RESEARCH PAPER

Assay of Empagliflozin in Empagliflozin Tablets by HPLC

Dr. Parag Das (Ph.D.)[1*], Mr. Updesh Singh[2], Mr. Animesh Maity (M.Sc.)[3], Mr. Amrit Kumar Rath[4], Prof. (Dr.) Sruti Ranjan Mishra[5], Mrs. Amrita Nayak[6], Mrs. Manashi Das[7]

[1]Vice President – Technical, Oman Pharmaceutical Products Co LLC, Sultanate of Oman.*
[2]Sr. Officer, Analytical Development Labs, Oman Pharmaceutical Products Co LLC, Sultanate of Oman.
[3]Sr.Manager, Analytical Development Labs, Oman Pharmaceutical Products Co LLC, Sultanate of Oman.
[4&6]Associate Professor, Danteswari College of Pharmacy, Borpadar, Raipur Road, Jagdalpur, Chhattisgarh, Pin: 494221.
[5]Professor, Danteswari College of Pharmacy, Borpadar, Raipur Road, Jagdalpur, Chhattisgarh, Pin: 494221.
[7]Free lance Pharma Professional, Pune, India.

ABSTRACT

Background: Empagliflozin is a member of the sodium-glucose co-transporter 2 (SGLT2) inhibitor class, which helps lower blood glucose levels by promoting the excretion of glucose through urine. It is indicated for type 2 diabetes and is not suitable for managing type 1 diabetes, where insulin production is absent. Persistent high blood sugar in diabetes may lead to serious complications, including cardiovascular disease, kidney damage, neuropathy, and vision problems. The use of empagliflozin, combined with lifestyle modifications such as diet, exercise, and smoking cessation, along with regular blood glucose monitoring, can aid in glycemic control and reduce the risk of diabetes-related complications. Each 10 mg tablet contains 10 mg of empagliflozin, and each 25 mg tablet contains 25 mg of the drug. Recent dosage forms include orally disintegrating tablets (ODTs) and films to improve patient compliance.

Aim: The study aims to develop and validate a simple, rapid, and cost-effective HPLC-UV method for the simultaneous estimation of empagliflozin in tablet formulations. The method involves sample dilution with a diluent and chromatographic separation using a Waters e2695 HPLC system with a Waters Symmetry C8 column (250 mm × 4.6 mm, 5 µm). The mobile phase consists of a 50:50 v/v mixture of buffer and acetonitrile, with a column flow rate of 1.0 mL/min, column temperature set at 40°C, and autosampler temperature at 20°C. Empagliflozin eluted at approximately 8.0 minutes.

Results and discussion: The developed method was validated according to the ICH guidelines and values of accuracy; method precision and other statistical analysis were found to be in good accordance with the specified acceptance criteria.

Conclusion: Validation of the method followed ICH guidelines. Accuracy, precision, linearity, and other statistical parameters were within the acceptable criteria, confirming the reliability of the method.

Keywords: Empagliflozin, HPLC-UV, PDA and Validation

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Conflict of interest: None

INTRODUCTION

Empagliflozin, available in 10 mg and 25 mg tablet formulations, is an SGLT2 inhibitor used primarily to improve glycemic control in adults with type 2 diabetes. The 10 mg tablet is oval, pale yellow, and biconvex with the imprint "10," while the 25 mg tablet has the same shape and color with the imprint "25." Its molecular formula is C23H27ClO7, molecular weight 450.91 g/mol, and melting point approximately 152°C. Structurally, empagliflozin contains aromatic rings and oxygen functional groups characteristic of many pharmaceutical compounds.

Empagliflozin is highly soluble in water and may also be used in its free base form. Clinically, it helps reduce cardiovascular risk in adults with established cardiovascular disease alongside improving blood glucose levels when combined with diet and exercise. Its effectiveness and safety profile have made it a commonly prescribed therapy for patients aged ten years and above with type 2 diabetes.

The current study focuses on developing a robust, economical, and validated HPLC-UV method for the quantitative determination of empagliflozin in tablet formulations. This method aims to provide a reliable tool for routine quality control and ensure accurate dosing in pharmaceutical preparations.

MATERIALS AND METHODS

Chemicals and reagents:

Empagliflozin standards and Empagliflozin tablets sample were provided by Oman Pharmaceutical Products Co L.L.C. Milli-q water was procured from Inhouse.

Solution preparations:

Mobile phase A:

Transfer accurately 1 mL of Triethylamine in a beaker containing 1000 mL water. Mix well and sonicate to degas. Filter it with 0.45µ filter.

**Author for Correspondence:*

Mobile phase A:
Prepare the mixture of Acetonitrile and buffer - A in the ratio of 50:50 (v/v) and mix well. Sonicate to degas.

