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Development and Validation of UV Spectroscopic Method for Estimation of Empagliflozin in Tablet Dosage Form

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Abstract

To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Empagliflozin in tablet dosage form. The drug is freely soluble in analytical grade methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Empagliflozin in tablet dosage form and also validated as per ICH guidelines. The drug is soluble in analytical grade methanol, slightly soluble in ethanol and very slightly soluble in water. So, the analytical grade methanol is used as a diluent in method. The melting point of Empagliflozin was found to be 151-152˚C (uncorrected). It showed absorption maxima 276 nm in analytical grade methanol. On the basis of absorption spectrum the working concentration was set on 50µg/ml (PPM). The linearity was observed between 10-90 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.75, 99.00 and 99.17% for three levels respectively. The % RSD for precision was found to be 0.90% and for Ruggedness is 0.56%. A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Empagliflozin in tablet dosage form. The method could be considered for the determination of Empagliflozin in quality control laboratories.

Keywords: Empagliflozin, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

Introduction

Empagliflozin is an orally administered selective sodium glucose cotransporter-2 (SGLT-2) inhibitor, which lowers blood glucose in people with type 2 diabetes by blocking the reabsorption of glucose in the kidneys and promoting excretion of excess glucose in the urine. Empagliflozin chemically, (1-chloro-4-[b-D-glucopyranos-1-

yl]-2-[4-([S]-tetrahydrofuran-3-yl-oxy) benzyl]-benzene [1]. Empagliflozin is a sodium-glucose co- transporter 2 (SGLT-2) inhibitor, which is found almost exclusively in the proximal tubules of nephronic components in kidney [2]. Inhibition of SGLT2 in patients with type 2 diabetes and hyperglycaemia leads to excess glucose excretion in the urine. Empagliflozin works by inhibiting the sodium-glucose co-transporter-2 (SGLT-2) found in the proximal tubules in the kidneys [3]. Through SGLT2 inhibition, empagliflozin reduces renal reabsorption of glucose and increases urinary excretion of glucose. The glucose-lowering effect of the drug is independent of insulin. Empagliflozin was approved for medical use in the United States and in the European Union in 2014. It is on the World Health Organization's List of Essential Medicines [4].

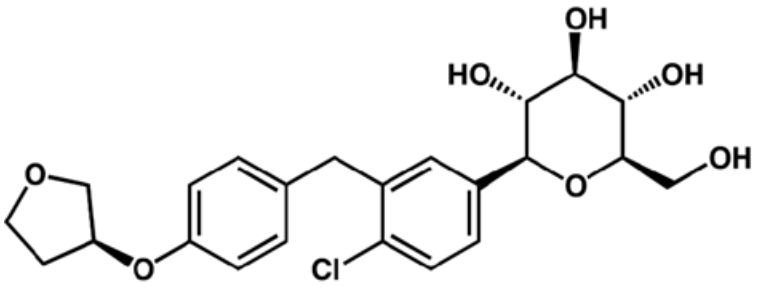

Figure No. 1: Chemical Structure of Empagliflozin

A survey of literature revealed that RP- HPLC [5, 6, 7], Human Plasma LC-MS [8], In-vivo marker study [9] and Pharmacokinetic study on volunteer [10, 11] these methods have been reported for determination of Empagliflozin in rat plasma, bulk and its dosage forms. There are also found some UV method for Empagliflozin [12, 13]. But, some of these methods lack adequate sensitivity, and some are expensive and time consuming. Therefore, it is important to develop new simple and sensitive methods for the UV spectrophotometric determination of Empagliflozin in tablet dosage form. So, the aim of work was to develop and validate an analytical method by UV-Visible Spectrophotometer for the estimation of Empagliflozin.

Method development

A. Determination of ƛ max (10 PPM) [15, 16, 17]

Materials and Methods

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair

Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

- **Materials**

Empagliflozin was obtained as a gift sample. Empagliflozin tablets were procured from local pharmacy. Methanol used was of analytical grade was used throughout the experiment. Freshly prepared solutions were employed.

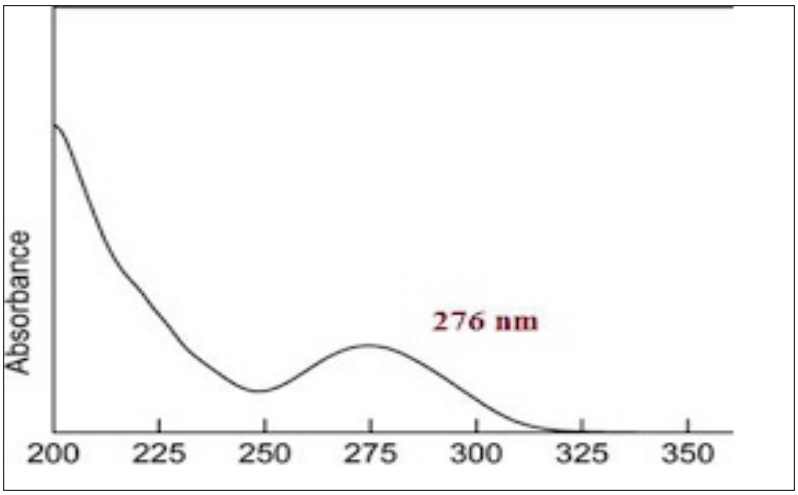

Figure No.2: Calibration Curve

50 mg weighed amount of Empagliflozin was dissolved into 100 ml of volumetric flask with analytical grade methanol. Pipette out 5 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with analytical grade methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

