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A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF EMPAGLIFLOZIN IN API AND TABLET DOSAGE FORM

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ABSTRACT

A simple, particular, fast and exact reversed phase high performance liquid chromatographic technique for analysis of Empagliflozin in API and tablet dosage form has been developed and validated. Chromatography was performed on a Symmetry C_{18}, 250nm×4.6mm and 5µm column with 0.02M phosphate buffer (PH-3.60 adjusted with Orthophosphoric acid): Acetonitrile in the ratio of 40:60 v/v as mobile phase at a flow rate of 1.0ml/min. UV detection was performed at 225nm. The run time was 6.0min. Empagliflozin was eluted with retention time of 2.790min. The method was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with USP and ICH guidelines. Validation acknowledge the method is particular, fast, accurate, precise, predictable and reproducible. Calibration plots were linear over the concentration range 0-35µgm/ml for Empagliflozin. The developed method showed better reproducibility and recovery with RSD <2%. The percentage recovery is within the limits 98-102% of Empagliflozin. The limit of detection (LOD) and the limit of quantification (LOQ) for Empagliflozin were found to be 0.06 and 0.18 µg/ml respectively. The proposed method was found to be simple, linear, precise, accurate and sensitive and can be used for routine quality control analysis for the estimation of Empagliflozin in API and tablet dosage form.

KEYWORDS

Empagliflozin, UV, RP-HPLC, Method development, Method Validation and ICH Guidelines.

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INTRODUCTON

Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 diabetes[1,2]. SGLT2 co-transporters are help for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic response resulting from SGLT2 inhibition reduces renal absorption and lowers the

renal threshold for glucose, therefore resulting in increased glucose excretion[3,4]. Additionally, it contributes to reduced hyperglycaemia[5] and also assists weight loss and blood pressure reduction.

Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor. SGLT2 co-transporters are help for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic effect[6] resulting from SGLT2 inhibition reduces renal absorption and lowers the renal threshold for glucose, resulting in increased glucose excretion. Additionally, it contributes to reduced hyperglycaemia, assists weight loss, and reduces blood pressure.

Chemically[7,8] it is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy] phenyl} methyl) phenyl]-6-(hydroxymethyl) oxane-3, 4, 5-triol with empirical[9] formula of $C_{23}H_{27}ClO_7$ and the molecular weight[10] of Empagliflozin is 450.91g/mol.

The structure of Empagliflozin is following

The main objective[11] of the study is method development and validation of Empagliflozin by RP-HPLC was accurate and precise. The objective of the present work is to develop a new precise method development and validation of parameters[12,13]. This new method was successfully developed and validated as per ICH guidelines[13-15] can be utilized for the validation of Empagliflozin in pharmaceutical dosage form.

MATTERIAL AND METHODS

Chemicals and reagents

All reagents and chemicals were used are analytical grade. The Empagliflozin standard (API) was received from Syncorp Clincare Technologies Pvt. Ltd[8]. Drug substance (25mg) from Eli Lilly and Company. Empagliflozin tablets 25 mg from the local market. HPLC grade water, Methanol, Dipotassiumhydrogen orthophosphate, Acetonitrile, Potassium dihydrogen orthophosphate and Ortho phosphoric acid were purchased from SD fine-Chem ltd; Mumbai.

Instrumentation

The experiment was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out with a symmetry C_{18}, (250mm x 4.6mm, 5µm) dimensions column at ambient temperature.

Chromatographic Conditions

The estimation was carried on HPLC Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Columnwith detection wavelength of 225nm. Injection volume is 20.0µl and maintaining flow rate at 1ml/min.

PREPATRATION OF SOLUTIONS

Buffer preparation

About 6.8 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and degassed in ultrasonic water bath and filtered through 0.45µm filter using vacuum filtration. The pH was adjusted to 3.60 with orthophosphoric acid.

Mobile phase preparation

A mixture of Acetonitrile 600ml (60%) and 400 ml of HPLC grade water (40%) were mixed and degassed in ultrasonic water bath for 15 minutes and filtered[16] through 0.45 µm filter under vacuum filtration.

Diluent

Mobile phase can be used as diluent[17].

Preparation of Standard Solution

Working concentration should be around 20µg/ml.

