Development And Validation Of RP-HPLC Method For Estimation Of Empagliflozin In Bulk Drug & Dosage Form

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Abstract

The proposed research study established and validated a RP-HPLC method for the estimation of Empagliflozin (EMG) in tablet formulation and bulk drug, that is new, sensitive, robust and appropriate. Developed RP-HPLC method utilized a mobile phase of Acetonitrile: 0.05% TFAA in Water (80:20), with a flow rate of 1.0 ml/min. The HPLC system included UV detector, Kromasil C18 column measuring 250 mm by 4.6 mm and having particle size of 5 µm. The detection was done at wavelength of 225 nm. The method produced an appropriate retention time of 3.04 minutes for EMG. The method was validated as per ICH guidelines. A simple and accurate method for assaying EMG in both tablet formulation and bulk drug was developed. This method is less time-consuming, making it suitable for regular use in the industry for analyzing bulk drug and marketed product of EMG.

1. Introduction

Empagliflozin (EMG), marketed as Jardiance is an oral antidiabetic drug that has received approval for the treatment of type 2 diabetes mellitus, heart failure and chronic kidney disease. It is part of the class of medications known as sodium-glucose co-transporter 2 (SGLT2) inhibitors. EMG works by selectively blocking SGLT2 proteins found in the proximal renal tubules of the kidneys. Roughly 90% of the glucose that has been filtered by the glomerulus, is reabsorbed into circulation with the help of SGLT2. EMG lowers blood glucose levels by blocking SGLT2, which prevents the reabsorption of glucose and leads to increased urinary glucose excretion (glucosuria). This mechanism does not rely on insulin secretion or sensitivity, which makes it appropriate for different stages of type 2 diabetes. EMG induces osmotic diuresis (increased urine output) and mild natriuresis (sodium excretion), which may play a role in its cardiovascular and renal benefits[1,2].

EMG is approved for minimizing the danger of cardiovascular mortality, failure of heart -related hospital admissions, advancement of chronic kidney disease in adults, irrespective of their diabetic status. It helps to reduce the risk of serious adverse cardiovascular events in patients of type 2 diabetes having cardiovascular disease. Other than glycemic control, EMG has been tested for various pharmacological activities. Clinical studies have shown that EMG reduces the risk of heart failure hospitalization and cardiovascular death of patients with heart failure, regardless of if they have diabetes. It decelerates the advancement of kidney disease

and lowers the risk of end-stage renal failure. EMG typically leads to a slight decrease in weight and blood pressure, owing to its diuretic properties. Typical side effects of EMG include urinary tract infections, genital mycotic (yeast) infections, hypotension and low blood sugar[3–6].

EMG in bulk drugs and pharmaceutical dosage forms has been estimated using a range of analysis methods, such as UV spectrophotometric, RP-HPLC, HPLC techniques. UV visible spectrophotometric approach is reported for the estimation of EMG in API as well as tablet where, the linearity was obtained within the range 2-10 µg/mL and 1.0–3.0 µg/mL with absorption maxima of 238.5 nm and 224 nm, respectively[7,8]. Sen et al (2022) established UV spectrophotometric method, ratio difference spectroscopy and derivative ratio spectrum zero-crossing method for instantaneous estimation of EMG, metformin hydrochloride and linagliptin at at 224.6, 226 and 237.2 nm, respectively[9].

Ahmad and Maryam (2023) were able to elute EMG at 5.0 min. The elution was performed on a C18 column utilizing isocratic mobile phase comprising 70:30 mixture of methanol and phosphate buffer at pH 3.0[10]. M Hanif et al (2021) employed mobile phase comprising a 0.1% trifluoroacetic acid pH 4.8 and acetonitrile (70:30) and EMG elution was observed at 3.6 min. Over the range of 0.025-30 µg/mL, calibration plot demonstrated a strong linear regression[11]. Patil et al (2016) utilized mobile phase consisting Methanol: Water (70:30) and EMG was detected at 224 nm. Linearity was in range 2-14 µg/mL and EMG eluted at 4.8 min[12]. Using a DAD detector, a stability-indicating method for EMG was developed with mobile phase of methanol/acetonitrile/0.1%OPA in a ratio of 75:20:5. The absorption maxima at 222.0 nm revealed that the peak occurred at 2.54 minutes[13].

Padmaja and Veerabhadram (2026) utilized mobile phase made up of methanol and acetonitrile in a (50: 50), which was adjusted to flow rate of 20 µl/min while detecting at 265 nm. EMG exhibited a retention time of 2.184 minutes[14]. Vijaya et al (2021) employed C18 column and mobile phase acetonitrile and water (50:50, v/v) with detection at 230 nm for simultaneous estimation of Metformin and EMG. The Rt value for Metformin and EMG was observed to be 2.20 ± 0.02 minute and 3.64 ± 0.02 minute, respectively[15]. Additionally, the chromatographic methods for simultaneous estimation of EMG and Metformin Hydrochloride[16,17]; EMG and Linagliptin[18] have been reported in the literature.