Diluent:
Prepare Mixture of Water, Acetonitrile in the ratio of 30:70 v/v/v. Sonicate it.

Preparation of Standard Stock Solution:
Weigh about 25 mg of Empagliflozin working standard, transfer into 50 mL volumetric flask, add 30 mL of diluent and dissolve by using sonication; dilute to 50 mL with diluent. Mix well

Preparation of Standard Solution:
Standard Solution:
Step-I: Further dilute 10 mL of Standard Stock solution into a 100 mL volumetric flask with diluent & mix well.
Test Solution: (Empagliflozin 10mg Tablet)
Weigh and transfer 5 tablets equivalent to 50 mg of Empagliflozin into 100 mL volumetric flask. Add about 70 mL of diluent and sonicate for 15 minutes with intermediate swirling, make up volume up to the mark with diluent and mix. Filter the solution with 0.45µ syringe filter (disregard about 4-5 mL of filtrate). Further dilute 10 mL of this solution to 50 mL with diluent & mixed well.

Test Solution (Empagliflozin 25mg Tablet)
Weigh and transfer 4 tablets equivalent to 100 mg of Empagliflozin into 200 mL volumetric flask. Add about 150 mL of diluent and sonicate for 15 minutes with intermediate swirling, make up volume up to the mark with diluent and mix. Filter the solution with 0.45µ syringe filter (disregard about 4-5 mL of filtrate). Further dilute 10 mL of this solution to 50 mL with diluent & mixed well.
Procedure:
After equilibrating the column inject diluent as blank, standard solution to test the system suitability criteria. Once the system suitability test passes then inject diluent, placebo solutions, standard solution, test solution and bracketing standard solution into the liquid chromatographic system and record the chromatograms. Report the results.

Chromatographic study:
Empagliflozin tablets content in all solutions was determined by HPLC by using the chromatographic conditions as mentioned in Table No- 1.
The Chromatographic data were analyzed and Specificity, Linearity and range, method precision, and accuracy were determined.

Table no-1

Column	**Symmetry C84.6x 250mm,5µm (Part No: WAT054270) (Mfg. By: Waters)**
Flow rate	1.0 mL/minute
Injection volume	5µl
Column Temperature	40°C
Sample Temperature	20°C
Wavelength	UV-225 nm
Elution:	Isocratic
Run Time	8 min

Results and discussion:
The developed method for estimation of Empagliflozin in Empagliflozin tablets by HPLC were validated by using the following parameters:

System suitability:
For establishing the system suitability, the procedure described in the methodology was followed before starting the analysis. System suitability data has been presented in Table No- 1.

Table No.1 – System suitability	
Injection #	Area of Empagliflozin
1	1268299
2	1270241
3	1273596
4	1273328
5	1268730
6	1267835
Mean	1270338
SD	2552.34272
%RSD	0.2

Specificity:
Specificity of the method was evaluated with respect to interference from blank at the retention time of each standard, Standard solution and sample preparation. Refer Fig. 2,3 for the chromatograms and specificity data has been presented in Table No- 1,2.

Table No. 2: Interference studies		
Observation		
Name of Solution	Interference	RT (Min)
Blank	No interference	NA
Placebo	No interference	NA
Standard	No interference	3.677
Sample	No interference	3.677

