



A New Simple Method Development, Validation and Forced Degradation Studies of Empagliflozin By Using RP-HPLC

V. Shirisha*¹, Bolle Krishnaveni¹, Santosh Illendula¹, K.N.V. Rao¹, and H. Rajeswar Dutt¹

Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Cherlapally (v), Nalgonda (Dt), Telangana (St), India, 508001

Received: 10 Oct 2018 / Accepted: 8 Nov 2018 / Published online: 1 Jan 2019

Corresponding Author Email: shirishabpharm62@gmail.com

Abstract

A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Empagliflozin in bulk and pharmaceutical dosage form dosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5 μ m, 15cm x 4.6mm i.d. column with UV detection at 228 nm and Phosphate Buffer: Acetonitrile = 45:55 (pH-2.8) at a flow rate of 1.0 ml/ min. The proposed method was successfully applied to the determination of Empagliflozin in bulk and pharmaceutical dosage form. The method was linear over the range of 0–50 μ g/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.07 μ g/ml and quantification was found to be 0.21 μ g/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.

Keywords

RP-HPLC, Empagliflozin, Method development and validation, ICH Guidelines.

INTRODUCTION:

Empagliflozin (trade name Jardiance) is a drug of the gliflozin class, approved for the treatment of type 2 diabetes in adults in 2014. It was developed by Boehringer Ingelheim and Eli Lilly and Company.[1] Empagliflozin is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), and causes sugar in the blood to be excreted by the kidneys and eliminated in urine. Empagliflozin is primarily used in type 2 diabetics to lower blood glucose levels. Empagliflozin in people with type 2 diabetes reduces the risk of cardiovascular death and congestive heart failure.[2][3]

Empagliflozin is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), which is found almost exclusively in the proximal tubules of nephronic components in the kidneys. SGLT-2 accounts for about 90 percent of glucose reabsorption into the blood. Blocking SGLT-2 reduces blood glucose by blocking glucose reabsorption in the kidney and thereby excreting glucose (i.e., blood sugar) via the urine.[4][5][6]

The IUPAC Name of Empagliflozin is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy] phenyl} methyl) phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol.

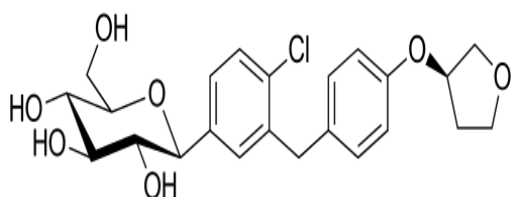


Fig 1: Chemical Structure of Empagliflozin

MATERIALS AND METHODS

HPLC Instrumentation & Conditions:

The HPLC system employed was HPLC with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer analysis:

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Empagliflozin, so that the same wave number can be utilized in HPLC UV detector for estimating the Empagliflozin. While scanning the Empagliflozin solution we observed the maxima at 228 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.^[7]

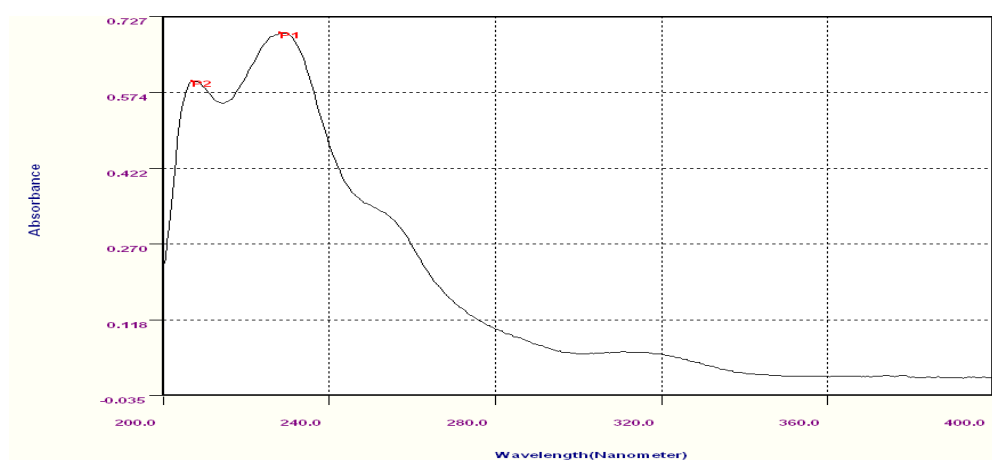


Fig 2: UV spectrum

Optimized Chromatographic Conditions:

Column: Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5 μ m.

Mobile Phase: Phosphate Buffer: Acetonitrile = 45:55 (pH-2.8)

Flow Rate: 1.0ml/minute

Wave length: 228nm

Injection volume: 20 μ l

Run time: 08 mins.