The reported methods were not found to be reproducible in different environmental conditions like changes in temperature and humidity. Thus, the rationale behind proposed research work was to develop a laboratory method for estimating EMG that fulfills all system suitability parameters within the accepted limits and meets validation parameter criteria according to ICH guidelines.

2. Materials

2.1. Instruments

2.2. Materials

3. Methods

3.1. Preliminary characterization of drug

Color, odor, and appearance parameters were utilized to evaluate EMG. In order to determine the solubility, EMG (20 mg) was dissolved in the appropriate solvent, sonicated 5-10 minutes to achieve a concentration of 2 mg/mL in 10 ml of water.

3.2. Analytical wavelength

3.2.1. Selection of solvent

Variety of solvents such as water, ethanol, methanol and hydroalcohol were examined for complete dissolution of EMG. Methanol was observed to be better solvent as compared to other solvents for dissolving EMG.

3.2.2. Standard stock solutions

10 mg of EMG was weighed with precision and placed into a 20 ml volumetric flask. Following this, 15 ml of methanol was added and the mixture was sonicated to ensure complete dissolution of the standard. The solution was then diluted with methanol to the calibration mark, resulting in a stock solution with a concentration of 500 µg/mL. 0.8 mL of the sample was further diluted to a final volume of 20 mL using methanol, resulting in a solution with a concentration of 20 µg/mL.

3.2.3. Analytical wavelength

Using methanol as the blank, EMG standard solution (20 µg/mL) was scanned in the range of 400 nm to 200 nm. It was noted that EMG exhibited maximum absorbance at 225 nm.

3.3. Method Development

3.3.1. Standard stock solution:

A 100 µg/mL solution was obtained by diluting 2 mL of a 500 µg/mL stock solution with mobile phase to a final volume of 10 mL. It was formulated in the mobile phase and injected during development trials.

3.3.2. Method Optimization

For method optimization, number of trials was run on HPLC system having principle of reversed phase chromatography and UV detection.

EMG standard solution of concentration 100 µg/mL was employed for each run. Kromasil C18 column with dimension of 250 mm by 4.6 mm i.d., and 5µm particle size, with column temperature of 40^{0C} was used for

each run. Injection volume and rate of flow was 20 µl and 1.0 ml/min respectively. Results were analyzed at wavelength of 225 nm and following mobile phases were tried as shown in Table --.

Table1 --: Mobile phase trials

Sr. No.	Mobile Phase	Composition
1.	Methanol: Water	(70:30)
2.	Methanol: Water	(80:20)
3.	Acetonitrile: Water	(70:30)
4.	Acetonitrile: 0.05% TFAA in Water	(70:30)
5.	Acetonitrile: 0.05% TFAA in Water	(80:20)

The mobile phase Acetonitrile: 0.05% TFAA in Water (80:20) with run time of 7 min was selected for chromatographic method development.

3.3.3. System suitability test

System suitability test was conducted by analyzing 5 duplicate EMG injections of concentration 10 µg/mL and the chromatograms were recorded.

3.3.4. Assay (Analysis of Marketed Test Sample)

Test sample tablets of Jardiance 25 mg that were marketed were chosen for analysis. All 20 tablets were weighed simultaneously, and their average weight was determined. Twenty tablets, which had been weighed previously, were placed in a mortar and pestle and ground into a fine powder. A 50 mL volumetric flask was used to weigh out the powder equivalent of 25 mg of EMG (273.6 mg of powder material) and transfer it into the flask. 35 mL methanol was introduced and subjected to sonication for 15 minutes, with shaking at intervals. After 15 minutes, it was permitted to cool at room temperature and then diluted to a total volume of 50 mL with methanol. Then, the solution was filtered using an appropriate 0.45 µ syringe filter, with 3-5 mL of the first filtrate being discarded. Additionally, 0.5 ml of the filtered stock solution was mixed with mobile phase to reach a total volume of 25 ml, resulting in an EMG concentration of 10 µg/mL. The resultant solution was injected, and obtained chromatograms were examined. The % assay was determined using the equation below:

$$\%\ Assay = \frac{EMG\ Spl\ area}{EMG\ Std\ avg\ area}\ X\ \frac{EMG\ STD\ wt\ (mg)}{50}\ X \frac{1.0}{20}\ X\ \frac{50}{Tablet\ sample\ weight\ (mg)}$$

$$\frac{20}{1.0}\ X\ \frac{Avg\ wt\ of\ sample\ (mg)}{Label\ claim\ of\ EMG}\ X\ 100$$

3.4. Validation

The method developed for estimating EMG was validated according to ICH guidelines for the following parameters.

3.4.1. Filtration study

The filtration study of an analytical procedure examines the interference of extraneous components from the filter, deposition on the filter bed, and the compatibility of the filter with the sample. This research utilized a sample from the EMG Test (Tablet solution). Filtration study performed using both filtered and unfiltered solution. During the filtration process, 0.45 µm PVDF and 0.45 µm Nylon syringe filters were utilized by discarding a 5 mL aliquot of the sample.

