

**ECO-FRIENDLY UV SPECTROPHOTOMETRIC ESTIMATION OF EMPAGLIFLOZIN:
A GREEN ANALYTICAL CHEMISTRY APPROACH**Priyanka K.*, Sania Muskaan¹, Kummari Madhu², Angarely Arun Kumar³, Uddagiri Swathi⁴

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DOI: <https://doi.org/10.5281/zenodo.20456773>**How to cite this Article:** Priyanka K.*, Sania Muskaan¹, Kummari Madhu², Angarely Arun Kumar³, Uddagiri Swathi⁴. (2026). Eco-Friendly Uv Spectrophotometric Estimation of Empagliflozin: A Green Analytical Chemistry Approach. World Journal of Pharmaceutical and Medical Research, 12(6), 294–300.

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Article Received on 02/05/2026

Article Revised on 23/05/2026

Article Published on 01/06/2026

ABSTRACT

A simple, rapid, economical, and green UV spectrophotometric method was developed for the estimation of Empagliflozin in bulk and tablet dosage form. The method was designed according to the principles of green analytical chemistry by minimizing the use of hazardous organic solvents. Empagliflozin was initially dissolved in a small quantity of methanol and further diluted with distilled water. The prepared solution was scanned in the wavelength range of 200–400 nm using a UV–Visible spectrophotometer and 224 nm was selected as the analytical wavelength based on suitable absorbance characteristics. The method exhibited good linearity within the selected concentration range with a correlation coefficient (R^2) of 0.999. The developed method was validated according to analytical validation parameters including selectivity, sensitivity, linearity, accuracy, precision, robustness, LOD, LOQ and assay. The results obtained for these parameters were found to be within acceptable limits, indicating the sensitive, accurate, reliability and reproducibility of the method. Assay studies demonstrated satisfactory estimation of the drug content. The greenness profile of the method was evaluated using AGREEprep and MoGAPI assessment tools. The AGREE prep evaluation showed an excellent greenness score of 0.86 due to reduced solvent consumption, minimal waste generation, simple sample preparation, and low energy requirement. The MoGAPI pictogram obtained score of 83 indicating that the method possesses good eco-friendly characteristics with minimal environmental burden. The proposed method was found to be simple, eco-friendly, cost-effective, accurate, precise and suitable for routine analysis of empagliflozin in pharmaceutical laboratories.

KEYWORDS: Empagliflozin, Method Validation, Green Analytical Chemistry, AGREEprep, MoGAPI.**INTRODUCTION**

Empagliflozin is a selective sodium-glucose co-transporter-2 (SGLT2) inhibitor widely used for the treatment of type 2 diabetes mellitus. The drug acts by inhibiting glucose reabsorption in the proximal renal tubules, thereby promoting urinary glucose excretion and improving glycemic control. In addition to its antihyperglycemic activity, empagliflozin has demonstrated beneficial effects in reducing cardiovascular and renal complications associated with diabetes mellitus.^[1]

Several analytical methods including HPLC, LC–MS/MS, HPTLC, and UV spectrophotometry have been reported for the estimation of empagliflozin either alone or in combination with other antidiabetic agents.

Chromatographic techniques provide excellent sensitivity and selectivity; however, they require expensive instrumentation, high energy consumption, longer analysis time, and large quantities of hazardous organic solvents. In contrast, UV spectrophotometric methods are simple, rapid, economical, and suitable for routine pharmaceutical analysis.^[2]

Recently, increasing attention has been directed toward Green Analytical Chemistry due to growing environmental and safety concerns associated with conventional analytical procedures. Green analytical chemistry aims to reduce the environmental impact of analytical methods by minimizing solvent consumption, decreasing hazardous chemical usage, reducing waste generation, lowering energy consumption, and improving

operator safety. The principles of green chemistry introduced by Paul Anastas and John Warner have significantly influenced the development of eco-friendly analytical methodologies.^[1]