Column temperature: Ambient

Sampler cooler: Ambient

MOBILE PHASE PREPARATION

About 2.72172grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with

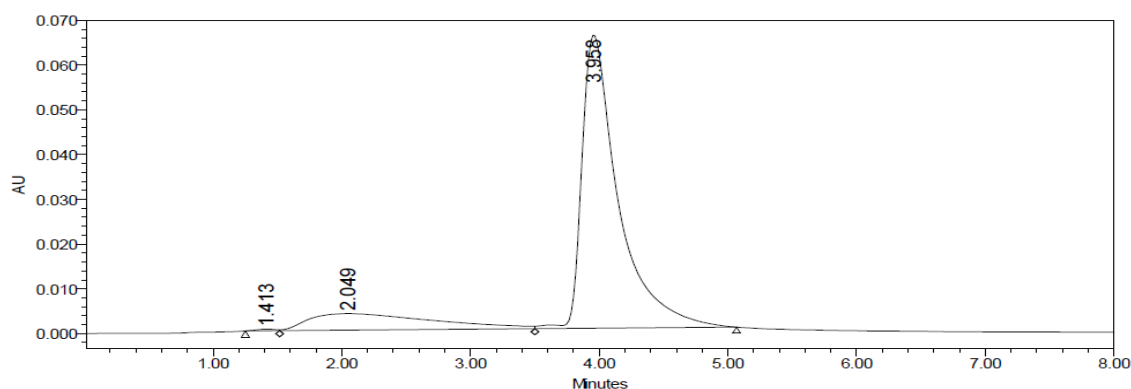
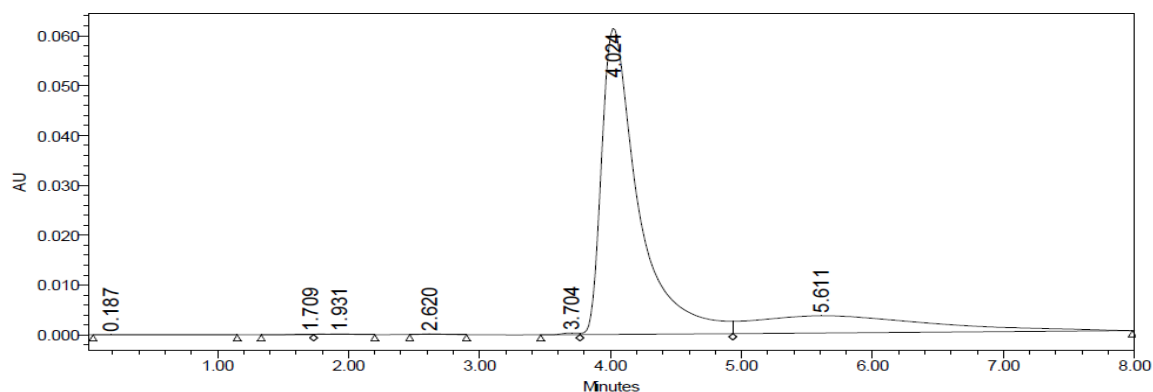
HPLC Grade water. The pH was adjusted to 2.80 with diluted orthophosphoric acid Solution. 450ml (45%) of above Phosphate buffer solution and 550ml of HPLC Grade Acetonitrile (55%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The resulted solution was filtered through 0.45 μ m filter under vacuum filtration.^[8]

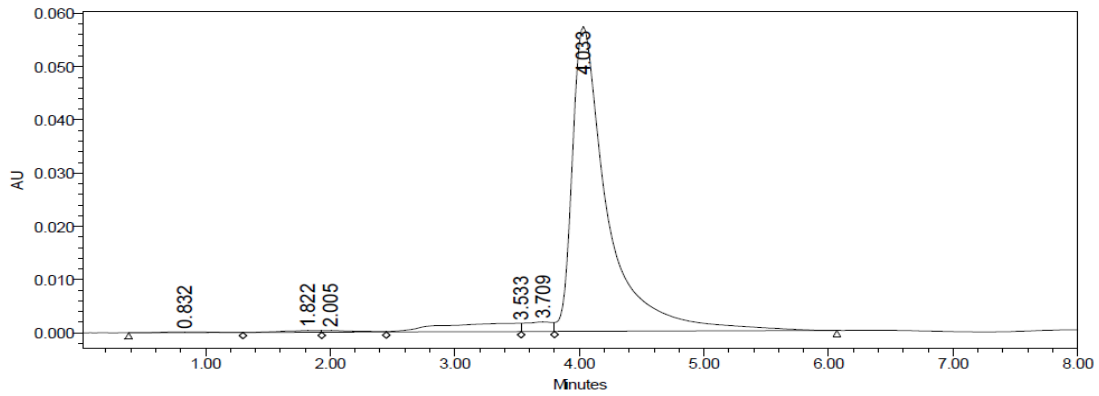
SAMPLE AND STANDARD PREPARATION FOR THE ANALYSIS

25 mg of Empagliflozin standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.3 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase.^[9]

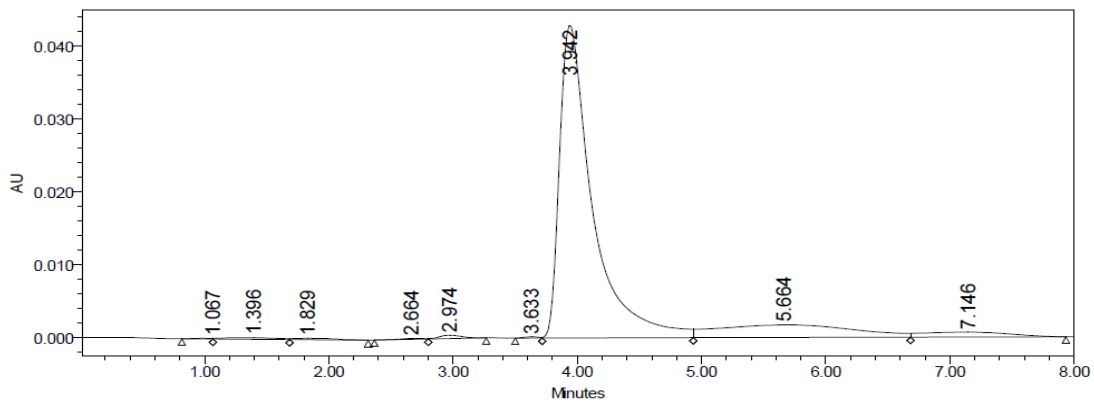
RESULT AND DISCUSSION:
Table-1: Trials for method development