3.4.2. Solution stability

An examination of the stability of the analytical solution was carried out for both standard and test sample solutions. The stability study was conducted under standard laboratory conditions. The solution was kept under normally illuminated laboratory conditions and analyzed after 12 and 24 hours. The stability study of the Standard and Test solutions was conducted by determining the difference between the test solution results at each stability time point and those of the initial measurement.

3.4.3. Specificity

For determination of specificity, blank and placebo solutions were analyzed. The mobile phase was injected as blank while for the placebo was prepared using the excipients as stated in Table 1.

Table 2: Placebo preparation

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	crospovidone	Disintegrants	5
Total			100 mg

The placebo solution was prepared as follows. A quantity of 248.6 mg of placebo material (corresponding to 25 mg of EMG) was measured and placed into a 50 mL volumetric flask. 35 mL methanol was introduced and sonicated for 15 minutes while being shaken intermittently. It was allowed to cool till room temperature after 15 minutes and was made up to a volume of 50 mL with methanol. A suitable 0.45 µ Nylon syringe filter was used to filter the solution, while discarding 3-5 mL of the initial filtrate. 0.5 ml of the filtered stock solution was further diluted to 25 ml using the mobile phase. The resulting solution was injected, and chromatograms were obtained and examined.

3.4.4. Linearity and range

To determine the linearity and range, the dilutions of concentration 1, 5, 10, 12, 25 and 15 µg/mL were prepared as given in Table 2. The dilutions were prepared in the range of 10 to 150%.

Table 3: Dilutions for Linearity study

Sr. No.	Level (%)	mL of stock solution	Diluted to with Mobile phase (mL)	EMG Concentration (µg/mL)
1	10	0.40	20	1.00
2	50	2.00	20	5.00
3	100	4.00	20	10.00
4	125	5.00	20	12.50
5	150	6.00	20	15.00

Each dilution was injected three times, and the mean area was documented. The calibration curve was graphically represented with the analyte concentration in µg/mL on the X-axis and the mean area on the Y-axis.

3.4.5. Limit of Detection and Quantitation:

According to ICH Q2R1 guidelines, LOD and LOQ were calculated using the Calibration Curve method. This involved calculating the residual standard deviation of a regression line and determining LOD and LOQ with the following formulas: LOD = 3.3 σ / S and LOQ = 10 σ / S Where σ represents the residual standard deviation of the regression line, and S is the slope of the regression line.

3.4.6. Accuracy (% Recovery) study

Accuracy was evaluated within the range of 50% to 150% of the working concentration. As demonstrated in Table 3, each solution corresponding to the different accuracy levels was prepared three times. For each sample, % Recovery was calculated, and for each level, % RSD was observed along with % RSD for overall recovery.

Table 4: Dilutions for Accuracy study

Level (%)	API (mg)	Placebo	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)	Conc (µg/mL)
50	12.7	248.2	50	0.5	25	5.08
	12.6	247.9	50	0.5	25	5.04
	12.7	248.5	50	0.5	25	5.08
100	25.1	248.1	50	0.5	25	10.04
	25.2	248.9	50	0.5	25	10.08
	25.3	248.4	50	0.5	25	10.12
150	37.5	249.2	50	0.5	25	15.00
	37.6	248.7	50	0.5	25	15.04
	37.6	247.8	50	0.5	25	15.04

3.4.7. Precision study

To determine the precision, repeatability and intermediate precision was performed by using six dilutions as stated in Table 4. All these dilutions were injected in triplicate and analyzed.

Table 5: Dilutions for Precision study

Sample	Powder wt (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
Sample 1	273.4	50	0.5	25
Sample 2	272.8	50	0.5	25
Sample 3	273.9	50	0.5	25
Sample 4	273.6	50	0.5	25
Sample 5	273.4	50	0.5	25
Sample 6	273.7	50	0.5	25

3.4.8. Robustness

Standard solution was injected under various chromatographic conditions, including changes in flow rate by ±10% (±0.1 ml/min), column oven temperature (±2ºC), and wavelength (±3 nm). The resulting chromatograms were analyzed.

4. Result and Discussion

4.1. Preliminary characterization and identification of drug

The parameters like color, odour and appearance for EMG were observed to be white, odorless and slightly amorphous powder. The solubility of EMG was found to be better in methanol as compared to water. Thus, methanol was finalized as the solvent for dissolving EMG and all dilutions were prepared using methanol.

4.2. Selection of analytical wavelength

A scan of 20 µg/mL of EMG was taken from 400 nm to 200 nm and UV spectrum as shown in Fig. 1 was obtained. EMG showed maximum absorbance at 225 nm.

