ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (366-372), Month: April - June 2019, Available at: www.researchpublish.com

Method development and validation for Estimation of Empagliflozin by UV spectrophotometry in human plasma

Dr.K. Bhavyasri*[1], M.V.S. Lavanya[2], Dr.D.Rambabu[3]

[*1]Associative Professor, Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad, India.
[2]Research Student, Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy,Barkatpura, Hyderabad, India.
[3] Sr.Q.C Manager, Gland Pharma Pvt,Ltd

Corresponding Author Email:-bhavya.khagga@gmail.com

Abstract: **a new reversed phase simple, economic and specific validated high-performance liquid chromatography method for estimation of Empagliflozin in human plasma. Mobile phase used is methanol: acetonitrile (50:50% v/v). uv detection at 225nm.the bioanalytical procedure involves deproteination of plasma with liquid-liquid extraction. The percentage of relative recovery and coefficient of variation of accuracy and precision were within acceptable limits. The method proved in simple, cost-effective, and sensitive foe estimation of Empagliflozin in human plasma.**

Keywords: **Empagliflozin, liquid-liquid extraction, human plasma, method development, validation, bioanalytical procedure.**

1. INTRODUCTION

Diabetes is one of the largest health problems in the world now-a-days. There is need for drugs with novel mechanism of action that can be used alone or in combination with other anti-diabetic agents to improve glycemic control and hyperglycemia is poorly controlled despite a number of therapeutic options [(1-4)]. By removing excessive glucose from the body through urine, Jardiance helps to improve glycemic control among patients with type 2 diabetes [(5-9).] This can result in improved HbA1clevels, which can reduce the risk of diabetes-related complications. It is used to control blood glucose levels in people with type 2 diabetes. This is achieved through helping the kidneys remove glucose from the bloodstream through the urine [(10-14)]. Empagliflozin is an SGLT2 inhibitor, a drug class which helps to stop sodium-glucose transport proteins that have been filtered out of the blood by the kidneys being reabsorbed back into the blood. Empagliflozin allows a significant amount of sugar to be removed through urination. The SGLT2 proteins are responsible for 90 per cent of the glucose that is reabsorbed into the blood [(15-17)].

IUPAC name of Empagliflozin: 1-chloro-4-(glucopyranos-1-yl)-2-(4-(tetrahydrofuran-3-yloxy) benzyl) benzene. Molecular weight is 450.912g/mol. Molecular formula.

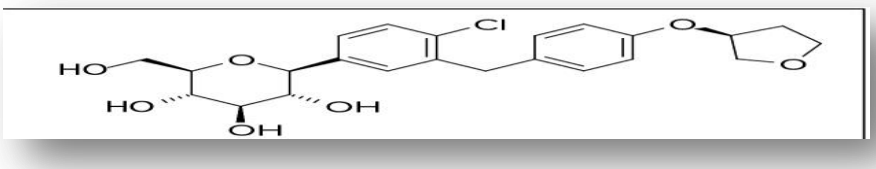

Figure 1: structure of Empagliflozin.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (366-372), Month: April - June 2019, Available at: www.researchpublish.com

EMPA is a white to yellowish, on-hygroscopic crystalline solid.it is commercially marketed under the name Jardiance and marketed by Boehringer Ingelheim, Germany. The drug was approved by USFDA on august 1 2014 and Europe in May 2104[18-20].

2. INTRODUCTION TO UV SPECTROSCOPY

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Any molecule has either n, π or σ or combination of these electrons. These bonding (σ and π) and non-bonding electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks and the nature of the electron present the molecular structure can be elucidated

UV spectroscopy obeys the Beer-Lambert law,

Beer law: This law can be stated as follows: "When a beam of monochromatic radiation is passed through a solution of absorbing substances, the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially".

$$I=I_0*e^{-k1*c} \quad \text{1}$$

Where, I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

C = molar concentration of solute

K_1=constant

Lambert's law: This law can be stated as follows "When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light".

$$I=I_0*e^{-k2*l} \quad \text{2}$$

Where, I0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

L = length of sample cell (cm.)

K_2=constant

After combining equation 1 and 2 and deriving we get the following equation 3 of Beer-Lambert law as:

$$A = \log (I_0/I) = Ɛcl \quad \text{3}$$

Where, A = absorbance

I_0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

C = molar concentration of solute

L = length of sample cell (cm.)

Ɛ = molar absorptivity

A literature search has shown that there are only few quantitative analytical methods for estimation of empagliflozin further, very few methods were available that shows the quantification of empagliflozin in biological fluids, these methods include LC-MS, GC-MS, which needs high end instrumentation which are costly and not available in conventional bioanalytical laboratory. Thus, the conclusion was to develop a rapid, simple and economical method which was based on liquid–liquid extraction (LLE) for sample preparation and UV detection for quantification of empagliflozin from spiked human plasma.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (366-372), Month: April - June 2019, Available at: www.researchpublish.com

Materials and methods:

Chemicals and reagents: empagliflozin were gifted by pharma company, Hyderabad, Telangana, India. HPLC grade methanol procured from Rankem chemicals limited, New Delhi, India. Human samples are procured from healthy volunteers.