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry ODS (C ₁₈) RP Column, 250 mm x 4.6 mm, 5µm	Methanol: Water = 50: 50	1.0ml/min	228nm	Very Low response	Method rejected
Symmetry ODS (C ₁₈) RP Column, 250 mm x 4.6 mm, 5µm	Acetonitrile: Water = 60: 40	1.0ml/min	228nm	Low response	Method rejected
Symmetry ODS (C ₁₈) RP Column, 250 mm x 4.6 mm, 5µm	Acetonitrile: Methanol = 70: 30	1.0ml/min	228nm	Tailing peaks	Method rejected
Symmetry ODS (C ₁₈) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer: Acetonitrile = 20:80 (pH-4.0)	1.0ml/min	228nm	Resolution was not good	Method rejected
Symmetry ODS (C ₁₈) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer: Methanol = 30:70 (pH-3.8)	1.0ml/min	228nm	Tailing peak	Method rejected
Symmetry ODS (C ₁₈) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer: Acetonitrile = 45:55 (pH-2.8)	1.0ml/min	228nm	Nice peak	Method accepted

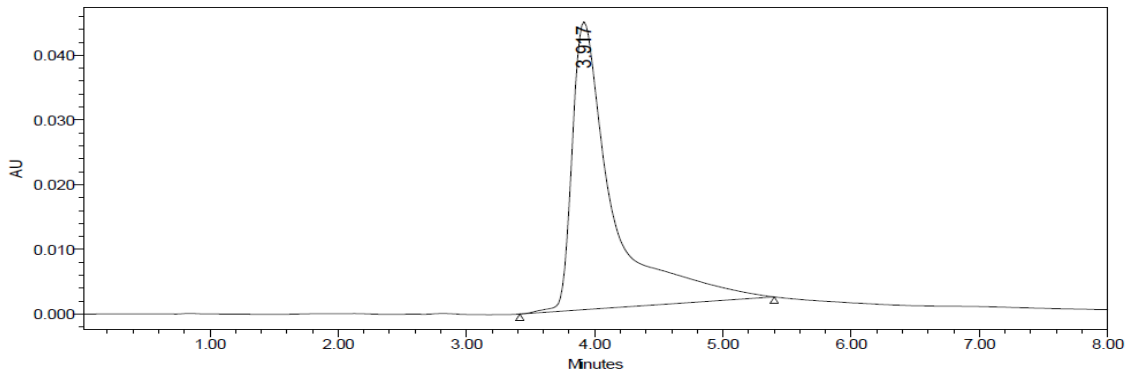

Trial-1

Trial-2



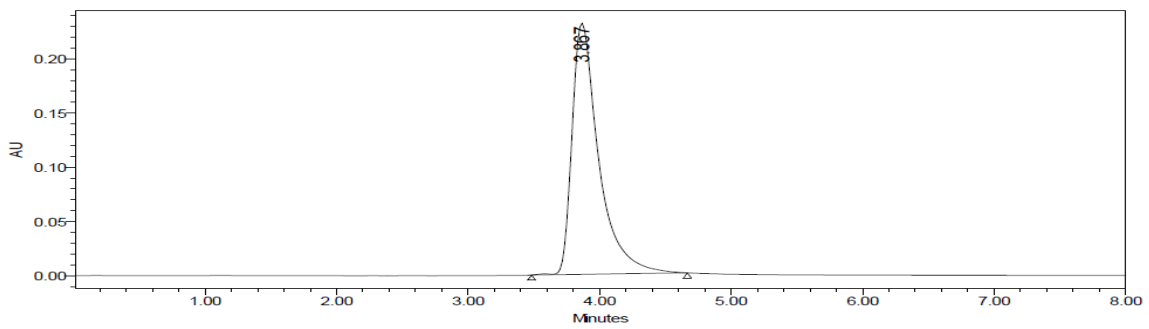
Trial-3



Trial-4



Trial-5



Trial-6

Table 2: Peak results

Rt	Peak Area	Tailing Factor	Plate Count
3.867	584624	1.42	4765

METHOD VALIDATION:

Accuracy: Recovery study: To determine the accuracy of the planned technique, recovery studies were distributed by adds completely different amounts (80%, 100%, and 120%) of pure drug of

Empagliflozin were taken and extra to the pre-analyzed formulation of concentration 30 μ g/ml. From that proportion recovery values were calculated.^[10]

Table-3: Accuracy Readings

Conc. In ppm	Conc. Found	Peak Area	% Recovery
24	24.022	472546	101.916
24	23.937	471121	101.736
24	24.206	475612	100.343
		Avg.	101.3317
		S.D	0.860928
		%RSD	0.849614
Conc. In ppm	Conc. Found	Peak Area	% Recovery
30	30.103	574216	100.113
30	30.521	581211	101.394
30	30.575	582121	101.547
		Avg.	101.018
		S. D	0.787478
		%RSD	0.779542
Conc. In ppm	Conc. Found	Peak Area	% Recovery
36	36.041	673514	100.858
36	36.502	681214	99.737
36	36.557	682132	100.091
		Avg.	100.2287
		S. D	0.57304
		%RSD	0.571732

Precision:
Repeatability

The exactitude of every technique was determined one by one from the height areas & retention times

obtained by actual determination of six replicates of a set quantity of drug. Empagliflozin (API). The % relative variance was calculated for Empagliflozin square measure bestowed within the table-4.^[11]