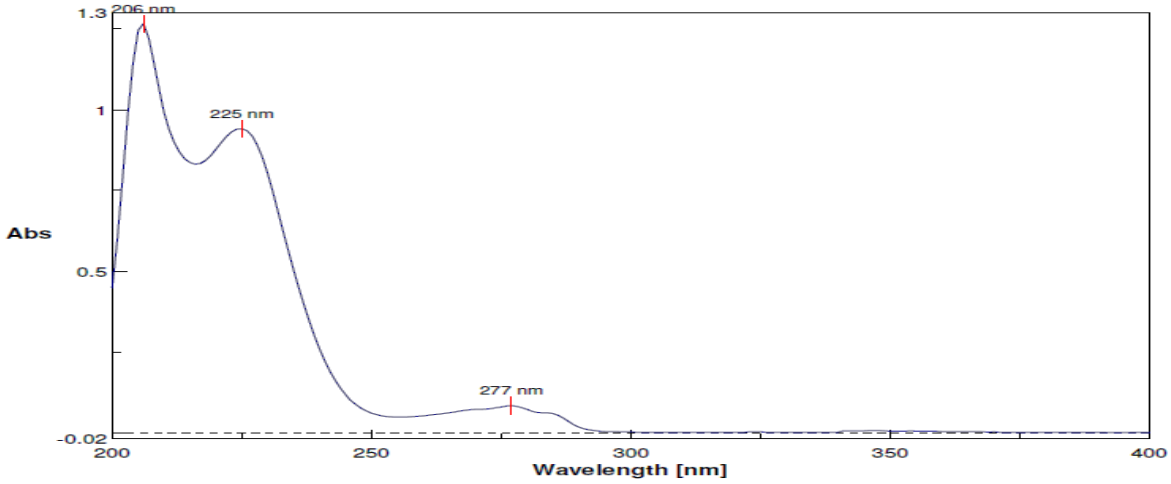


Fig. 1: UV spectrum of EMG

4.3. Method Development

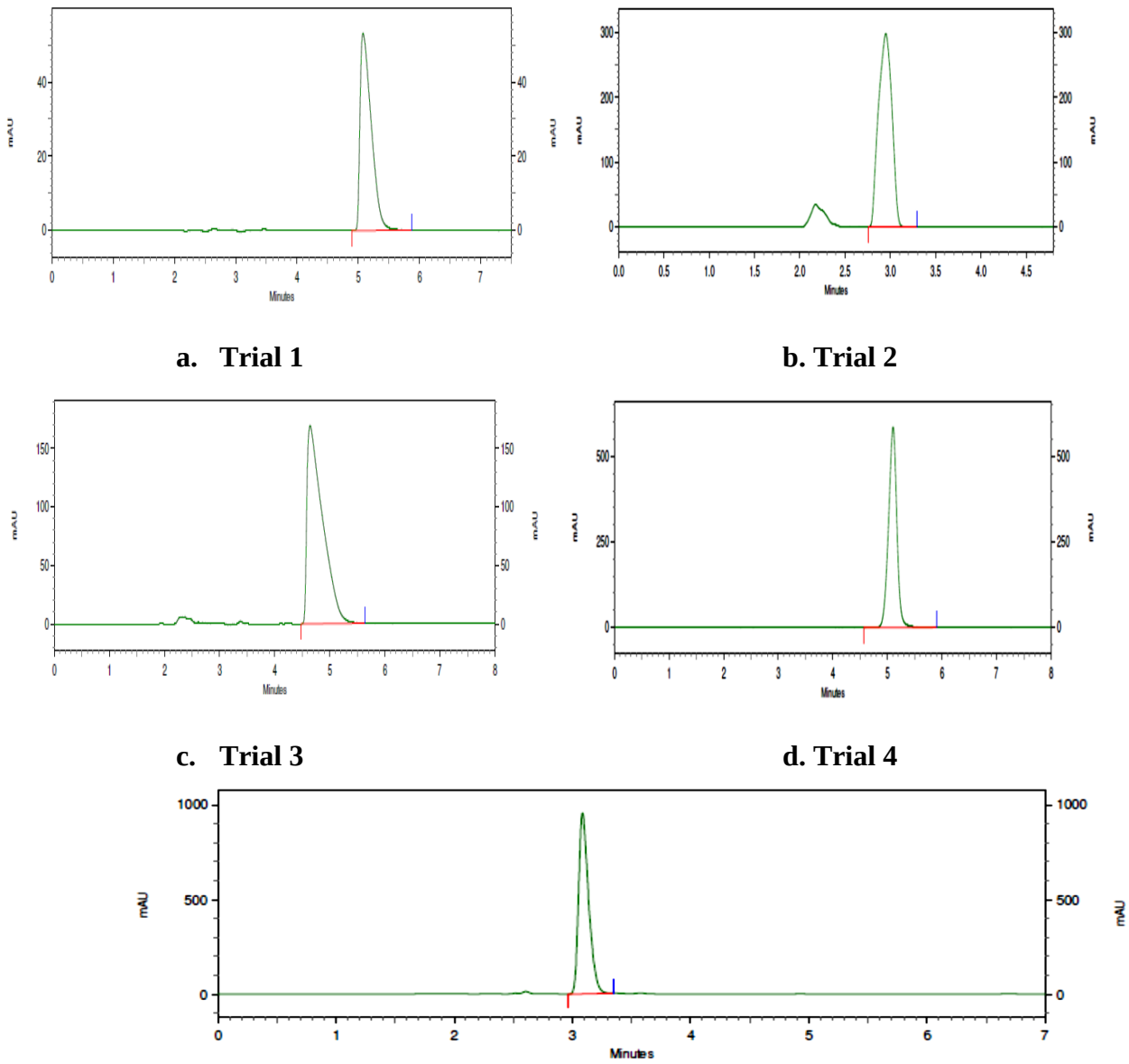
4.3.1. Method Optimization

Various mobile phases were tried for method optimization for estimation of EMG. The chromatograms obtained and the observations for trial mobile phases are shown in Fig. 2 and Table 5 respectively.

Table 6: Optimization of RP-HPLC method

Trial no.	Mobile phase	Observation	Conclusion
1	Methanol: Water (70:30)	EMG eluted at Rt 5.08 min, with Unacceptable Chromatography. (Asymmetry: 2.32, Theoretical Plates: 1465)	Method Rejected
2	Methanol: Water (80:20)	EMG eluted at Rt 2.95 min, but Chromatography not Acceptable. (Asymmetry: 0.82, Theoretical Plates: 1613)	Method Rejected
3	Acetonitrile: Water (70:30)	EMG eluted at Rt 4.64 min, with Unacceptable Chromatography. (Asymmetry: 3.27, Theoretical Plates: 1158)	Method Rejected
4	Acetonitrile: 0.05% TFAA in Water (70:30)	EMG eluted at Rt 5.06 min, with good chromatography. (Asymmetry: 1.49, Theoretical Plates: 5836)	Try to reduce EMG Rt in next trial by

			change in mobile phase composition
5	Acetonitrile: 0.05% TFAA in Water (80:20)	EMG eluted at Rt 3.04 min, and good chromatography Observed. (Asymmetry: 1.34 & Theoretical Plate: 7354)	Method Accepted



a. Trial 1 **b. Trial 2**

c. Trial 3 **d. Trial 4**

e. Trial 5

Fig. 2: Typical chromatograms obtained from trial mobile phases

Based on the trial observations, it was concluded that the conditions in trial four resulted in a better peak, improved retention time, and tailing factor. Consequently, these conditions were used for method validation.

The optimized analytical conditions for developing a chromatographic method to estimate EMG are shown in Table 6.