B. Preparation of working concentration

Preparation of Standard stock solution

Standard stock was prepared by dissolving 50 mg of Empagliflozin in 100 ml of analytical grade methanol to get concentration of 500 µg/ml (PPM).

Preparation of Standard solution

Pipette out 5 ml from standard stock solution and diluted up to 50 ml with analytical grade methanol to get concentration of 50 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations [15, 16, 17]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Tofactinib was accurately weighed and transferred into the 100 ml of volumetric flask, added 70 ml analytical grade methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with analytical grade methanol. Filtered the solution through nylon syringe filter 0.45 µ. Pipette out 5 ml of the filtered solution and diluted up to 50 ml with analytical grade methanol. The absorbance was measured at 276 nm. The absorbance was recorded.

Table No.1: Absorbance of Dosage Form

MSN Laboratories Pvt. Ltd. (25 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0000
2	Standard	0.5374
3	Sample	0.5321

Table No.2: Dosage Form Specifications

No.	Brand / Company	M.D.	E.D.	Batch No.	Avg wt (g)	Assay (%)
1	EMPAONE - 25 MSN Laboratories Pvt LTD (10mg)	06/2023	08/2026	A-5142	0.5065	99.01

E. Method of validation [14, 16]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 10 - 90 PPM at 276 nm. The calibration data showed a linear relationship between concentrations.

Table No.3: Linearity Studies

Sr. no.	Sample Concentration	Absorbance
1	10 PPM	0.1161
2	30 PPM	0.3277
3	50 PPM	0.5325
4	70 PPM	0.7418
5	90 PPM	0.9412
Correlation coefficient		0.999

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100 ml of volumetric flask with analytical grade methanol and measuring the absorbance at 276nm.

Table No.4: Accuracy Studies

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.78	98.75
100	1	0.99	99.00
120	1.2	1.19	99.17

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the %RSD was calculated.

Table No.5: Precision studies

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5304
2	Sample Solution 2	0.5254
3	Sample Solution 3	0.5311
4	Sample Solution 4	0.5326
5	Sample Solution 5	0.5212
MEAN		0.5281
SD		0.0047
% RSD		0.8953

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table No. 6: Results for Ruggedness Studies

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5319 0.5326	0.5323	99.05	0.5546
2	Analyst 2	0.5345 0.5384	0.5365	99.83	

Results Solubility test was passed as per criteria

1. Solubility of Empagliflozin

Table No.7: Results for solubility studies

Sr. no.	Title	Result
1	Methanol	Soluble
2	Acetonitrile, Ethanol	Slightly Soluble
3	Water	Very Slightly Soluble

2. Melting point of Empagliflozin

The melting point of Empagliflozin was found to be 151-152˚C (uncorrected).

3. Results for linearity for assay method of Empagliflozin

The linearity of method was determined at concentration level ranging from 10 to 90 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.999**