Table-4: Repeatability Results of Precision

HPLC Injection Replicates of Empagliflozin	Retention Time (Minutes)	Peak Area
Replicate – 1	3.873	598647
Replicate – 2	3.867	586484
Replicate – 3	3.866	594624
Replicate – 4	3.865	588642
Replicate – 5	3.865	584213
Replicate – 6	3.867	589874
Average		590414
Standard Deviation		5344.816
% RSD		0.905266

Intraday & Inter day: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & %

RSD (% RSD < 2%) within a day & day to day variations for Empagliflozin revealed that the proposed method is precise. [12]

Table-5: Results of Intra day & Inter day

Conc. Of Empagliflozin (API) (µg/ml)	Observed Conc. Of Empagliflozin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
24	23.86	0.95	24.79	0.86
30	30.09	0.64	29.89	0.43
36	36.07	0.87	36.12	0.91

Linearity and Range

The calibration curve showed good linearity in the range of 0 – 50 µg/ml, for Empagliflozin (API) with

correlation coefficient (r^2) of 0.999. A typical calibration curve has the regression equation of $y = 16721x + 70860$ for Empagliflozin. [13]

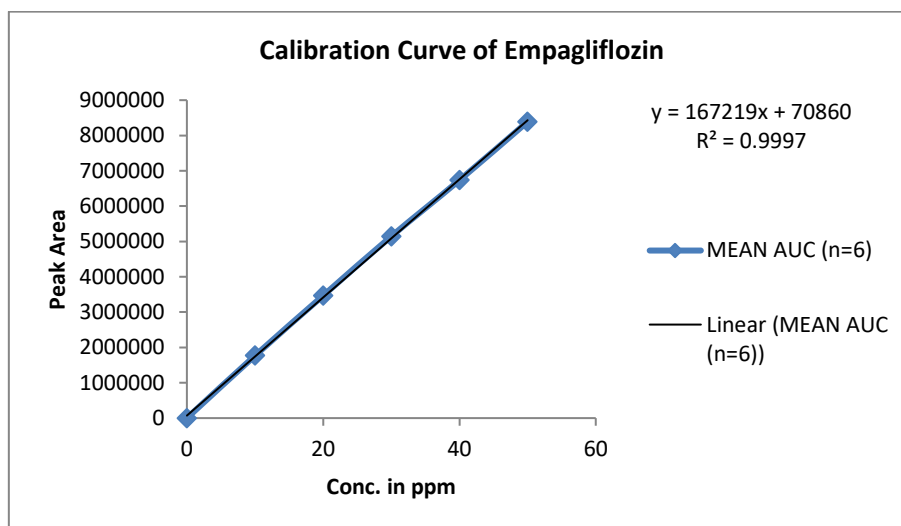


Fig-3: Calibration curve of Empagliflozin (API)

Table-6: Linearity Results of Empagliflozin

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
10ppm	1768452
20ppm	3468421
30ppm	5146243
40ppm	6735124
50ppm	8389756