Table 7: Optimized Chromatographic Conditions

Parameter	**Description**
Mode	Isocratic
Column Name	Kromasil C18**,** 250 mm X 4.6mm ID, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	225 nm
Column Oven temp	40ºC
Mobile Phase	Acetonitrile: 0.05% TFAA in Water (80:20 %V/V)
Flow Rate	1.0 ml/min
Diluent	Mobile phase
Run time	7 Minutes
Retention time	3.04 Minutes

4.4. System suitability test

Table 7 shows the results obtained from the system suitability test. For each of the five standard samples run, area, asymmetry, and theoretical plates were observed. The acceptance criteria specify that the relative standard deviation of the analyte peak areas must not exceed 2.0%, the theoretical plates of the analyte peak must be a minimum of 2000, and the tailing factor (asymmetry) of analyte peaks must be below 2.0. The method was found to meet the parameters for system suitability. It follows that the chromatographic method is suitable for the intended analysis.

Table 8: Results for System Suitability Test of EMG

Sr No.	**Standard solution**	**Area**	**Asymmetry**	**Theoretical plates**
1	7564012	7564012	1.36	6234
2	7581379	7581379	1.35	6253
3	7596907	7596907	1.35	6248
4	7560304	7560304	1.36	6228
5	7578970	7578970	1.36	6245
Mean		**7576314**	**1.36**	**6242**
STD Dev		**14700.6761**		
% RSD		**0.19**		

4.5. Assay (Analysis of Marketed Test sample)

The average weight of 20 tablets (Jardiance 25 mg Tablet) was found to be 273.6 mg. By weighing this amount, stock solution and further dilutions were prepared. The assay has been carried out on two samples by using optimized chromatographic conditions and the mean was calculated. The assay result and respective chromatogram are shown in Table 8 and Fig.3 respectively.

