Research Article

A New Simple RP-HPLC Method Development and Validation of Empagliflozin in Bulk and it's Tablet Dosage Form

Ramreddy Godela[1]*, Kranthi Kumar Pola[1], Venkatarayudu Ganta[2], Ashwin Kumar Jangam[3], Dr. Srinivasa Rao Avanapu[1]
1. Bhaskar College of Pharmacy, Moinabad, Hyderabad, India.
2. Research Associate, VIMTA Labs, Hyderabad, India.
3. Drug Inspector, Dept. of Health, Medical and Family Welfare, Telangana, India.
***Corresponding author's E-mail:** ramreddy.godela@gmail.com

Received: 18-05-2019; **Revised:** 26-06-2019; **Accepted:** 05-07-2019.

ABSTRACT

The primary important objective of the present research work is to develop simple, specific, rapid, accurate and sensitive reverse phase HPLC method and validated for the qualitative and quantitative determination of empagliflozin in its active pharmaceutical ingredient and tablet dosage form according to ICH guidelines. An isocratic separation was done by using Phenomenex C18 column possess 75 x 4.6 mm, 2.6 µ,100 A0 dimensions with a mobile phase composition of water: acetonitrile (10:90% v/v) at a flow rate of 1ml/min and response detected by using 261 nm wavelength as absorption maximum. The Retention time of empagliflozin was found to be 2.84 minutes, LOD and LOQ were observed at 1.5 µg/ml and 4.6 µg/ml concentration respectively, linear curve was observed in the concentration range of 10-60 µg/ml with correlation coefficient of 0.99. The percentage recovery (accuracy) was in the range of 98.3- 102% and the % RSD was observed to be less than 2%. The proposed method was validated for accuracy, precision, sensitivity, linearity and robustness and successfully employed for quantitative determination of empagliflozin in tablet dosage form in quality control department of pharmaceutical industry.

Keywords: RP-HPLC, Retention Time, Limit of detection, Limit of quantification, Robustness.

INTRODUCTION

Chemically empagliflozin is (1S)-1,5-Anhydro-1-(4-chlor-3-{4-[(3S)-tetrahydro-3-furanyloxy]benzyl} phenyl)-D-glucitol works as sodium-glucose co-transporter 2 (SGLT2) inhibitors offer an insulin-independent component for improving blood glucose levels, since they advance urinary glucose discharge (UGE) by restraining glucose reabsorption in the kidney. Notwithstanding glucose control, SGLT2 inhibitors are related with weight reduction and circulatory strain decreases, and don't build the danger of hypoglycemia[1].

On extensive literature review revealed that, different analytical methods have been reported for the qualitative and quantitative analysis of empagliflozin in bulk and pharmaceutical dosage forms using UV–visible spectroscopy[2,3] and reverse phase- high performance liquid chromatography (RP-HPLC). In depth literature survey reveals that even though so many numbers of RP-HPL C methods were reported, but there is no RP- HPLC method with less retention time with simple mobile phase system was not reported for quantitative estimation of empagliflozin in bulk drugs and pharmaceutical dosage forms[4,5,6].

The objective of the present research work was to develop and validate simple, precise, sensitive and accurate analytical method with less retention time and simple cost-effective solvent system for the estimation of empagliflozin in pure and commercially available tablets for regular analysis in pharmaceutical industry. Chromatographic method is the most effective popular method for the analysis of drug substance and drug product; hence a new RP- HPLC method was developed and validated for the estimation of empagliflozin.[7]

MATERIALS AND METHODS

The empagliflozin reference standard (claim 99.18%) was provided by HETERO Drugs. Tablets of empagliflozin (JARDIANCE -10mg) were purchased from a local pharmacy. HPLC grade acetonitrile was obtained from Finar Chemicals Limited, Ahmadabad, India. All the glass wares used in this research work were made of Borosilicate glass and the solvents and prepared solutions were filtered by using Nylon (0.45µm) filters.

Chromatography

RP-HPLC method was performed with Cyberlab HPLC equipment with UV detector and manual injector with a 10 µL loop. The equipment was connected to data-processing system (LC- Solution software). The chromatographic system was performed using C_{18} (250 x 4.6mm, 2.6µ,100 A^0) column. Separation was successfully achieved using a mobile phase composition of Acetonitrile: Water (90:10 v/v) at a flow rate of 1 ml/min. The eluent was measured using UV detection at a wavelength of 261nm.The column temperature was maintained at 25°C±2 and the injection volume of 10 µL was injected. The prepared mobile phase was filtered through a 0.45 µm nylon filter prior to use.

Preparation of standard solution

30 mg of empagliflozin weighed accurately dissolved in 100 ml of diluents. Pipette out 10 ml of this solution into 100 ml of volumetric flask dilute to the volume with diluents, concentration of the empagliflozin was found to be 30µg/ml.