Accurately weighed around 25mg of Empagliflozin working standard, taken into a 25ml volumetric flask, then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 µg/ml.

Further dilutions have been made to get the final concentration of 20µg/ml.

Preparation of Test Solution

An accurately measured volume of label claim solution was diluted with diluents to obtain a solution containing about a linear range.

Method development selection of wavelength

Stock solution of 1000µg/ml was prepared for Empagliflozin and further diluted to get the concentration of 20µg/ml of Empagliflozin was prepared with mobile phase. The ʎ max was selected by scanning the above standard solution between 200 to 400nm. The scanned results showed that reasonable maximum absorbance was recorded

at 225nm. Therefore, 225 nm was selected as the detection wavelength for the RP-HPLC investigation.

Construction of Calibration Curve

Different concentrations of standard solution were prepared and their chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration were calculated from the chromatograms. Then the linearity plot was made using the mean peak areas at their respective concentrations.

METHOD VALIDATION

The present developed method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and system suitability parameters as described in ICH guidelines.

Linearity of Empagliflozin

The calibration curve showed good linearity in the range of 0-35 µg/ml, for Empagliflozin (API) with correlation coefficient (r^2) of 0.998 (Figure No.2). A typical calibration curve[21] has the regression equation of y = 5667.x + 4089 for Empagliflozin.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Empagliflozin were taken and added to the pre-analyzed formulation of concentration 20µg/ml. From that percentage recovery[22] values were calculated. The results were shown in Table No.2.

The mean recovery for 80%, 100%, 120% level was found to be 101.382, 99.906 and 99.83. %RSD was found to be 0.325, 0.652 and 0.246respectively. They are within the limits.

Precision

Precision[23] is the measure of the degree of repeatability of an analytical method under normal process and is normally expressed as the %RSD for a statistically significant number of samples.

System Precision (Repeatability)

The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Empagliflozin (20µl). The percent relative standard deviations were calculated for Empagliflozin are presented in the Table No.3.

The repeatability study which was conducted on the solution having the concentration of about 60µg/ml of Empagliflozin showed a RSD of 0.849997%. It was concluded that the analytical technique said to be good repeatability.

Intermediate Precision

For intra-day studies[24] the drug having concentration value 80%, 100 % and 120% of the target concentration (n =3), were injected in triplicate into the HPLC system for intra-day and for inter-day studies the drug at above three concentrations for three days. Data were subjected to statistical treatment[25] for the calculation of SD and % RSD. The data are shown in Table No.4.

Robustness

Aof small changes in chromatographic conditions[26] such as change in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) and Acetonitrile content in mobile phase (±2%) studied to determine the robustness[27] of the method are also in favour. (Table No.6, % RSD < 2%)[22]. The developed RP-HPLC method for the analysis of Empagliflozin (API).

Limit of detection

The limit of detection[18] is defined, as the minimum concentration of an analyte in a sample solution that can be detected not quantified by using experimental conditions[19] of the method.

LOD = 3.3 σ /S

Limit of quantification

The limit of quantification[20] is defined as the minimum concentration of an analyte in a sample solution that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

LOQ = 10 σ/s

The Limit of detection was found to be 0.06µg/ml and Limit of quantification was found to be 0.18µg/ml for Empagliflozin. LOD and LOQ both measure based on signal to noise ratio.

System suitability parameter

System suitability was examined by giving six replicates and evaluated the chromatographic

parameters like retention time, tailing factor, theoretical plates and peak area the results of system suitability was reported in the Table No.6. The chromatogram of Empagliflozin standard was shown in Figure No.5. And the optimized chromatographic conditions were shown in Table No.7.

RESULTS AND DISCUSSION

The present study was designed to establish an accurate, precise and linear RP-HPLC method for estimation of Empagliflozin and in API as per ICG guidelines. The method was found to be linear in the range of 0-35µg/ml with a correlation coefficient (r^2) of 0.9984. The LOD and LOQ of the method were calculated to be 0.6 and 0.18µg/ml respectively.

