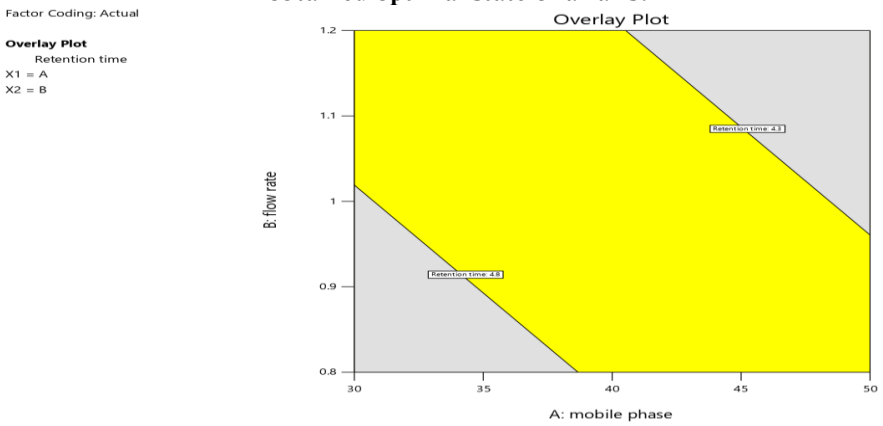

Fig 5. The sweet spot present between the desired responses in an overlay plot.

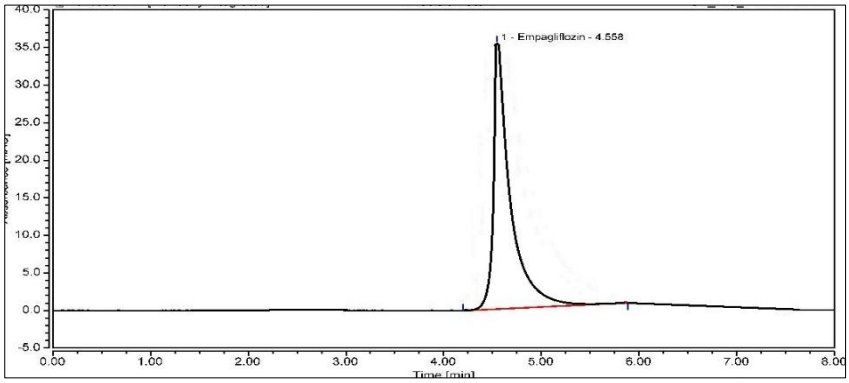

Fig 6.Optimized chromatogram of empagliflozin

Validation Parameter:

Specificity

No additional or co-eluted peaks were observed in the chromatograms at observed the retention times of the analytes. As a result, the peaks were declared pure, confirming the approach's specificity, and there was no interference found in the Standard & Sample owing to the blank.

Table 5 : Specificity result for empagliflozin

Sr.No.	Empagliflozin	Retention Time
1	Blank	0.000
2	Standard Solution	4.534
3	Sample Solution	4.551

Precision

1.System Precision : The assay values of three preparations of empagliflozin 25 mg is having a% Assay of 100.05, which is NLT 90.0% and NMT 110.0%; thus, the procedure for assay of empagliflozin 25 mg tablet is precise.

2.Intermediate Precision : The assay results of three preparations of empagliflozin is having % Assay, 100.05 which is NLT, 90.0%; and NMT, 110.0% So the procedure for assay of empagliflozin and tablet is precise.

Accuracy

The % recovery of the empagliflozin for each injection of each concentration is 99.36%, 100.80%, 99.39% and mean recovery is 100.83 %, which is within the acceptance standards, consequently the method for % assay determination of empagliflozin is accurate.

Table 6 : Accuracy result for empagliflozin

Sr.No.	Level	mg of drug spiked	Area	mg of drug Recovered	% Recovery
1	80%	0.040000	12.101	0.039745	99.36
2	100%	0.050000	15.345	0.050400	100.80
3	120%	0.060000	18.157	0.059635	99.39
Average					100.83
Std.dev					0.90
%RSD					0.90

Linearity

Calibration curves were established by five injections and it was observed to be linear between the range of 80 and 120. The obtained results were within the range, and the coefficient of correlation for the standard curve was 0.9939;, hence the method was linear within a given range.