Preparation of sample solution

Weigh and transfer powder equivalent to 30 mg of empagliflozin in to 100ml volumetric flask, add 60ml of diluent and sonicate for 15minutes and diluted to the volume with diluent, filter the solution through 0.45µm Nylon filter. Pipette out 1ml of this solution in to 100ml volumetric flask and diluted to the volume with diluent to get the concentration about 30µg/ml.

$$\text{Assay\%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AVG\ Wt}{LableClaim} \times 100$$

Where:

AT = Peak Area of test preparation.

AS = Peak Area of standard preparation.

WS = Weight of working standard in mg

WT = Weight of sample in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Method validation

Validation is establishing documented evidences, which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics[9].

System suitability Test

Test for system suitability was performed by injecting blank solution once and standard solution of 30µg/ml test concentration (prepared as per the assay method) for six times into HPLC system. The system suitability was established by evaluating the system suitability parameters from the chromatograms thus obtained. Typical system suitability parameters include % RSD, Tailing factor (T) and Theoretical plates (N)[9].

Linearity

The linearity of an analytical method aims to elicit whether the test result are directly proportional to concentration. This is well explained by plotting a graph with peak area vs. concentration. The linearity of the drug was established by constructing the calibration curve with concentration on x-axis and absorbance on y-axis over a concentration range of 10µg/ml-60µg/m.

Accuracy

Accuracy of the method was determined by performing recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%). A known amount of empagliflozin added separately to pre-analyzed samples and percent recoveries were calculated at each level

Precision

Intra-day precision was determined by analyzing empagliflozin for three times in the same day (intra-day). Inter-day precision was determined by analyzing empagliflozin daily for three days and % RSD was calculated

Sensitivity (LOD and LOQ)

Limit of detection (LOD) is defined as the lowest amount of an analyte that can reliably bedetected. Limit of quantification (LOQ) of an analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where σ is the standard deviation of y-intercepts of regression lines

S is the slope of the calibration curve

Robustness

Robustness of an analytical procedure was performed by slightly changing the mobile phase composition and flow rate.

RESULTS AND DISCUSSION

Initially, the solubility of empagliflozin was checked in various solvents. The drug was found to be slightly soluble in methanol, freely acetonitrile and water.

Method Optimization

Optimization of the chromatographic conditions was carried out by running several trials to obtain retention time, peak symmetry, plate count and relative standard deviation within the limits and possible optimal. After several trails, a method using mobile phase composition of acetonitrile and water in the ratio of 90:10 at a flow rate of 1ml/min, on Phenomenex C_{18} (250 x 4.6mm, 2.6µ,100 A^0) column at 261nm, was found to be the most suitable and acceptable. Optimized method resulted in chromatogram with empagliflozin eluting at 2.84min (fig.no.1).

Method validation

The proposed method was validated according to Q2 specifications of the ICH guidelines[9].

System Suitability

The system suitability was established by assessing the system suitability parameters from the chromatograms thus obtained. Typical system suitability parameters include % RSD, Tailing factor (T) and Theoretical plates (N). All the parameters measured values (table-1) were satisfying the acceptance criteria.

Linearity

The linearity of detector response was determined by preparing a series of solution of the working standards over the range of 10µg/ml to 60µg/ml of concentration. These solutions were injected onto the chromatographic system and response area were recorded. Calibration curve was constructed by plotting area against concentration and regression equation was computed.

r^2 value of linearity curve was 0.999, plots with values were shown in fig.no.2.

Accuracy

The percentage recovery of drug from the spiked sample solutions were found to be in the range of 98.3 – 102 % (Table-2) indicates the accuracy of proposed analytical method was within the acceptance criteria of the ICH guidelines.