To evaluate the environmental friendliness of analytical methods, several greenness assessment tools such as NEMI, Analytical Eco-Scale, GAPI, AGREE, and MoGAPI have been developed. Among these, AGREEprep and MoGAPI provide comprehensive evaluation of analytical procedures based on solvent usage, waste generation, sample preparation, energy consumption, and occupational hazards. Recent studies have emphasized the importance of using multiple greenness assessment tools to ensure reliable evaluation of environmentally sustainable analytical methods.^[3]

Therefore, the present study was aimed at developing and validating a simple, accurate, economical, and eco-friendly UV spectrophotometric method for the estimation of empagliflozin using minimal organic solvent consumption. The developed method was further assessed for its environmental sustainability using AGREEprep and MoGAPI greenness assessment tools to establish its suitability for routine pharmaceutical analysis.

2. MATERIALS AND METHODOLOGIES

Chemicals and reagents

Empagliflozin was purchased from Dhamech Pharma, Mumbai. Methanol and distilled water were supplied by Virat Lab, Mumbai.

Instruments

For the experiment, a Lab India Analytical UV-Visible spectrophotometer(double-beam)- with a pair quartz cell having a 1cm length was used. Weighing was carried out using CONTECH electronic balance.

Preparation of Standard solution of Empagliflozin

Empagliflozin(10mg) was weighed carefully and transferred to 10ml volumetric flask and dissolved with 2ml methanol and diluted upto 10ml with water to get the concentration of 1000 μ g/ml. This concentration is diluted in a separate volumetric flask to attain 100 μ g/ml. This solution is used as a stock solution. The stock

solution is further diluted with water to attain desired concentrations.

Preparation of sample solutions

Marketed formulation (Empanorm 25mg- Alkem) was broken into powder and separately transferred 10mg equivalent weight(72mg) of tablet powder into 10ml volumetric flask and dissolved it in 2ml methanol and make up the solution up to 10 ml with water. From this further dilutions were made to get the desired concentrations(10 μ g/ml).

METHODOLOGY

The greenness profile of the developed method was evaluated using:

- i. **AGREEprep:** It has twelve windows to consider each principle. The result is a pictogram with 12 sections around it, associated with each parameter, and a circle in the middle, giving the final score between 0 and 1. It is also possible to interpret the results with colours such as red, yellow, and green tones in the pictogram. In the final score scale, 0 indicates un-satisfactory, and 1 indicates satisfactory.^[4-5]
- ii. **MoGAPI:** GAPI assessment are demonstrated as a pictogram consisting of four additional pentagons surrounding a centre pentagon. They are related to five parameters: method's general type, sample handling, sample preparation, solvents/reagents, and instrumentation. According to the evaluation results, the pictogram contains red, yellow, and green colours. In total, fifteen different criteria are assessed with GAPI. The main advantage of this tool is that it is a simple tool that includes many different parameters of an analytical method. Even though it overcomes the significant disadvantages of the NEMI tool, giving qualitative results and providing a complex representation are the drawbacks of GAPI.^[6]

3. RESULTS AND DISCUSSIONS

Selection of wavelength

The absorbance of the solution containing Empagliflozin10 μ g/ml was scanned in the UV range 200-400nm using water as blank.

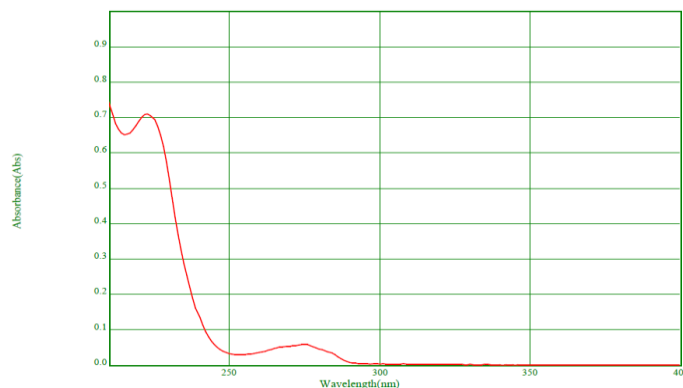


Figure no. 1: Figure showing λ_{max} of Empagliflozin.