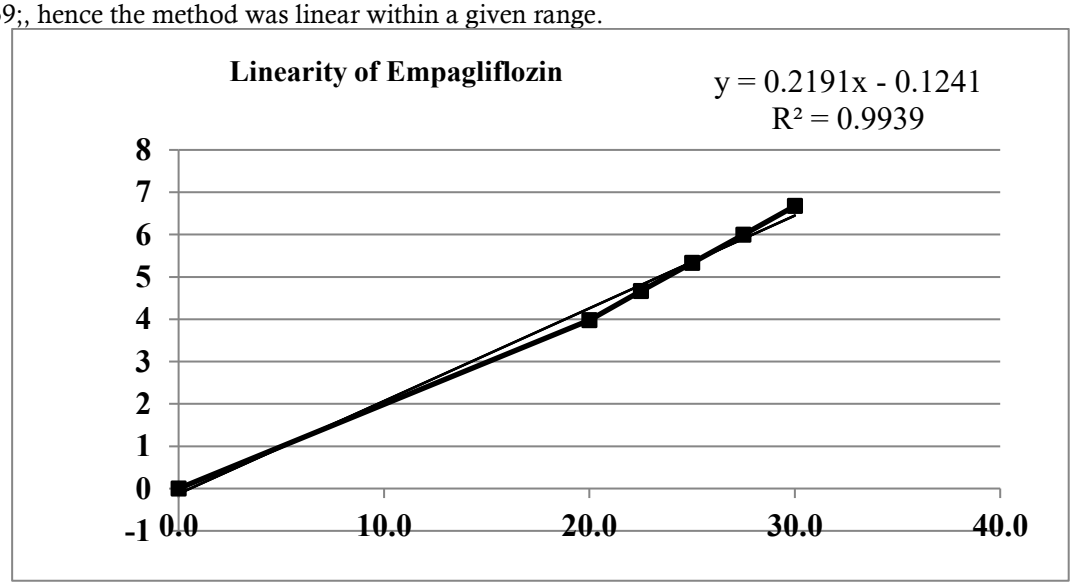

Fig 4 : Calibration curve for empagliflozin

Table 7 : Linearity result for empagliflozin

Sr. No.	Concentration in ppm	Concentration of Solution	Diluted to	Area
1	20.0	80	50	11.884
2	22.5	90	50	13.969
3	25.0	100	50	16.258
4	27.5	110	50	18.409
5	30.0	120	50	20.767

Solution Stability

% RSD was found to be less than 2 hence the sample solutions of empagliflozin 50 mg are stable up to 4 hours at room temperature.

Table 8 : Solution stability result for empagliflozin

Sr.No.	Hours	Area	% Assay	%Assay Difference
1	Initial	17.000	100.05	-----
2	8 Hour	5.199	29.93	70.12
3	24 Hour	5.154	29.80	70.25

Table 9: Validation results of empagliflozin

Parameters	Empagliflozin
Specificity	There was no interference observed in Standard & Sample due to Blank
Linearity	
Range (µg/mL)	80-120 %
Y-Intercept	1.0223
Slope	1.4623
Correlation coefficient	0.9939
Accuracy	
% recovery	99.85
Precision	
System Precision	RSD - 0.67 %
Method Precision	% Assay - 100.05
Intermediate precision	% Assay – 100.05
Stability of solution	RSD < 2%

CONCLUSION

To develop an effective and efficient RP-HPLC system to quantify empagliftozin, QbD approach was embraced to build a strong and effective system. The multivariate regression analysis was successfully achieved.

to determine important implications of the two parameters to retention time. CCD aided the chromatographic environment in determining the interaction and quadratic effect of the key components on the preferred responses.We had conducted our validation based on the ICH Q2R2 guidelines. It is an accurate, strong, specific and exact technique which may be effectively utilized in analysis of empagliftozin in normal analysis.

Declarations:

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Informed consent : None
Data availability : None

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