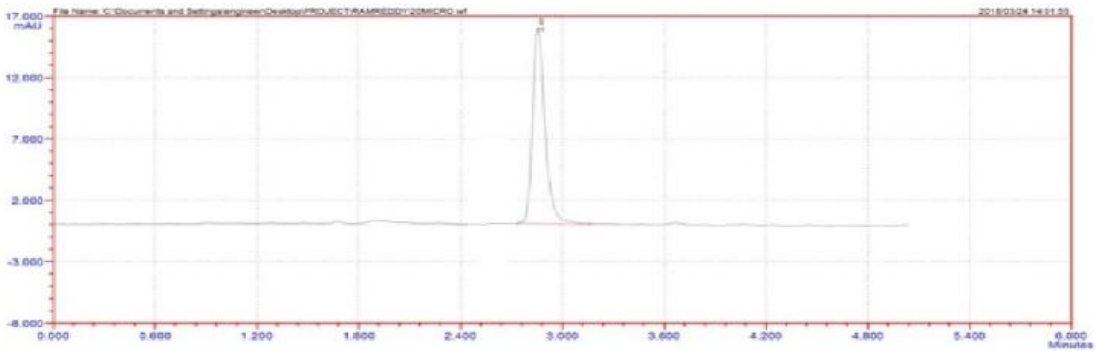

Figure 1: Optimized Chromatogram of empagliflozin

Table 1: Results of System Suitability Test for empagliflozin 30µg/ml

Injection	Retention Time (min)	Peak area (µV x sec)	USP Plate count	USP Tailing factor
1	2.81	**13235**	6532	1.12
2	2.79	**12960**	5532	1.08
3	2.79	**12801**	6209	1.12
4	2.80	**13506**	6461	1.1
5	2.84	**13102**	5087	1.12
6	2.83	**12932**	5913	1.04
Mean		**13089.3333**	5955.666667	**1.09**
Standard Deviation	**252.82695**			
%RSD	**1.93154948**			

*Average of six determinations. RSD: Relative standard deviation, SD: Standard deviation

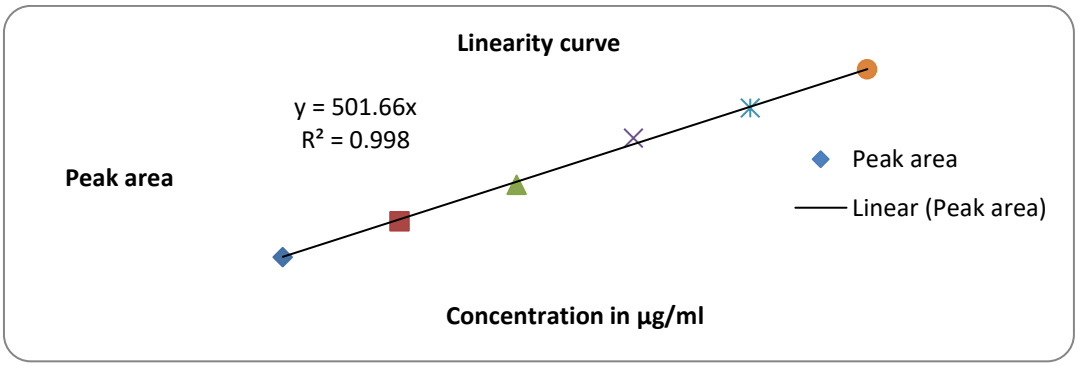

Figure 2: Calibration curve of empagliflozin

Table 2: % Recovery data for empagliflozin

% Level	Amount added (µg/ml)	Standard solution peak area	Spiked Average peak area	% Recovery
50%	10	4772	4654	98.3
100%	20	8762	8103	102
150%	30	13570	13756	99

Precision

Variation of results of the concentration (30 µg/ml) within the same day (intra-day), variation of results concentrations (10-60 µg/ml) between days (inter-day) was analyzed % RSD of peak area values of empagliflozin working standard solutions were found to be in the range of 1.8**%** and 0.50-1.57%, for intra-day (Table-3) and inter-day precision respectively (Table-4). The low values of (≤2) these statistical parameters represents the method with good precision.

Sensitivity and robustness

LOD and LOQ were found to be 1.5µg/ml and 4.6µg/ml, which indicate the method has good sensitivity. The % RSD values (Table-5) was below 2.0% by changing the parameters like flow rate (±0.1ml) and mobile phase composition ratio (±1), hence the method was said to be robust.

Assay

Percentage purity of the tablet dosage form was determined by injecting the 30µg/ml standard solution anda sample solution equivalent to 30µg/ml of empagliflozin. Based on the data (Table-6) the percentage purity of empagliflozin in tablet dosage form was found to be 99.6%. The proposed analytical method can be successfully applied for the routine analysis of tablet dosage forms.

Table 3: Results of Intra-day Precision

No. of injections (20µg/ml)	Peak area
injection-1	13205
injection-2	12960
injection-3	12901
injection-4	13506
injection-5	13032
injection-6	12837
Mean	13073.5
SD	246.8366
%RSD	**1.888068**

*Average of six determinations. RSD: Relative standard deviation, SD: Standard deviation

Table 4: Results of Intra-day Precision

Concentration	Peak area			MEAN	SD	%RSD
	DAY-1	DAY-2	DAY-3			
10	4903	4812	4968	4894.333	78.36028	1.601041
20	9738.3	9538.3	9861	9712.533	162.8857	1.677067
30	14637	14407	14457	14500.33	120.9683	0.834245
40	20445	20345	20245	20345	100	0.491521
50	24673	24854	24578	24701.67	140.2153	0.567635
60	30073.6	31072	30117	30420.87	564.3154	1.855027