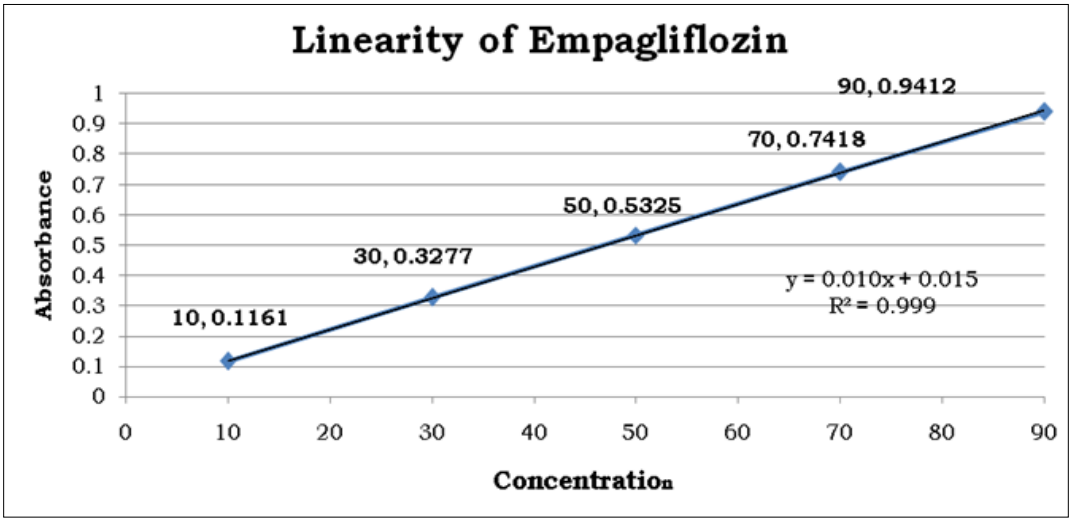

Figure No.3: Empagliflozin Standard Curve

4. Results for accuracy for assay method of Empagliflozin

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Empagliflozin

The % RSD for different sample of precision was found to be 0.8953 ~ 0.90 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Empagliflozin

The % RSD for different sample of ruggedness was found to be 0.5546 ~ 0.56 and it is within acceptance criteria represented in Table – 6

Conclusion

A method for the estimation of Empagliflozin in tablet form has been developed. From the spectrum of Empagliflozin, it was found that the maximum absorbance was 276 nm in analytical grade methanol. A good linear relationship was observed in the concentration range of 10-90 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Empagliflozin in solid dosage forms. Hence, the method could be considered for the determination of Empagliflozin in quality control laboratories.

References

1. *en.wikepedia.org/wiki/Empagliflozin accessed on 20-12-2023.*
2. *https://go.drugbank.com/drugs/DB09038 accessed on 20-12-2023.*
3. *Grempler R, Thomas L, Eekhardt M, Himmeslsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T and Eickelmann P: Empagliflozin in, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterization and comparison with other SGLT-2 inhibitors. Diabetes Obes Metab 2012, 14(1): 83-90.*
4. *Frampton JE: Empagliflozin: a review in type 2 diabetes drugs. Springer 2018; 78(10): 1037-48.*
5. *Shyamala, Nirmala K, Mounika J, Nandini B. Validated stability-indicating RP-HPLC method for determination of empagliflozin. Der Pharm Lett. 2016;8(2): 457-464.*
6. *Siridevi MP, Kumar HT, Rao SY, Rao VPK. RP-HPLC Method for Quantification of Empagliflozin in Pharmaceutical Formulation. Asian J Pharm Technol. 2019;9(3):208-211.*
7. *Sreenivas SKG and SA. A new validated RP-HPLC method for the determination of Metformin HCl and Empagliflozin in its bulk and pharmaceutical dosage forms. International Journal of Pharmaceutical Sciences and Research.*
8. *Jagadeesh, M., & Kumar, G. (2022). Development and validation of empagliflozin in human plasma using nevirapine as internal standard by liquid chromatography-tandem mass spectrometry. International Journal of Health Sciences, 6(S6), 272–281.*
9. *Seman, L. et al. Empagliflozin (BI 10773), a potent and selective SGLT2 inhibitor, induces dose-dependent glucosuria in healthy subjects. Clin. Pharmacol. Drug Dev. 2, 152–161 (2013).*
10. *Friedrich, C. et al. A Randomized, Open-Label, Crosover Study to Evaluate the Pharmacokinetics of Empagliflozin and Linagliptin After Coadministration in Healthy Male Volunteers. Clin. Ther. 35, 33–42 (2013).*
11. *Brand, T., MacHa, S., Mattheus, M., Pinnetti, S. & Woerle, H. J. Pharmacokinetics of empagliflozin, a sodium glucose cotransporter-2 (SGLT-2) inhibitor, coadministered with sitagliptin in healthy volunteers. Adv. Ther. 29, 889–899 (2012).*
12. *Padmaja N, Veerabhadram G, Development and validation of analytical method for simultaneous estimation of empagliflozin and linagliptin in bulk drugs and combined dosage forms using UVvisible spectroscopy, Scholars Research Library Der Pharmacia Lettre, 2015, 7 (12), 306-312.*
13. *Banik S, Karmakar P, Miah MAH, Development and validation of UV spectrophotometric method for the determination of linagliptin and vildagliptin in bulk and Pharmaceutical dosage form, Bangladesh Pharmaceutical Journal, 2015, 18(2), 163-168.*
14. *ICH draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology, Federal Register, 60, IFPMA, Switzerland, 1995, pp. 1276.*
15. *Beckeet .A.H, Stenlak .J.B, "Practical pharmaceutical chemistry edn 4th CBS Publisher & Distribution, New Delhi, 2004, 275-337.*
16. *United States Pharmacopoeia. In Validation of Compendial Methods. 26th edn: Pharmacopoeial Convention Inc., Rockville, 2003, pp. 2439-2442.*
17. *Indian Pharmacopoeia .Volume II. Ministry of Health and Family Welfare Government of India: Published by Indian Pharmacopoeia Commission, Ghaziabad, 2007, pp. 692- 693.*