The Precision was estimated by make use of repeatability; intra-day and inter-day studies and the results were estimated as %RSD values and were found to be within the limits. Recovery of Empagliflozin was found to be in the range of 99.83-100-% which confirms the accuracy of the method. Intraday and interday studies shows that the % RSD was found to be within acceptance limit (≤2%) (1.07%), the retention time was 2.790min with run time of 6 minutes. So it was concluded that there was no significant difference for the assay, which was tested within day and between days. The system suitability was studied with six replicates standard solution of API and results were found to be consent criteria.

Table No.1: Linearity Results of Empagliflozin

S.No	CONC.	AUC (n=6)
1	0	0
2	10	62895
3	15	91302
4	20	120283
5	25	146794
6	30	172745
7	35	199734

Table No.2: Data of Recovery Studies

S.No	Sample ID	Concentration (µg/ml)		Peak Area	%Recovery of Pure drug	Statistical Analysis
		Amount Added	Amount Found			
1	S_1: 80 %	16	16.263	96254	101.643	Mean= 101.3827%
2	S_2: 80 %	16	16.162	95682	101.012	S.D. = 0.329652
3	S_3: 80 %	16	16.239	96121	101.493	% R.S.D.= 0.325156
4	S_4: 100 %	20	19.834	116493	99.170	Mean= 99.90667%
5	S_5: 100 %	20	20.082	117895	100.410	S.D. = 0.652099%
6	S_6: 100 %	20	20.028	117589	100.140	R.S.D.= 0.652708
7	S_7: 120 %	24	23.902	139542	99.591	Mean= 99.837%
8	S_8: 120 %	24	23.961	139878	99.837	S.D. = 0.246
9	S_9: 120 %	24	24.020	140213	100.083	% R.S.D. = 0.246402

Table No.3: Data Showing Repeatability Analysis

S.No	HPLC Injection Replicates of Empagliflozin	Peak Area
1	Replicate – 1	117856
2	Replicate – 2	119854
3	Replicate – 3	116985
4	Replicate – 4	117895
5	Replicate – 5	118547
6	Replicate – 6	117454
7	Average	118098.5
8	Standard Deviation	1003.834
9	% RSD	0.849997

Table No.4: Data for Empagliflozin analysis

S.No	Conc. of Empagliflozin (API) (µg/ml)	Observed Conc. Of Empagliflozin (µg/ml) by the proposed method			
		Intra-Day		Inter-Day	
		Mean (n=6)	% RSD	Mean (n=6)	% RSD
1	16	16.08	0.97	16.03	0.97
2	20	20.04	0.44	20.03	0.45
3	24	23.97	0.37	24.05	0.19

Table No.5: Result of Method Robustness Test

S.No	Change in parameter	% RSD
1	Flow (1.1 ml/min)	0.09
2	Flow (0.9 ml/min)	0.07
3	Temperature (27^{o}C)	0.06
4	Temperature (23^{o}C)	0.14
5	Wavelength of Detection (227 nm)	0.24
6	Wavelength of detection (223 nm)	0.28

Table No.6: System suitability parameters of Empagliflozin

S.No	Parameters	Values
1	λ max (nm)	225
2	Correlation coefficient (r2)	0.9984
3	Theoretical plates	4126
4	Tailing factor	1.12
5	Retention time	2.790
6	LOD	0.06µg/ml
7	LOQ	0.18µg/ml

Table No.7: Optimized Chromatographic Conditions

S.No	Condition	Result
1	Column	Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column
2	Elution	Isocratic
3	API concentration	20µg/ml
4	Detector	UV detector
5	Mobile Phase	Phosphate buffer (0.02M) (pH-3.6): Acetonitrile = 40: 60
6	Flow Rate	1.0ml/minute
7	Wave length	225 nm
8	Injection volume	20 µl
9	Run time	6.0 minutes
10	Column temperature	Ambient
11	Sampler Temperature	Ambient
12	Peak area	117895
13	Retention time	2.790