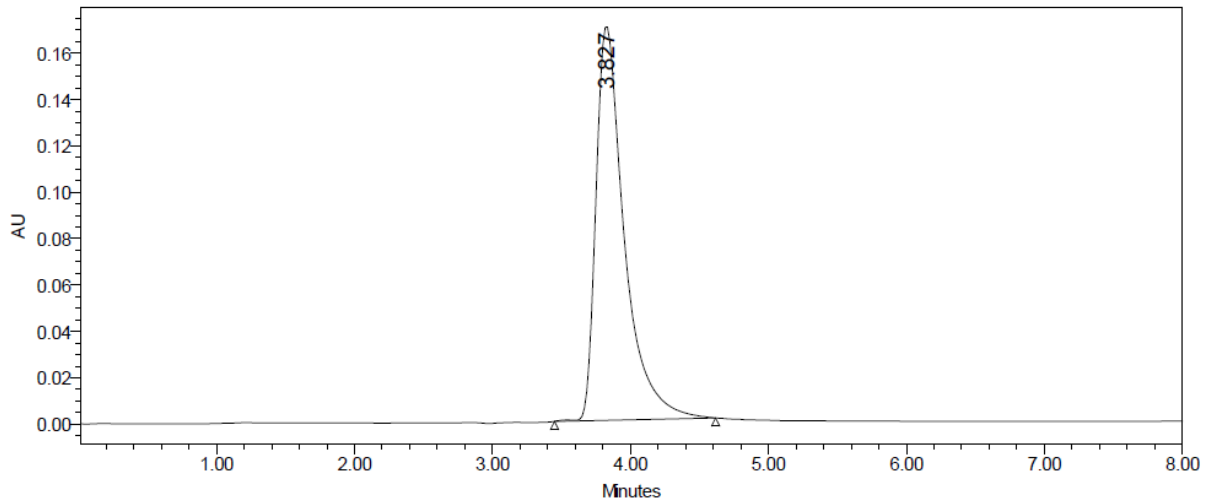


Fig 4: Calibration of Empagliflozin concentration in 10 ppm

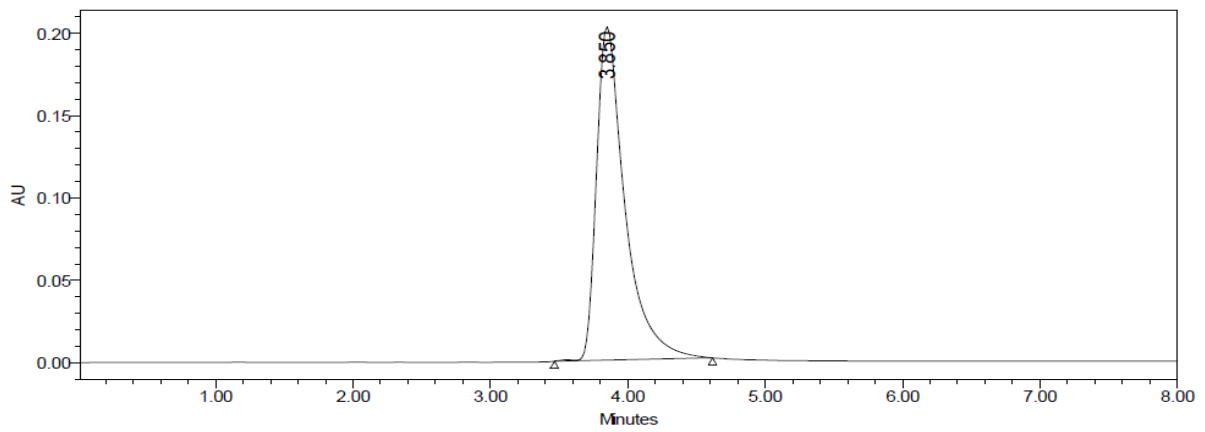


Fig 5: Calibration of Empagliflozin concentration in 20 ppm

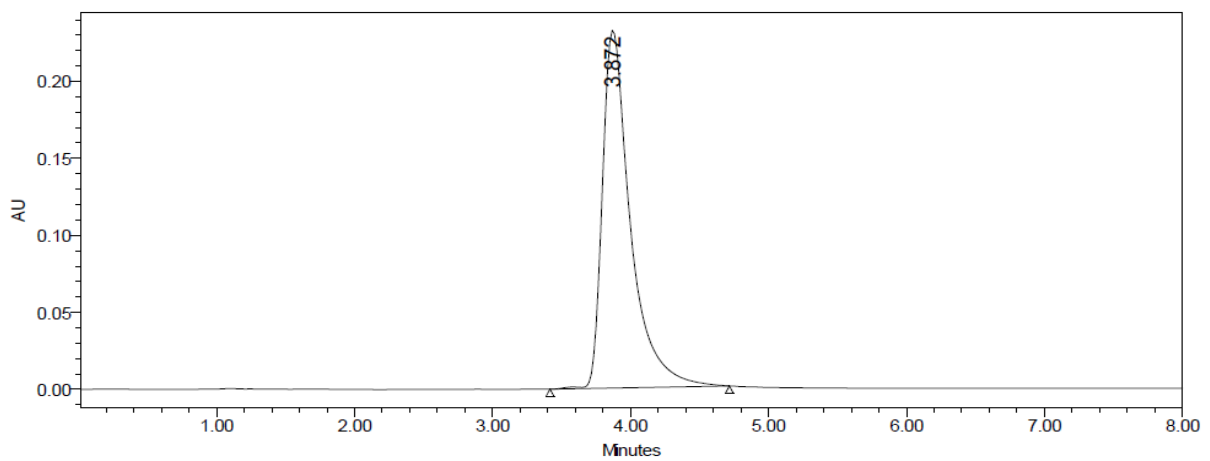


Fig 6: Calibration of Empagliflozin concentration in 30 ppm

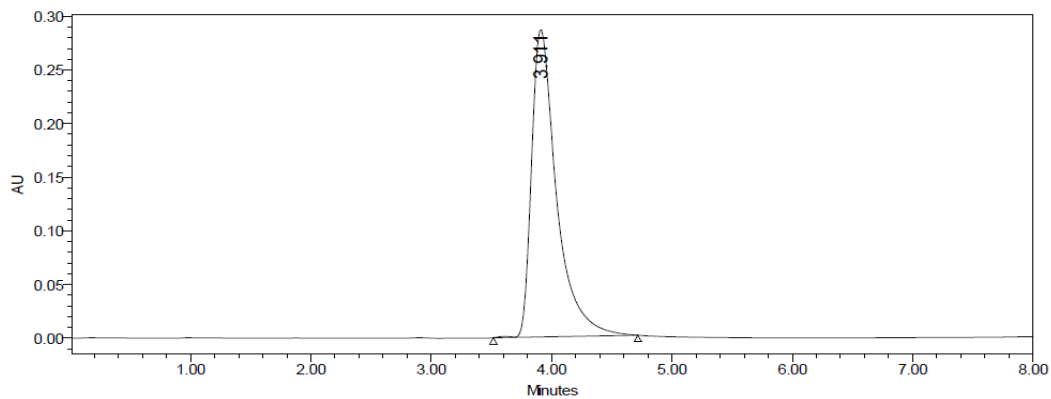


Fig 7: Calibration of Empagliflozin concentration in 40 ppm

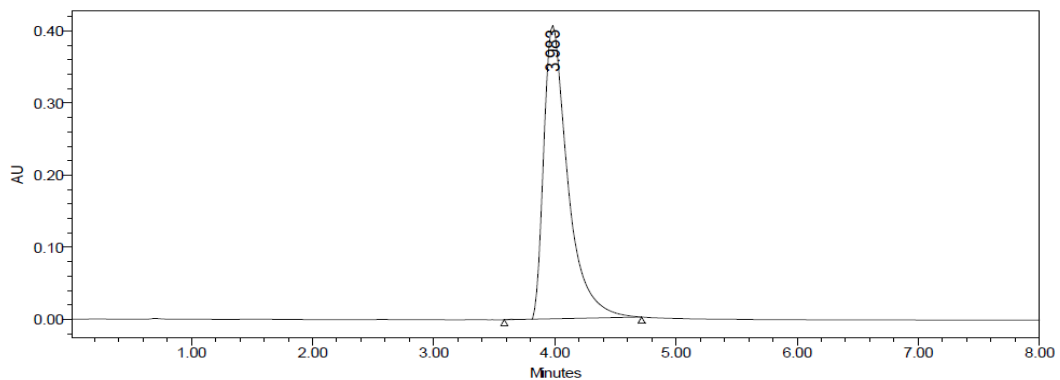


Fig 8: Calibration of Empagliflozin concentration in 50 ppm

LOD & LOQ: The Minimum concentration level at which the analyte can be reliably detected (LOD) & quantified (LOQ) were found to be 0.07 & 0.21 $\mu\text{g/ml}$ respectively.