Figure 1 Chemical structure of Empagliflozin

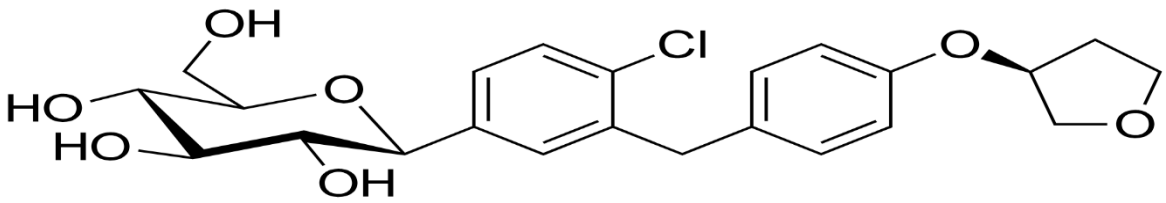


Figure 2: Blank Chromatogram

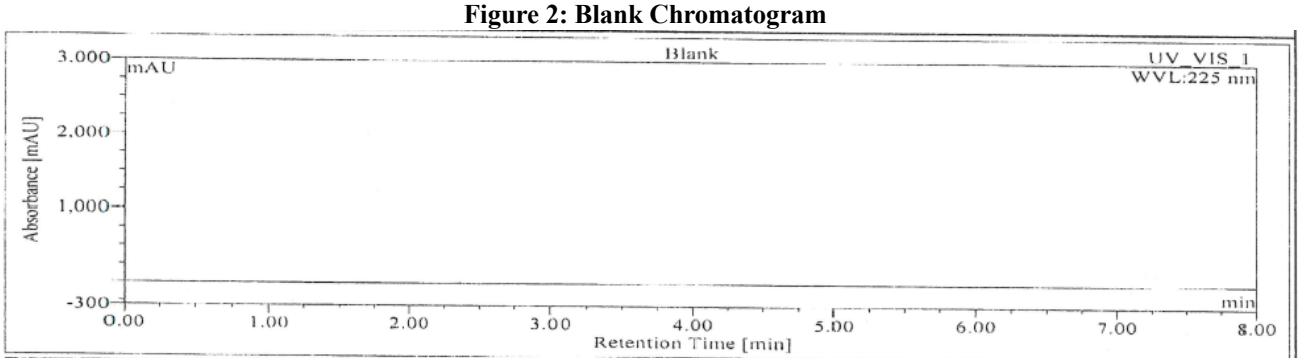


Figure 3: Standard Chromatogram

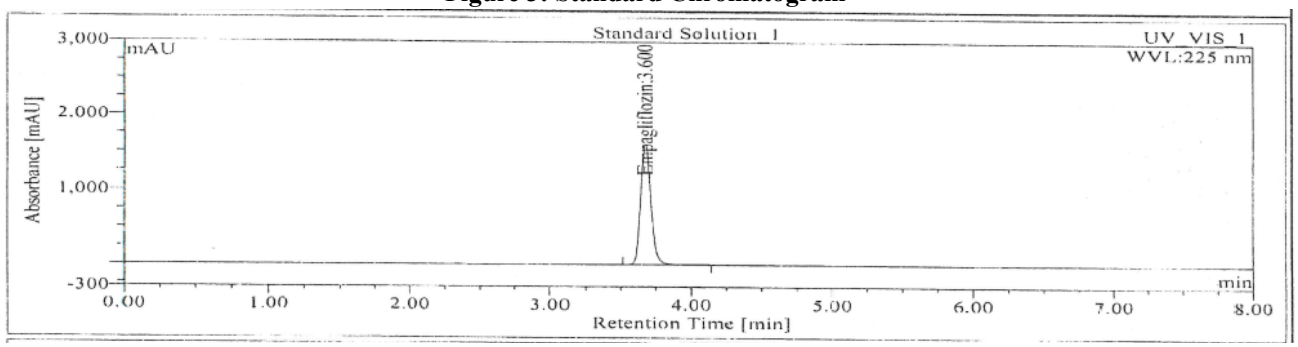
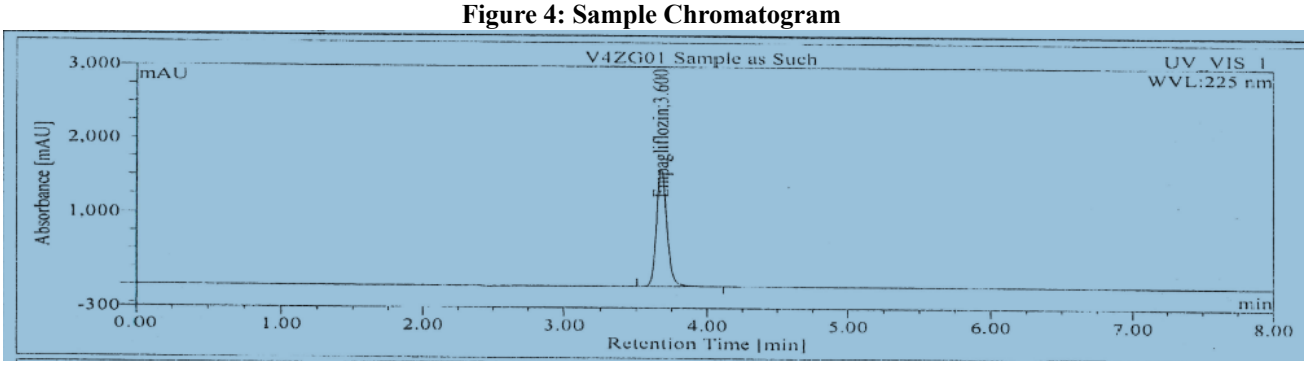


Figure 4: Sample Chromatogram



Linearity and range:

Standard solution containing Empagliflozin was prepared. Linearity study was demonstrated for Empagliflozin in the

concentration range from six different concentrations LOQ, 50%, 80%, 100%, 120%, and 150% the targeted concentrations with respective Empagliflozin concentration in test sample (Empagliflozin tablets contains 250mg and 10mg of Empagliflozin). Considering the The recommended dosage of Empagliflozin is 10 mg empagliflozin once daily in the morning, taken with or without food. Empagliflozin may be increased to 25 mg empagliflozin once daily for additional glycemic control. The linearity was evaluated using the calibration curve plotted by average peak areas against concentrations to calculate coefficient of correlation and slope. In general, a value of correlation coefficient (r) > 0.990 is considered as the evidence of an acceptable fit for the data to the regression line.