Table 9: Assay results of Jardiance 25 mg Tablet

Sample	Area	% Assay	Mean Assay
Sample 1	7596802	100.38	99.74
Sample 2	7516976	99.11	

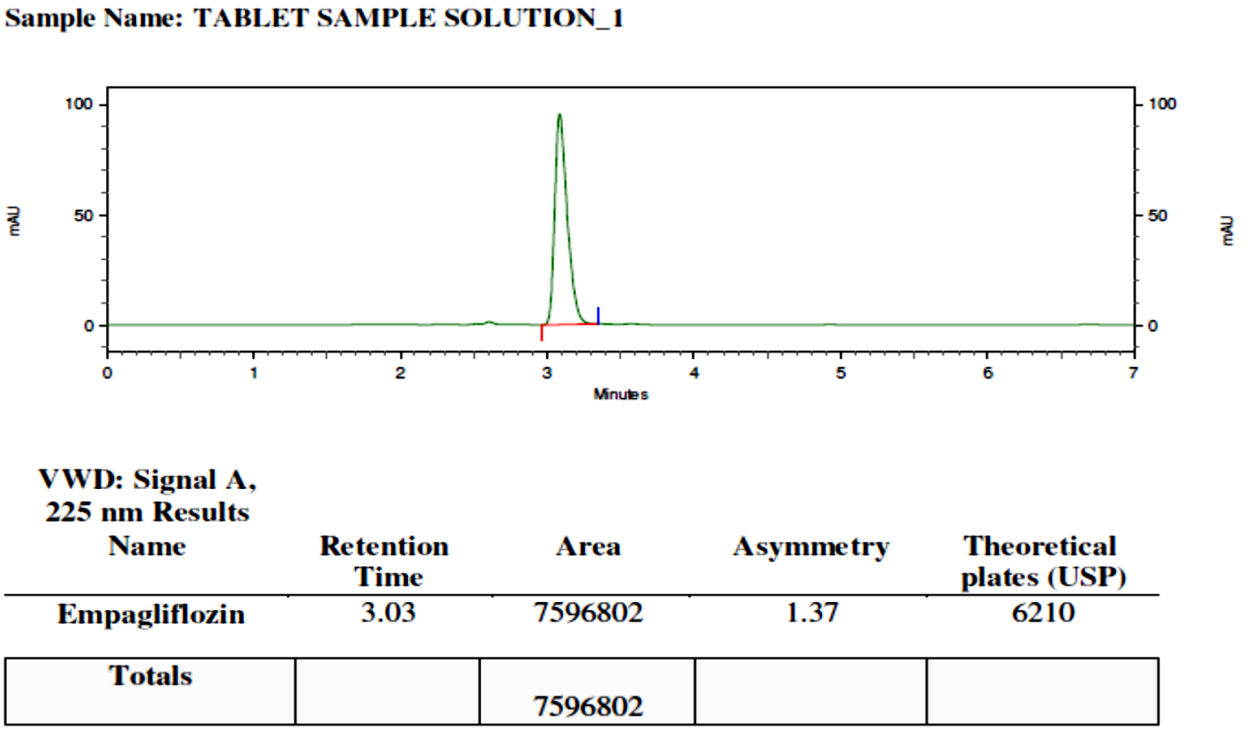


Fig. 3: Typical chromatogram Jardiance 25 mg Tablet sample.

For the assay, the acceptance criterion is that the percentage falls within 90-110%. The assay result falls within the limits for the chosen marketed test sample, and thus the sample is suitable for validation.

4.6. Validation

4.6.1. Filtration study

Filtration study was conducted on tablet test sample and the results are shown in Table 9.

Table 10: Results of Filter study

Sample description	Area	% Absolute difference
Unfiltered	7557023	NA
0.45 µ PVDF filter	7512971	0.58
0.45 µ Nylon filter	7503696	0.71

The acceptance criterion stipulates that the absolute % difference between filtered samples and the unfiltered sample must not exceed 2.0. It was noted that both the PVDF and Nylon filters meet the criteria for filter study, making either suitable for use. Therefore, the PVDF filter was utilized for the proposed research because it demonstrated a smaller absolute difference compared to the Nylon filter.

4.6.2. Solution stability

Analytical solutions were subjected to a stability study for both standard and test samples. The stability study was conducted under standard laboratory conditions. The solution was kept under normally illuminated laboratory conditions and analyzed at the start, after 12 hours, and after 24 hours. Table 10 displays the results.

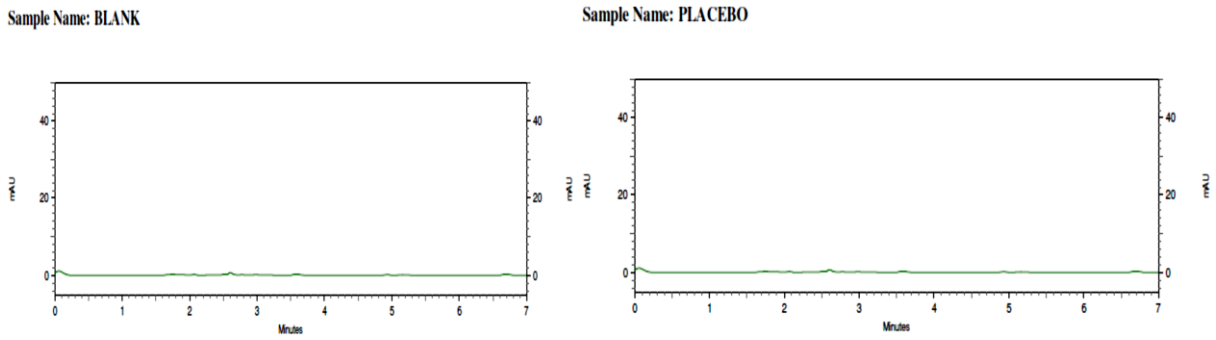
Table 11: Results of Solution stability

Sample solution			**Standard solution**		
Time point	**Area**	**% Absolute difference**	**Time point**	**Area**	**% Absolute difference**
Initial	7543912	NA	**Initial**	7568025	NA
12 Hours	7501903	0.56	**12 Hours**	7531972	0.48
24 Hours	7476680	0.89	**24 Hours**	7509630	0.77