STABILITY STUDIES

ACID DEGRADATION

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base flagon. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water shower at 60°C for 4 hours.

Permitted to cool to room temperature. The sample was then neutralized using dilute NaOH solution & final volume of the sample was made up to 100ml with water to prepare 100 $\mu\text{g/ml}$ solution. It was infused into the HPLC framework against a clear of portable stage (subsequent to advancing the versatile stage pieces). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile. The typical chromatogram shown below is the degradation profile of Minocycline in 0.1N HCl. ^[14]

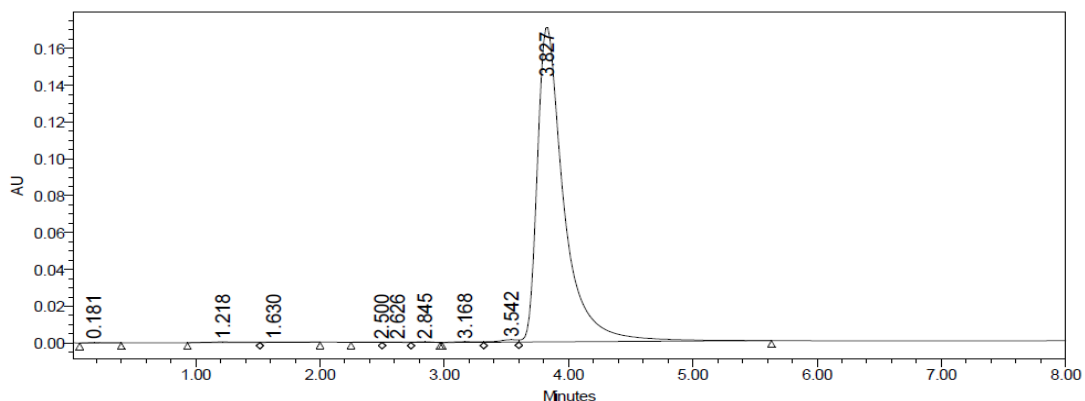


Fig-9: Acidic degradation

BASIC HYDROLYSIS:

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base flagon. 30 ml of 0.1N NaOH was added to it. & it was refluxed in a water bath at 60°C for 4 hours. Allowed to cool to room temperature. The example was then killed utilizing 2N HCl arrangement and last volume of the example was made up to 100ml to plan

100 µg/ml arrangements. It was infused into the HPLC framework against a clear of portable stage in the wake of enhancing the versatile stage arrangements. This experiment was repeated several times using same concentration of NaOH such as 0.1N to observe its degradation profile. The chromatogram shown below is the degradation profile of Minocycline in 0.1N NaOH. ^[15]

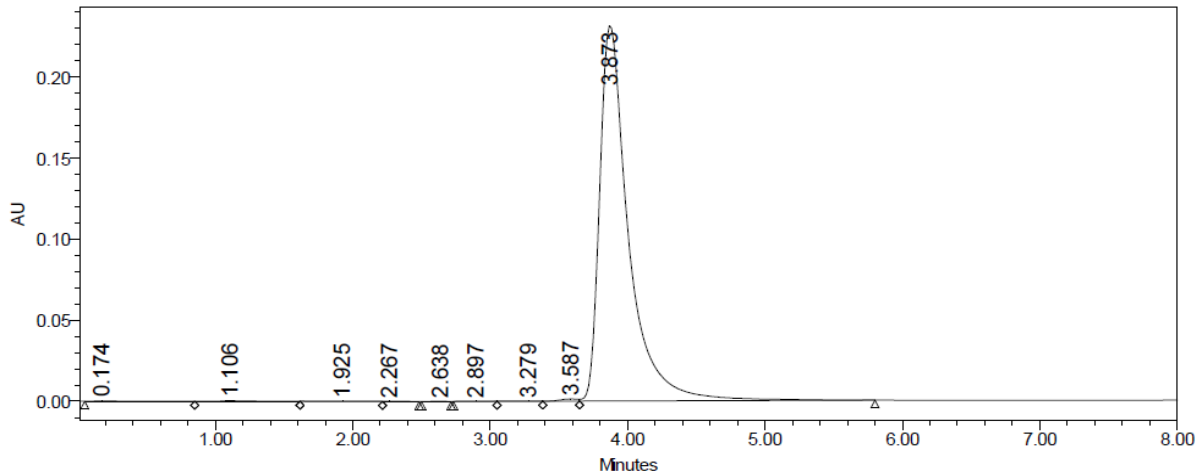


Fig-10: Basic degradation

THERMAL DEGRADATION

Precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base carafe. 30 ml of HPLC water was added to it. Then, it was refluxed in a water bath at 60° c for 6 hours uninterruptedly. After the reflux was over, the

drug became soluble and the mixture of drug & water was allowed to cool to room temperature. Last volume was made up to 100 ml with HPLC water to plan 100 µg/ml arrangement. It was infused into the HPLC framework against a clear of versatile stage/mobile phase. ^[16]