*Average of three determinations. RSD: Relative standard deviation, SD: Standard deviation

Table 5: Results of robustness

Variation of Parameter		System suitability parameters			
		Retention time (min)	%RSD	USP Tailing factor	USP Plate count
Mobile phase Ratio (±1) Water: Acetonitrile	9:91	2.80	0.65	1.12	4589
	10:90	2.8150	0.54	1.04	6823
	11:89	2.84	0.63	1.10	3561
Flow rate (±0.1ml)	1.1ml	2.79	0.59	1.04	5423
	1ml	2.78	0.91	1.04	2963
	0.9 ml	2.79	0.72	1.06	4327

Table 6: System suitability parameters of empagliflozin (standard and test) in assay method

Peak name	Retention time	Peak Area	%Area	USP Tailing	USP Plate count
Empagliflozin (Standard)	2.810 min.	12723	100.00	1.23	4527
Empagliflozin (Test)	2.810 min.	13235	100.00	1.13	5791

Assay %=

$$\frac{13235}{12723}\times\frac{20}{100}\times\frac{10}{100}\times\frac{100}{41}\times\frac{100}{10}\times\frac{99.1}{100}\times\frac{81}{40}\times 100 = 99.6\%$$

The RP-HPLC assay method plays vital role in both qualitative and quantitative analysis of drug in its pure and tablet dosage. Till date, many RP-HPLC methods have been developed for qualitative and quantitative analysis of empagliflozin. However, the retention times in the reported studies were in a range 7 - 5min. But, the method with high retention time could not be treated as economical as it requires more amount of solvents and needs to be run for long time. If the retention time could be reduced solvent consumption and run time for sample analysis can be lowered, hence rapid analysis of more number samples can be done. In previously reported methods very expensive solvents like glacial acetic acid, buffers etc..were used and more effective linearity range could not be accomplished. In the present study RP-HPLC method with retention time of 2.84 min was observed by a simple mobile phase composition of acetonitrile : water(90:10), better accuracy and huge linearity range of 10-60µg/ml was attained with this simple mobile phase composition disclose the cost effectiveness of the method as compared to previously reported methods. The statistical results of the validated parameters within the limits stated by international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. Compared to previously reported methods the proposed method has great advantage in terms of retention time, linearity and sensitivity, hence this method could be used for better qualitative and quantitative analysis of empagliflozin.

CONCLUSION

The isocratic HPLC method was developed for study of empagliflozin in pharmaceutical dosage form. The validated method is very rapid, accurate, and precise. Moreover, it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence this method can be conveniently used for routine quality control analysis of empagliflozin in its pure and tablet dosage forms .

REFERENCES

1. Neumiller JJ, Empagliflozin: a new sodium-glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes, Drugs in Context, vol.3, 2014, pp-1-18 .
2. Padmaja N, Veerabhadram G, Mulagiri Sharath Babu, Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs and combined dosage forms, Der Pharmacia Lettre vol.8(13), 2016, pp-207-213.
3. Jyothirmai N, Anil Kumar M, Nagaraju B, Novel UV and Visible Spectrophotometric methods for the analysis of Empagliflozin a type 2 diabetic drug in bulk and pharmaceutical formulations, journal de afrikana, vol.3(1), 2016, pp-177-187.
4. Shyamala, Soumika M.et al, Method Development and Validation of Empagliflozin by RP-HPLC in Bulk and Pharmaceutical Dosage Form,Pharmanest, vol.7(1), 2016, pp-3040-3042.
5. Padmaja N, Veerabhadram G, Method Development and Validation of RP-HPLC Method for the Estimation of Empagliflozin in API, Int J Pharm Sci Res vol.7(2), 2016, pp-724-727.
6. Geetha Susmita A, Rajitha G, Ramya Yadav Y, Uma P, Analytical Method Development And Validation Of New Stability-Indicating Reverse-Phase High-Performance Liquid Chromatography Method For Simultaneous Estimation Of Metformin Hydrochloride And Empagliflozin In Tablet Dosage Form, Asian J Pharm Clin Res, Vol 12(1), 2019, PP-241-244.
7. Shyamala KN, Mounika J, Nandini B. Validated stability-indicating RP-HPLC method for determination of Empagliflozin, Pharm Lett, vol.8, 2016, pp-:457-464.
8. ICH. Q2B Validation of Analytical Procedures: Methodology. International Conference on Harmonisation. Geneva: IFPMA; 1996.
9. Sethi PD. HPLC quantitative analysis of pharmaceutical formulations. 1st ed.CBS Publishers; Delhi, 2001,pp-5-10.

Source of Support: Nil, **Conflict of Interest:** None.