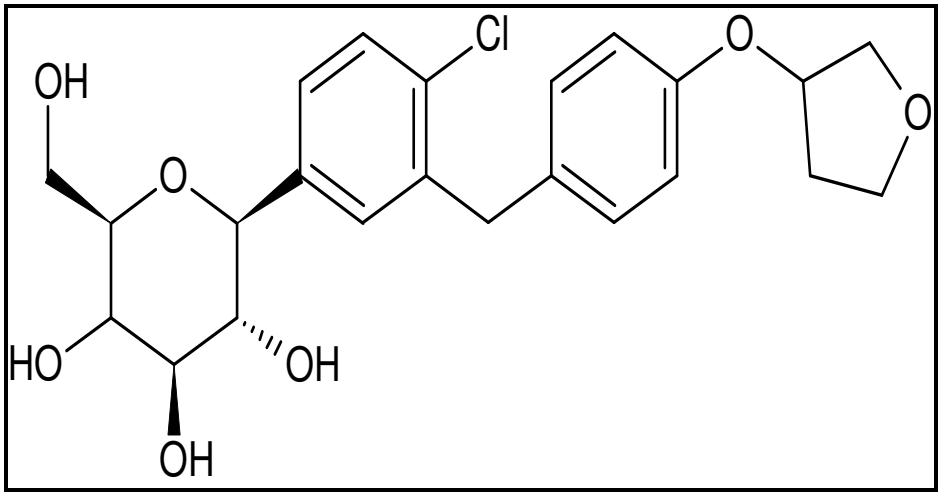

Figure No.1: Chemical Structure of Empagliflozin

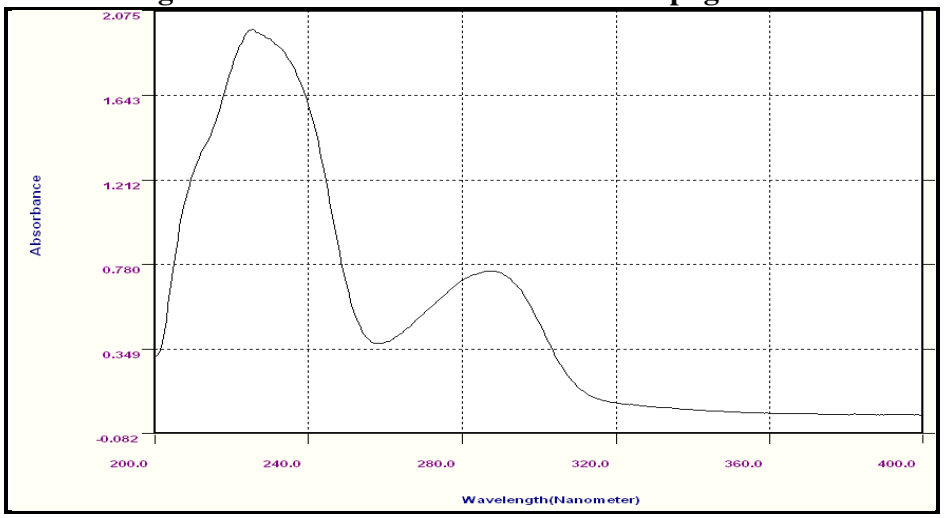

Figure No.2: UV-Spectrum for Empagliflozin

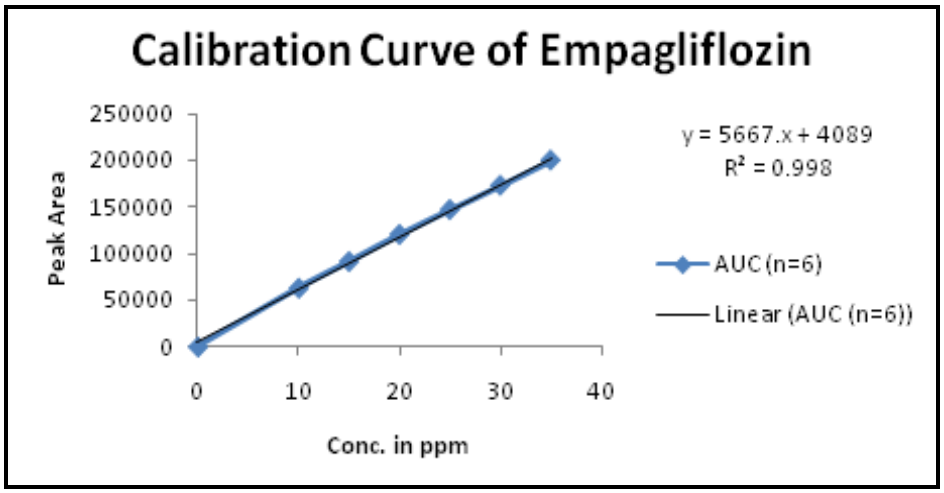

Figure No.3: Calibration Curve for Empagliflozin

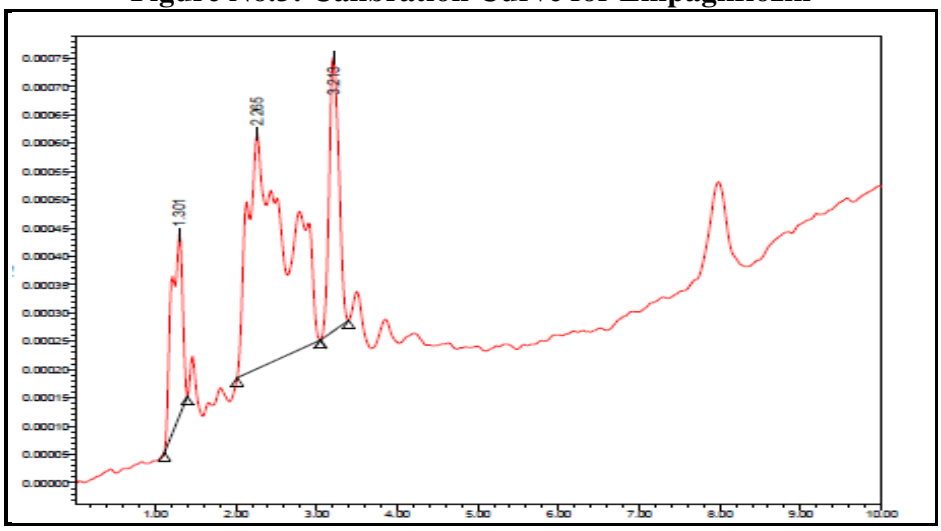

Figure No.4: Chromatogram for Blank Solution

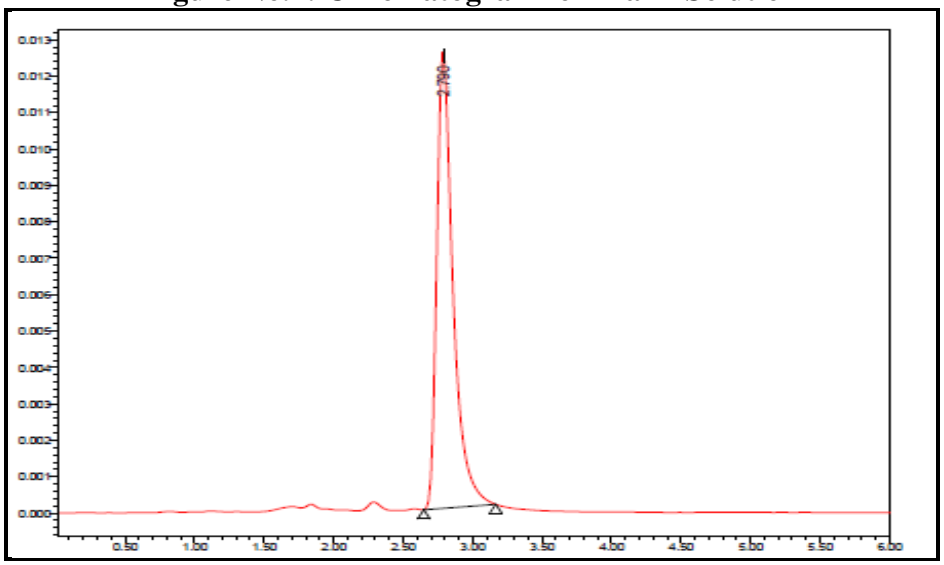

Figure No.5: Chromatogram for Empagliflozin

CONCLUSION

In this study, we have established a new, rapid RP-HPLC method and validated for different parameters linearity, accuracy, precision and LOD, LOQ, Robustness and system suitability. By studying all these validation parameters, we have concluded that the method was linear, accurate, precise, robust and rapid for the determination of Empagliflozin in API. So, the method can be successfully used for the estimation of Empagliflozin in API.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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