For the Empagliflozin maximum wavelength was found at 224 nm, and maximum absorbance was found to be 0.7093.

ASSAY

Placed the working standard solution and sample solution into the UV spectrophotometric system and measured the absorbance and calculated the % Assay

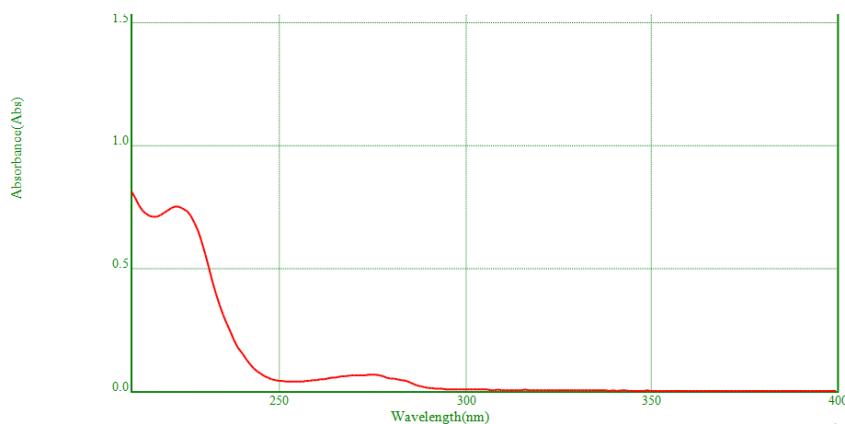


Figure no. 2: Figure showing absorbance of Empagliflozin tablets with absorbance of 0.7236.

Absorbance is converted into concentration by using the linearity curve data. From the linearity graph

$$y=mx+c$$

By using above equation the concentration was found to be 10.1.

Now by using the below formula assay of Empagliflozin was determined

$$= (\text{Concentration} \times \text{average weight in mg}) / (\text{Weight of powder equivalent to 10mg} \times \text{label claim of Empagliflozin}) \times 10$$

The percentage purity of Empagliflozin in tablet dosage form was found to be 101%.

VALIDATION PARAMETERS

SPECIFICITY

The blank solution i.e., water was placed in the UV and spectrum was recorded.