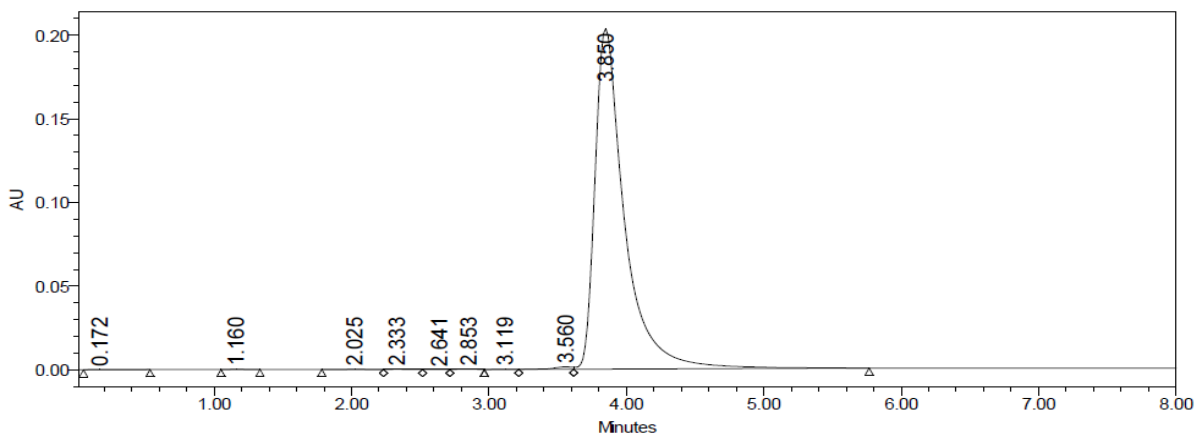
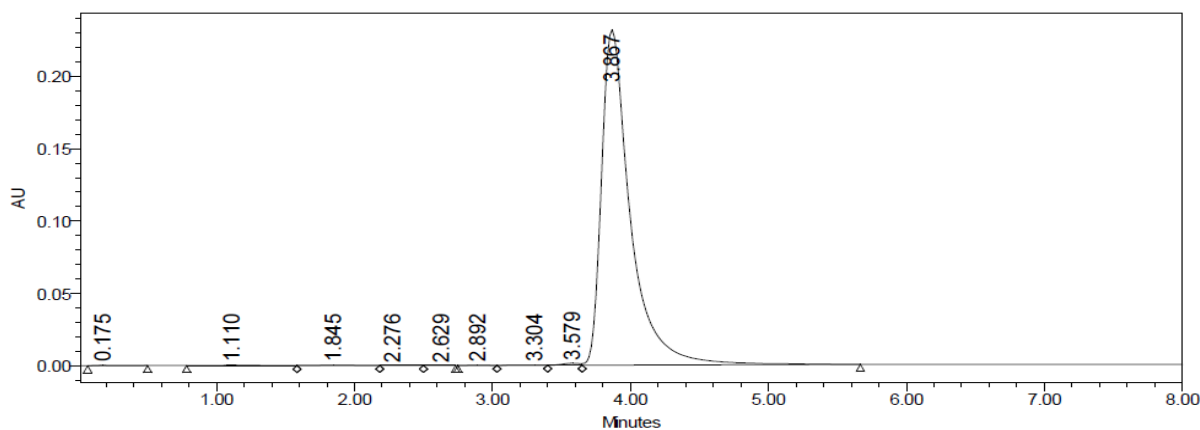


Fig-11: Thermal degradation studies

PHOTOLYTIC DEGRADATION:

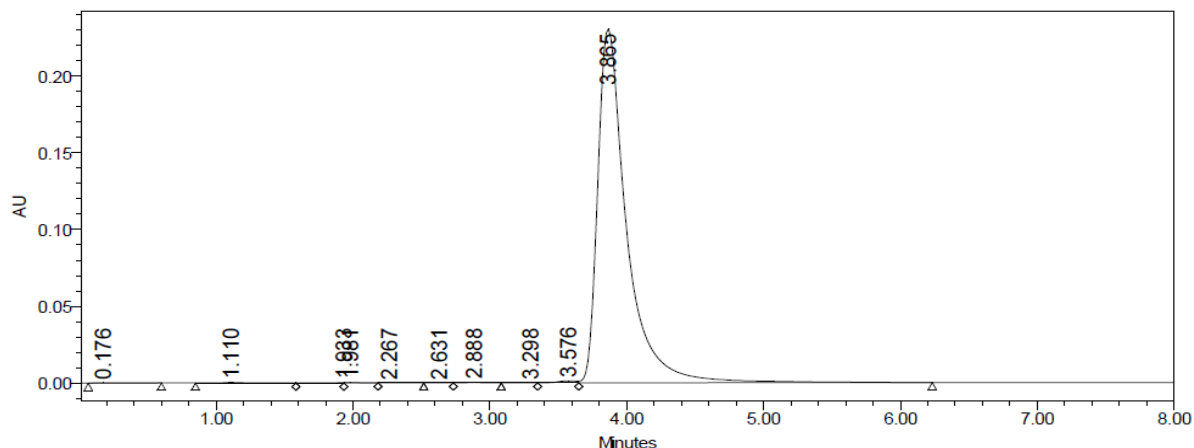
Around 10 mg of unadulterated medication was taken in a clean and dry Petri dish. It was kept in an UV bureau at 254 nm wavelength for 24 hours without interference. Precisely measured 1 mg of the UV uncovered medication was exchanged to a clean

and dry 10 ml volumetric cup. First the UV exposed drug was dissolved in methanol & made up to the mark with mobile phase to get 100 µg/ml solution. At long last this arrangement was infused into the HPLC framework against a clear of portable stage and chromatogram was gotten. ^[17]