The acceptance criterion is that the absolute difference percentage of filtered samples must not exceed 2.0 in relation to the initial solution. The Standard and Test solution demonstrated stability for up to 24 hours. Therefore, both solutions can be utilized for up to 24 hours.

4.6.3. Specificity:

Specificity refers to the capability to unambiguously access the analyte when components that might be expected to occur alongside it are also present. Blank and placebo solutions were prepared and injected to assess interference at the EMG's Rt.



a. Blank solution **b. Placebo solution**

Fig. 4: Typical chromatograms of specificity study

The acceptance criteria stipulate that both the blank and placebo solution chromatograms must not show any interference at the EMG retention time. There was no interference at the EMG's Rt with either the blank or the placebo. Thus, the developed chromatographic method met the specificity criteria.

4.6.4. Linearity and Range

The linearity refers to its capacity to produce test results that correspond proportionally to the concentration of the analyte in samples within a specified range. The areas were recorded (Table 11) for linearity solutions and calibration graph was constructed as shown in Fig. 5. The linearity study data obtained from the calibration graph has been summarized in Table 12.

Table 12: Linearity Data for EMG

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	1.00	754069	754090	0.107
		754904		
		753298		
50%	5.00	3812690	3815725	0.083
		3819025		
		3815460		
100%	10.00	7581687	7580564	0.064
		7575241		
		7584765		
125%	12.50	9479691	9478580	0.106

		9468008		
		9488041		
150%	15.00	11286042	11271872	0.119
		11259469		
		11270104		

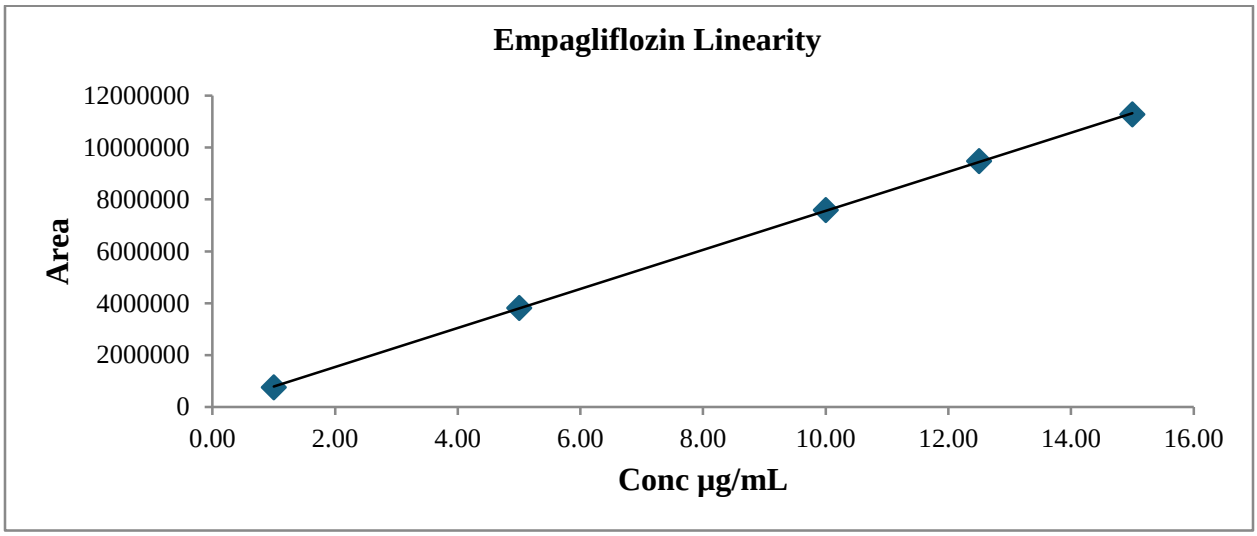


Fig. 5: Calibration curve of EMG.

Table 13: Data of linearity of EMG

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	1.0 -15.0 µg/mL	NA
2	Correlation coefficient (R^2)	0.99996	NLT 0.98
3	Intercept	30751	To be report
4	Slope	752806.33	To be report
5	% RSD for area at each level	NA	NMT 2.0

The calibration curve indicated that the EMG exhibits a linear response within the range of 1.0-15.0 µg/ml. The regression value was noted to fall within the limit.

4.6.5. Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ values for EMG were determined to be 0.167 µg/mL and 0.506 µg/mL, respectively. These limits guarantee the identification and measurement of EMG in commercial formulations.