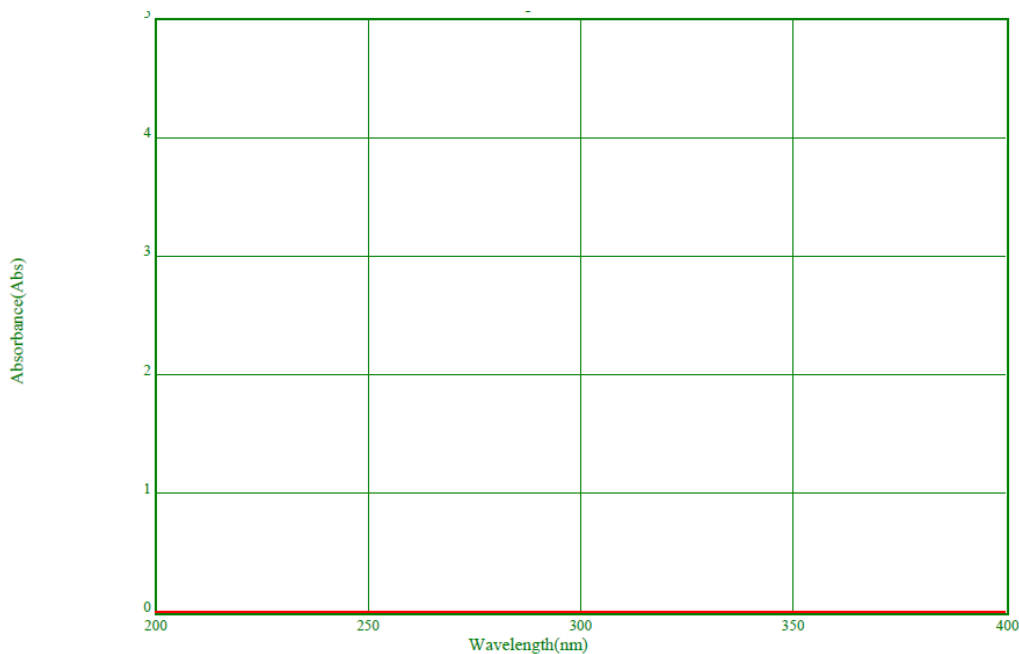


Figure no.3: Spectrum showing blank.

From the spectrum we can conclude that excipients or solvents are not interfering the spectrum of Empagliflozin.

absorbances were noted by placing in UV. Then graph was plotted by taking Absorbances vs concentrations. It showed Linear graph.

LINEARITY

Five different concentrations were prepared and

Table No. 1: Observation values for Linearity.

S.No	Concentration [$\mu\text{g/ml}$]	Absorbance of Empagliflozin
1	6	0.25
2	8	0.488
3	10	0.716
4	12	0.9413
5	14	1.1399

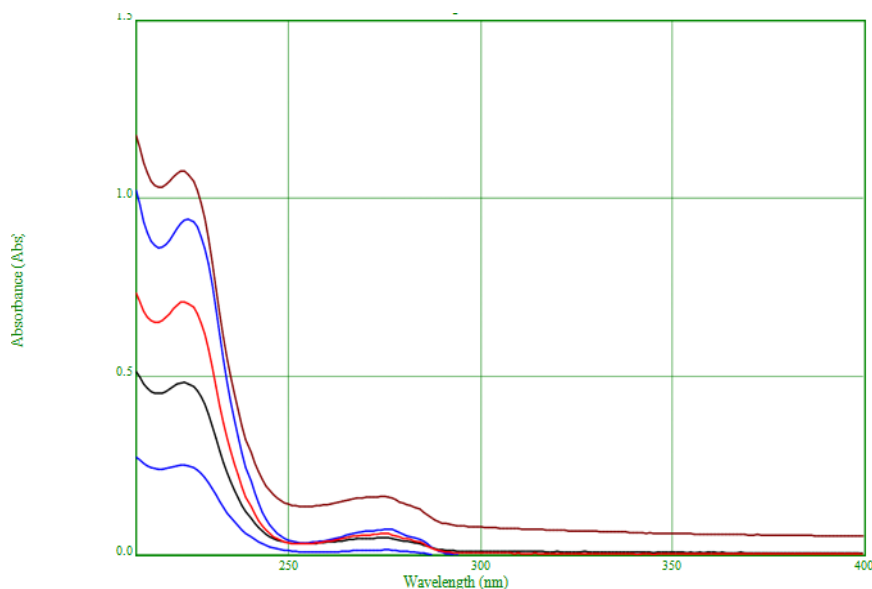


Fig. no. 4: Spectrum showing Linearity.

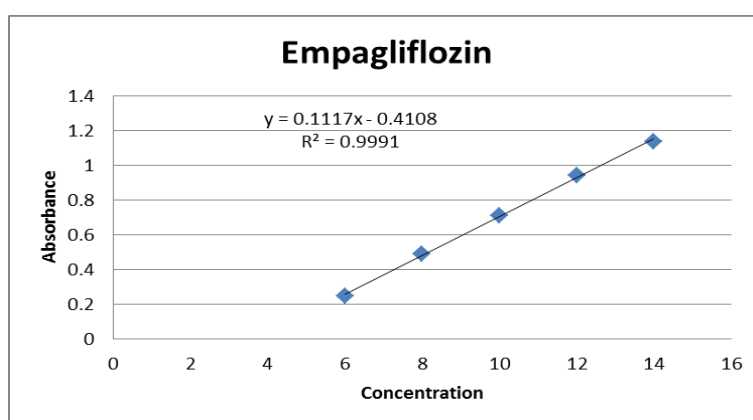


Fig. no. 5: Calibration Curve of Empagliflozin.