Fig-12: Photolytic degradation.
OXIDATIVE HYDROLYSIS (3% H₂O₂):

Precisely measured 10 mg. of unadulterated medication was taken in a clean and dry 100 ml volumetric jar. 30 ml of 3% H₂O₂ and a little methanol was added to it to make it dissolvable and

then kept in that capacity in dim for 24 hours. Last volume was made up to 100 ml. utilizing water to get ready 100 µg/ml arrangement. The above example was infused into the HPLC framework.^[18]


Fig-13: Peroxide degradation
Table-7: Results of forced degradation studies of Empagliflozin API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	73.616	26.384	100.0
Basic Hydrolysis (0.1 M NaOH)	24Hrs.	95.475	4.525	100.0
Thermal Degradation (50 °C)	24Hrs.	91.106	8.894	100.0
UV (254nm)	24Hrs.	97.124	2.876	100.0
3 % Hydrogen Peroxide	24Hrs.	96.343	3.657	100.0

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Empagliflozin API. Facilitate the proposed RP-HPLC strategy has magnificent affectability, exactness and

reproducibility. The result shows the developed method is yet another suitable method for assay, purity & stability which can help in the analysis of Empagliflozin in different formulations.

REFERENCES

1. Grempler R, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T, Eickelmann P (January 2012). "Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors". *Diabetes, Obesity & Metabolism*. 14 (1): 83–90. doi:10.1111/j.1463-1326.2011.01517.x. PMID 21985634.
2. Usman MS, Siddiqi TJ, Memon MM, Khan MS, Rawasia WF, Talha Ayub M, Sreenivasan J, Golzar Y (January 2018). "Sodium-glucose co-transporter 2 inhibitors and cardiovascular outcomes: A systematic review and meta-analysis". *European Journal of Preventive Cardiology*: 2047487318755531. doi:10.1177/2047487318755531. PMID 29372664.
3. Office of the Commissioner. "FDA approves Jardiance to reduce cardiovascular death in adults with type 2 diabetes". Press Announcement. United States Food and Drug Administration. Retrieved 12 December 2016.
4. Abdul-Ghani MA, De Fronzo RA (September 2008). "Inhibition of renal glucose reabsorption: a novel strategy for achieving glucose control in type 2 diabetes mellitus". *Endocrine Practice*. 14 (6): 782–90. doi:10.4158/ep.14.6.782. PMID 18996802.
5. Nair S, Wilding JP (January 2010). "Sodium glucose cotransporter 2 inhibitors as a new treatment for diabetes mellitus". *The Journal of Clinical Endocrinology and Metabolism*. 95 (1): 34–42. doi:10.1210/jc.2009-0473. PMID 19892839.
6. Bays H (March 2009). "From victim to ally: the kidney as an emerging target for the treatment of diabetes mellitus". *Current Medical Research and Opinion*. 25 (3): 671–81. doi:10.1185/03007990802710422. PMID 19232040.
7. Giddings, J. Calvin (1965) *Dynamics of Chromatography, Part I. Principles and Theory*. Marcel Dekker, Inc., New York. p. 281.
8. Ettre, C. (2001). "Milestones in Chromatography: The Birth of Partition Chromatography" (PDF). *LCGC*. 19 (5): 506–512. Retrieved 2016-02-26.
9. Martin, A J P; Synge, R L M (1941). "Separation of the higher monoamino-acids by counter-current liquid-liquid extraction: the amino-acid composition of wool". *Biochemical Journal*. 35 (1–2): 91–121.
10. Lindsay, S.; Kealey, D. (1987). *High performance liquid chromatography*. Wiley. from review Hung, L. B.; Parcher, J. F.; Shores, J. C.; Ward, E. H. (1988). "Theoretical and experimental foundation for surface-coverage programming in gas–solid chromatography with an adsorbable carrier gas". *J. Am. Chem. Soc.* 110 (11): 1090–1096.
11. Displacement Chromatography. *Sacheminc.com*. Retrieved 2011-06-07. Archived September 15, 2008, at the Wayback Machine.
12. Snyder, Lloyd R.; Dolan, John W. (2006). *High-Performance Gradient Elution: The Practical Application of the Linear-Solvent-Strength Model*. Wiley Interscience.
13. Majors, Ronald E. (2010-09-07) *Fast and Ultrafast HPLC on sub-2 μm Porous Particles — Where Do We Go from Here? — LC-GC Europe*. *Lcgeurope.com*. Retrieved 2011-06-07.
14. Xiang, Y.; Liu Y.; Lee M.L. (2006). "Ultra-high pressure liquid chromatography using elevated temperature". *Journal of Chromatography A*. 1104 (1–2): 198–202.
15. Horváth, Cs.; Preiss B.A.; Lipsky S.R. (1967). "Fast liquid chromatography. Investigation of operating parameters and the separation of nucleotides on pellicular ion exchangers". *Analytical Chemistry*. 39 (12): 1422–1428.
16. 1290 Infinity Quaternary Pump. Agilent
17. Waters. "Trademarks: Waters". www.waters.com.
18. K., Robards, (1994). *Principles and practice of modern chromatographic methods*. Haddad, P. R., Jackson, P. E. Amsterdam: Elsevier/Academic Press.