4.6.6. Accuracy (%Recovery):

The accuracy refers to how close the test results it produces are to the true value. To determine the accuracy of an analytical method, the method is applied to samples that have been analyzed and to which known quantities of analyte have been added. Table 13 shows the results of the accuracy study conducted at 50%, 100%, and 150%.

Table 14: Result and statistical data of Accuracy of EMG

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	3821503	5.04	5.08	99.21	**99.61**	**1.0473**
	3847125	5.08	5.04	100.79		
	3806972	5.02	5.08	98.82		
100	7590203	10.02	10.04	99.80	**99.24**	**0.4913**
	7553697	9.97	10.08	98.91		
	7592314	10.02	10.12	99.01		
150	11300402	14.92	15.00	99.47	**100.31**	**0.8631**
	11528963	15.22	15.04	101.20		
	11424720	15.08	15.04	100.27		

The total recovery rate was determined to be 99.72%, with a % RSD of 0.864. The criteria for accepting accuracy is that the recovery percentage at each level and the overall recovery must lie between 98.0% and 102.0%, while the recovery percentage RSD at each level and the overall recovery must not exceed 2.0. As a result, the recovery was determined to be acceptable at all three levels. % recovery remains unaffected by variations in analyte concentration.

4.6.7. PRECISION

The precision refers to the extent to which individual test results agree with one another when the method is applied multiple times to different samples from a homogeneous population. Analytical methods typically express precision using standard deviation or relative standard deviation. Test sample precision was executed, and the outcomes are shown in Table 14.

Table 15: Result of Intra- day and Inter- Day Precision for EMG

	Sample	**Test Sample (mg)**	**Area**	**% Assay**
Repeatability	Sample 1	273.4	7505203	99.13
	Sample 2	272.8	7416972	98.18
	Sample 3	273.9	7649206	100.85
	Sample 4	273.6	7521647	99.28
	Sample 5	273.4	7405730	97.82
	Sample 6	273.7	7469242	98.55
	Mean			**98.97**
	STD DEV			**1.0751**
	% RSD			**1.086**
Intermediate precision (Inter-Day)	Sample 1	273.8	7490251	98.79
	Sample 2	274.1	7548758	99.45
	Sample 3	273.2	7410140	97.95
	Sample 4	272.9	7513117	99.42
	Sample 5	273.4	7470694	98.68
	Sample 6	274.2	7420142	97.72
	Mean			**98.67**
	STD DEV			**0.7220**
	% RSD			**0.732**
Repeatability Plus Inter-day	**Mean**			**98.818**
	STD DEV			**0.8870**
	% RSD			**0.898**

The % assay value acceptance criteria range from 90% to 110%. In the same way, the acceptance criteria for % RSD should not exceed 2.0. Therefore, the % Assay and % RSD were found to be within the acceptance limits, indicating that the method is precise and reproducible.

4.6.8. Robustness:

The robustness reflects its ability to remain unchanged by small, intentional alterations in method parameters, serving as a gauge of its reliability under typical conditions. Modifications were applied to the wavelength, flow rate, and column oven temperature, and the results are displayed in Table 15.

Table 16: Result of Robustness study of EMG

Change in Parameter	Rt	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (228 NM)	2.98	7287602	1.32	6843
Wavelength by -3 NM (222 NM)	2.99	7387102	1.34	6480
Flow rate by +10% (1.1mL/min)	2.71	6736250	1.38	5893
Flow rate by -10% (0.9mL/min)	3.25	8250632	1.32	6917
Column oven temp by +2ºC (42 ºC)	3.02	7545691	1.34	6538
Column oven temp by -2ºC (38 ºC)	3.03	7539203	1.35	6379

For robustness study, the system suitability acceptance criteria must be met. Based on the results mentioned above, it was concluded that the outcome of the system suitability test fell well within the limits and that the analytical method proved robust.

5. Conclusion

This research work details the development of a simple, precise, and accurate RP-HPLC method. Using a mobile phase of Acetonitrile: 0.05% TFAA in Water (80:20 %V/V) at a flow rate of 1.0 ml/min, the analyte was resolved with a developed RP-HPLC method on an HPLC system featuring a UV-visible detector and Openlab EZ-Chrome Software, utilizing a Kromasil C18 column measuring 250 mm X 4.6 mm with a particle size of 5 µm. Detection occurred at 225 nm. Results produced by the developed method were validated through analysis of linearity, accuracy, precision, robustness, limit of detection and limit of quantification. The developed method offers several benefits, such as reproducibility of results, fast analysis, straightforward sample preparation, and enhanced selectivity and sensitivity. The regression coefficient (r2) for all analytes is at least 0.999, indicating a strong linearity. The % recovery for the tablet dosage form was within the acceptable range. With a value of %RSD below 2%, the proposed method demonstrated a high degree of precision. The developed method is robust, reproducible, and less time-consuming, making it suitable for routine analysis in the pharmaceutical industry for bulk EMG and pharmaceutical dosage forms.

6. References

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