3. INSTRUMENTATION

Double beam UV spectrophotometer; Model: SL 210; Make: ELICO. The data was obtained using Spectra Treats 3.11.01

Vortex mixer; Model: CM 101; Make: REMI

The analysis was performed using UV SL120 using UV detector used for method development and validation. The output signal was checked and the acquisition and integration of data was performed using spectral threats. Software on a computer. The diluents are filtered through 0. 25µm.detection was monitored at 223nm.

3.1. Procedure:

Selection of wavelength:

10mg of Empagliflozin drug was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with methanol as diluent .Then from this 0.1 ml was pipetted out and transferred into another 10 ml volumetric flask and the volume was made up to the mark with methanol to give 10ppm solution and this was scanned between 200 to 400nm and its absorbance was measured at 225nm.(Figure-2).

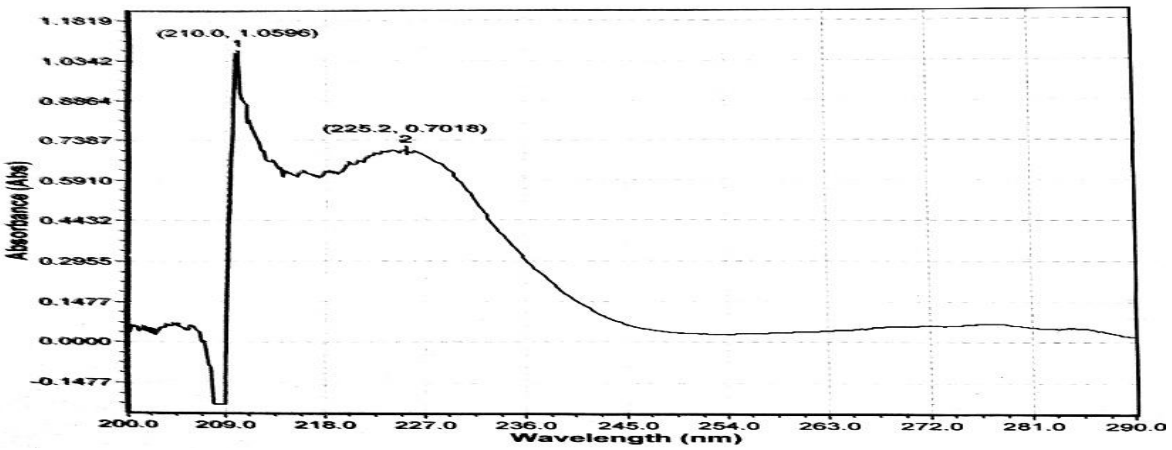

Figure 2: UV spectrum of Empagliflozin.

3.2. Assay:

3.2.1. Standard preparation:

10mg of Empagliflozin drug was accurately weighed and transferred into 10ml of volumetric flask and the volume was made up to the mark with methanol to get concentration of 1000ppm.From this 0.1 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark to get 10ppm solution and its absorbance was measured at 225nm.

3.2.2. Test preparation:

20 tablets were weighed and powdered. Powdered tablet equivalent to 10 mg of Empagliflozin was weighed accurately and it was taken into 10ml volumetric flask then volume was made up to the mark with methanol. From the above solution 0.1 ml of solution was pipetted out and taken in 10ml volumetric flask. The volume was made up to 10ml to get 10ppm solution and its absorbance was measured at 225nm.

The % Assay is calculated by using the following formula

% Assay= ((absorbance of the sample/absorbance of the standard) *(concentration of the standard/concentration of the sample)) *100

3.2.3. Preparation of standard stock solutions:

Preparation of standard solution: accurately weighed 10mg of empagliflozin was transferred into 10ml volumetric flask, dissolved and made up to the mark with diluent. This was the solution having strength of 1000µg/ml of Empagliflozin.

3.2.4. Extraction of plasma from blood:

Blood was collected into an EDTA containing tube and then it was centrifuged for10min at 3000rpm.blood was separated into layers after centrifugation. The supernatant which contains stray yellow color (plasma) was collected and used for sample preparation.

3.2.5. Preparation of plasma solution:

3ml plasma was deproteinated with 20µl of 30% ethyl acetate and to this 3ml of empagliflozin was added and contents of the tube were mixed on a cyclomixer for 3 min. the tube were kept in an inclined position on cyclomixer for 35mins and centrifuged at 4000rpm for 20mins.the supernatant was transferred to polypropylene tube and washed with one ml of ethyl acetate by mixing for one min and centrifuging at 1000rpm for 2mins. At 5°c.the supernatant layer of ethyl acetate was discarded and an aliquot was collected and absorbance was measured at 223nm.