ACCURACY

Accuracy should be assessed using a minimum of nine

determinations over a minimum of three concentrations levels the specified range(80%, 100%, 120%)

Table No. 2: Observation values for Accuracy.

S. No	Accuracy level	Conc. taken ($\mu\text{g/ml}$)	Absorbance	Conc. found ($\mu\text{g/ml}$)	Recovery	% Mean
1	80	18	1.5902	17.9	99.4	100.7
2	80	18	1.6506	18.4	102.2	
3	80	18	1.6215	18.1	100.5	
4	100	20	1.8497	20.2	101.0	100.5
5	100	20	1.8389	20.1	100.5	
6	100	20	1.8265	20.0	100	
7	120	22	2.0743	22.2	100.9	99.5
8	120	22	2.0058	21.6	98.1	
9	120	22	2.0456	21.9	99.5	

The results obtained for recovery at 80%, 100%.120% are within the limits(98-102%). Hence method is accurate.

PRECISION

Precision was determined by analyzing standard preparations (10ug/ml) for five times.

The spectrograms were recorded and results were summarized

i. Repeatability

Table No. 3: Observation values for Repeatability.

Concentration	Absorbance of Empagliflozin at 224nm
10 µg/ml	0.7093
10 µg/ml	0.7091
10 µg/ml	0.7087
10 µg/ml	0.7090
10 µg/ml	0.7092
Average	0.70906
SD	0.00023022
%RSD	0.032

ii. Intermediate precision

The inter-day precision of the proposed method was determined on samples of drug solutions at different days.

Table No. 4: Observation values for Inter-Day Precision.

Concentration	Empagliflozin	
	Day-1	Day-2
10 µg/ml	0.7093	0.7160
10 µg/ml	0.7091	0.7162
10 µg/ml	0.7087	0.7167
10 µg/ml	0.7090	0.7206
10 µg/ml	0.7092	0.7165
Average	0.70906	0.7172
SD	0.00023022	0.00192
%RSD	0.032	0.26

The %RSD obtained is within the limits(<2%). Hence the method is precise.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION(LOQ)

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

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

Table No.5:- Observation values for LOD & LOQ.

Drug Name	Standard Deviation	Slope	LOD	LOQ
Empagliflozin	0.00023022	0.1117	0.0067	0.0206

ROBUSTNESS

robustness of the method was demonstrated by placing prepared standard solution in the UV and spectrums and absorbance values were recorded by changing the

parameters like wavelength and temperature. There was no significant change in absorbance values.

Table No. 6: Observation values for Robustness.

Parameter	Empa
Wavelength	
222nm	0.7092
224nm	0.7093
226nm	0.7091
Temperature	
26 ⁰ C	0.7063
28 ⁰ C	0.7093
30 ⁰ C	0.7085

From the absorbance values we can confirmed that, there was no significant change in absorbance values.

AGREEprep

The AGREE prep assessment indicated an excellent greenness score of 0.86. The GAPI assessment indicated predominantly green zones corresponding to low solvent consumption, simple sample preparation, and minimal waste generation. A few light green zones were observed due to the use of a small quantity of methanol and off-line sample preparation. Overall, the method demonstrates a favorable environmental profile.

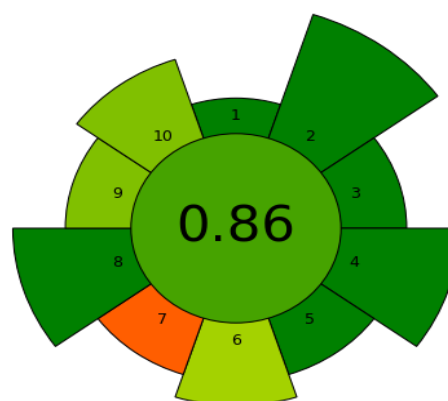


Fig. no. 5: Analytical Greenness Metric for Sample Preparation.