The results obtained are presented in Table No-3 which demonstrates that the current method was linear for the three analytes in the range specified above with a correlation coefficient better than 0.990. The plots have been represented in Fig. 3.

Table No. 3 - Linearity of Empagliflozin

Level No.	Concentration (µg/mL)	Mean area
1	10.042	128261
2	50.209	631851
3	80.334	1022593
4	100.418	1276876
5	120.501	1537425
6	150.627	1914691
Slope		12741.246
Intercept		-2248.378
CC		1.0000
R^2		1.000

Method Precision:

Precision was determined by preparing the standard sample as per the methodology. The sample was prepared in six replicates and injected into the chromatography system. The content of each preparation was calculated and finally the %RSD of the six replicate preparations data has been presented in Table No- 4.

Table No. 4 - Method Precision

Analysis	**27.06.2024**	
Instrument	**QC/INS/162**	
Column	**ARND-663**	
Analyst	**Updesh Singh**	
Preparation #	**% Assay**	
	Empagliflozin	**Empagliflozin**
	Batch No:V4ZE01	**Batch**
1	100.9	99.8
2	101.5	100.2
3	101.3	100.3
4	101.2	100.1
5	100.8	100.4
6	101.1	100.4
Mean	**101.1**	**100.2**
SD	**0.25820**	**0.22804**
%RSD	**0.3**	**0.2**

Accuracy:

The Accuracy of the methodology at LOQ%, 100% and 150% of the specification limit, samples were analyzed as per methodology and % recovery at each spiked level was calculated. Prepare LOQ%,100% and 150% concentration levels in triplicate Results are presented below in Table No-.5&6.

Table No. 5 - Accuracy of Empagliflozin Tablets 10mg

Sr. No.	Level	Wt. of Empagliflozin WS (mg)	Wt. of Placebo (mg)	Sample Area	Amount recovered (µg/mL)	Amount added (µg/mL)	% Recovery		
1	10%-1	5.1055	1235.10	125861	10.284	10.180	101.0	Avg:	101.6
2	10%-2	5.0885	1236.30	126657	10.349	10.146	102.0	SD:	0.55076
3	10%-3	5.0850	1235.90	126414	10.329	10.139	101.9	%RSD	0.5
4	100%-1	50.46	1235.80	1248441	102.009	100.617	101.4	Avg:	101.5
5	100%-2	50.40	1235.20	1249203	102.071	100.498	101.6	SD:	0.11547
6	100%-3	50.46	1234.70	1250742	102.197	100.617	101.6	%RSD	0.1
7	150%-1	75.47	1234.40	1864935	152.383	150.487	101.3	Avg:	101.3
8	150%-2	75.47	1234.60	1862296	152.167	150.487	101.1	SD:	0.20000
9	150%-3	75.39	1235.80	1866945	152.547	150.328	101.5	%RSD	0.2

Table No. 6 - Accuracy of Empagliflozin Tablets 25mg									
Sr. No.	Level	Wt. of Empagliflozin WS (mg)	Wt. of Placebo (mg)	Sample Area	Amount recovered (µg/mL)	Amount added (µg/mL)	% Recovery		
1	10%-1	10.1400	724.40	125518	10.256	10.110	101.4	Avg:	101.1
2	10%-2	10.1535	724.90	124882	10.204	10.123	100.8	SD:	0.30000
3	10%-3	10.1320	725.40	124958	10.210	10.102	101.1	%RSD	0.3
4	100%-1	100.48	738.00	1228635	100.391	100.179	100.2	Avg:	100.3
5	100%-2	100.45	735.80	1227295	100.281	100.149	100.1	SD:	0.32146
6	100%-3	100.45	736.30	1233820	100.815	100.149	100.7	%RSD	0.3
7	150%-1	150.48	736.00	1840560	150.391	150.029	100.2	Avg:	100.6
8	150%-2	150.50	735.20	1861671	152.116	150.049	101.4	SD:	0.72342
9	150%-3	150.53	735.70	1839014	150.265	150.078	100.1	%RSD	0.7

CONCLUSION

This intended study concludes that the proposed method is economical, simple, sensitive and reliable. Also, it is found to be specific, linear, precise and accurate. Hence, it can be employed for the routine Estimation of Empagliflozin in Empagliflozin tablets Related Substances by HPLC.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest

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