4. METHOD VALIDATION PARAMETERS

Method validation: ICH guidance for industry was followed for validation of the method. Linearity, Accuracy, Robustness, LOD, LOQ were assessed during method validation.

4.1. Linearity:

Calibration standard solutions were prepared in plasma from the working solutions. Five calibration curves ranging from the 2 to 10 ppm were run to establish the linearity by using linear regression analysis. From the stock solution 0.2ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml with methanol to give 2ppm, 4ppm, 6ppm. 8ppm and 10ppm concentration. respectively and absorbance was measured at 223nm using methanol as blank and the calibration curve is plotted.

4.2. Precision:

10ppm standard solution of Empagliflozin pure drug is selected for Precision study. From the standard stock solution 0.1ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml using methanol to give 10ppm solution. This procedure is repeated 6 time and observances of all were measured at 223nm using methanol as blank and its %RSD was calculated by using the formula

%RSD = (standard deviation of the measurement / mean value of measurement) *100

4.3. Accuracy:

Quality control of samples was prepared at four different levels. The concentration of empagliflozin was calculated from a standard calibration curve that was concurrently obtained. Accuracy was analyzed at each level by comparing the observed concentration as a mean relative percentage recovery. Standard quantity equal into 50%, 100% and 150 % is to be added in sample.2ml of standard solution was spiked with 4ml of sample solution,2ml of standard solution was spiked with 6ml of sample solution,2ml of standard solution was spiked with 8ml of sample solution. Absorbance was measured for three times at 223nm.Repeated three times and their absorbance is measured at 223nm and the %recovery is calculated by using the formula:

% Recovery = (amount found / amount added) *100

4.4. Limit of detection:

The detection limit (DL) may be expressed as:

$$\mathbf{DL = 3.3*\sigma / S}$$

where σ = the standard deviation of the response S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (366-372), Month: April - June 2019, Available at: www.researchpublish.com

4.5. Limit of quantification

The quantitation limit (QL) may be expressed as:

$$QL = 10*\sigma/ S$$

where σ = the standard deviation of the response S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

4.6. Robustness:

Robustness:6 aliquots of 6ppm of standard solution was prepared and it was scanned at wavelength at (±)1nm of λmax. The absorbance was noted down.

5. RESULTS AND DISCUSSION

Method development and optimization of chromatographic condition:

The %assay was found to be 99%.

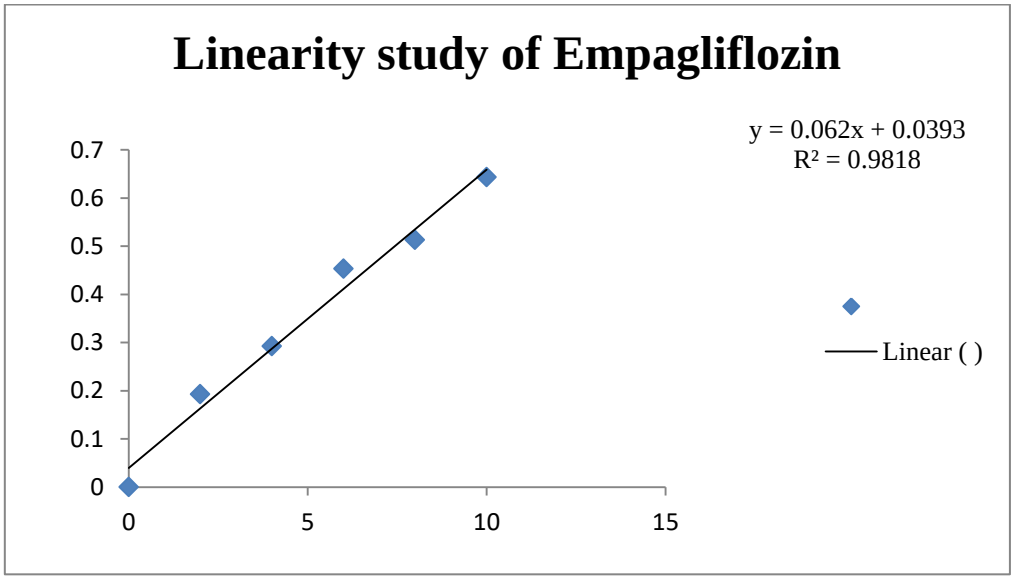

Figure 2: linearity curve of Empagliflozin.

Table 1: Conc. Vs Abs. table for Linearity Study.

Concentration(ppm)	Absorbance(nm)
0	0
2	0.1536
4	0.2797
6	0.4591
8	0.5708
10	0.7462

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (366-372), Month: April - June 2019, Available at: www.researchpublish.com

Table 2: Evaluation data of precision study.