MoGAPI

The environmental impact of the developed method was evaluated using the MoGAPI assessment tool. The obtained score of 83 indicated that the proposed UV spectrophotometric method possesses good eco-friendly characteristics with minimal environmental burden. The predominance of green regions in the MoGAPI pictogram confirmed reduced solvent consumption, low waste generation, simple sample preparation, and low energy utilization.

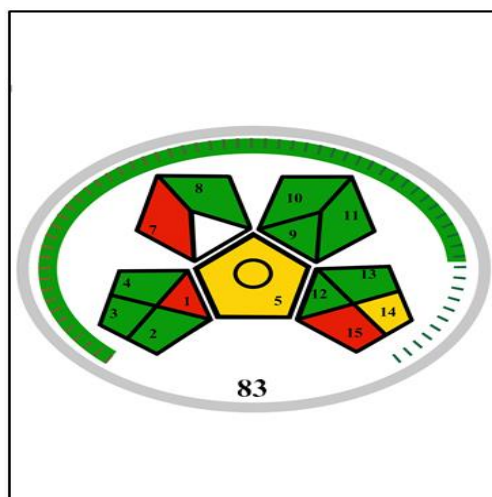


Fig. no. 6: Assessment of Greenness by using MoGAPI.

4. SUMMARY AND CONCLUSION

SUMMARY

A Green Analytical UV spectrophotometric method has been developed and validated for the estimation of Empagliflozin in its pure and tablet dosage form.

The process was done by using Green Analytical Principles with the detection wavelength set at 224nm with the absorbance of 0.7093. The method was linear with the correlation coefficient 0.99 in the concentration range of 6-14ug/ml. The limit detection and Limit of Quantification were found to be 0.0067ug/ml and 0.0206 ug/ml respectively. The repeatability and Inter-day precision were satisfactory and the relative standard deviation did not exceed 2%. The accuracy of the method from the recovery studies is within the limits. The method is sensitive, precise, accurate and robusted. The method met the ICH regulatory requirements.. The greenness profile of the method was evaluated using AGREEprep and MoGAPI assessment tools with a score of 0.86 and 83 respectively indicating reduced solvent consumption, minimal waste generation, simple sample preparation, and low energy requirement.

CONCLUSION

A simple, rapid, economical, and green UV spectrophotometric method was developed for the estimation of Empagliflozin in bulk and tablet dosage form. The method was designed according to the principles of green analytical chemistry by minimizing the use of hazardous organic solvents. Empagliflozin was initially dissolved in a small quantity of methanol and further diluted with distilled water. 224 nm was selected as the analytical wavelength for the prepared solution based on suitable absorbance characteristics.

The developed method was validated according to analytical validation parameters including selectivity, sensitivity, linearity, accuracy, precision, robustness, LOD, LOQ and assay. The results obtained for these parameters were found to be within acceptable limits,

indicating the sensitive, accurate, reliability and reproducibility of the method. Assay studies demonstrated satisfactory estimation of the drug content.

The greenness profile of the method was evaluated using AGREEprep and MoGAPI assessment tools. The AGREE prep evaluation showed an excellent greenness score of 0.86 due to reduced solvent consumption, minimal waste generation, simple sample preparation, and low energy requirement. The MoGAPI pictogram obtained score of 83 indicating that the method possesses good eco-friendly characteristics with minimal environmental burden.

The proposed method was found to be simple, eco-friendly, cost-effective, accurate, precise and suitable for routine analysis of empagliflozin in pharmaceutical laboratories.

ACKNOWLEDGMENT

Our sincere thanks to our SSRCP management for providing us power and strength to overcome all the hurdles and hindrances that come in the way of doing project.

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