Sample no.	%RSD
1	0.3597
2	0.3595
3	0.3589
4	0.3672
5	0.3572
6	0.3664
Mean	0.3582
SD	0.001390
%RSD	0.3881

Table 3: Evaluation data of accuracy study.

% Recovery level	%Recovery	Mean % recovery
50%	99.55	99.56
	99.65	
	99.50	
100%	99.68	99.71
	99.79	
	99.68	
150%	99.86	99.86
	99.75	
	99.90	

The limit of detection was found to be 0.51 ppm and limit of quantification found to be 1.57ppm.

Table 4: Evaluation data of robustness study

Sample no.	222nm	223nm	224nm
1	0.7289	0.7462	0.7490
2	0.7289	0.7461	0.7492
3	0.7285	0.7458	0.7487
4	0.7284	0.7458	0.7484
5	0.7285	0.7453	0.7486
6	0.7281	0.7456	0.7485
Mean	0.7286	0.7458	0.7487
SD	0.0003082	0.0003286	0.0003077
%RSD	0.04230	0.04406	0.04110

ACKNOWLEDGMENT

We are thankful that RBVRR Women's College of Pharmacy has provided us the opportunity to write this research article.

REFERENCES

[1] Nair S et al., and Wilding JP et al., Sodium glucose co transporter 2 inhibitors as a new treatment for diabetes mellitus. Clinical Endocrinal Metab 2010; 95 pg.: 34-42.

[2] Mcgill JB et al., The SGLT2 inhibitor Empagliflozin for the handling of type 2 diabetes mellitus. Diabetes Ther 2014; vol -5 pg.: 43-63.

[3] KD Tripathi et al., Essentials of medical pharmacology. New Delhi, India Edition 7th, 2013 pg.: 278-279.

[4] Wild S Roglic G et al., Green A et al., Sicree R et al., and King H et al., Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; vol 27 pg.: 1047-1053.

[5] Foneca VA et al., Kikman MS et al., Darsow T et al., and Robert E et al., The American Diabete Research Perspective Research Perspective Diabetes. Diabetes 2102; 61 vol (6) pg.: 1338-1345.

[6] Neumiller JJ et al., Empagliflozin: a new sodium-glucose co transporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. Drugs in context 2014; vol-3 pg.: 212-262.

[7] Nair Set al., and Wilding JP et al., Sodium glucose co transporter 2 inhibitors as a new treatment for diabetes mellitus. Clinical Endocrinal Metab 2010; 95 pg.: 34-42.

[8] Dailey G et al., Empagliflozin for the treatment of type 2 diabetes mellitus: An overview of safety and efficacy based on Phase 3 trials. Journal of Diabetes 2015; vol-7 pg.: 448-461.

[9] McGill JB et al., The SGLT2 inhibitor Empagliflozin for the handling of type 2 diabetes mellitus. Diabetes Ther 2014; vol-5 pg.: 43-63.

[10] Duckworth W et al., Abraira C et al., Moritz T, et al., VADT Investigators. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 2009 pg.; 360:129–139.

[11] Gerstein HC et al., Miller Meet al., Byington RP et al., Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008 pg.; 358:2545–2559.

[12] Patel A et al., MacMahon S et al., Chalmers J et al., ADVANCE Collaborative Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N ngl J Med 2008 pg.; 358:2560–2572.

[13] The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J et al., Med 1993; pg.: 329:977–986.

[14] UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonyl ureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998; pg-352:837–853.

[15] Volino LR et al., Pan EY et al., and Mansukhani RP et al., Sodium Glucose Co-Transporter 2 (SGLT2) Inhibitors in Type 2 Diabetes: A Literature Review of Approved Products. Pharmacology & Pharmacy 2014; vol-5 pg.: 1029-1058.

[16] Verma S et al., McMurray JJ et al., (October 2018). "SGLT2 inhibitors and mechanisms of cardiovascular benefit: a state-of-the-art review". Diabetologia. 61 vol (10) pg.: 2108–2117.

[17] Zimmermann J et al., (December 2016). "Empagliflozin (Jardiance) for Type 2 Diabetes Mellitus". American Family Physician. 94 vol (12) pg.: 1014–1015.

[18] Anderson JE et al., Wright EE et al., Shaefer CF et al., (February 2017). "Empagliflozin: Role in Treatment Options for Patients with Type 2 Diabetes Mellitus". Diabetes Therapy. 8 vol (1) pg.: 33–53.

[19] McGill JB et al., The SGLT2 inhibitor Empagliflozin for the handling of type 2 diabetes mellitus. Diabetes Ther 2014; vol- 5 pg.: 43-63.

[20] Elizabeth Mechatie et al., for Clinical Endocrinology News Digital Network August 1, 2014 FDA approves empagliflozin for adults with type 2 diabetes.