Stability indicating HPLC method development and validation for estimation of Empagliflozin in bulk and marketed formulation

Mayuri S. Hande[1], Vishal S. More[2], Payal S. Gadakh[1], Mahesh M. Deshpande[1], Shubham S. Ambilwade[3], Anagha C. Chitte[3], Ajay R. Kotkar[3]

Corresponding authors' details;

Mayuri S. Hande
Department of Pharmaceutical Quality Assurance, Amrutvahini College of Pharmacy, Sangamner, Maharashtra, India.
mayurihande1105@gmail.com
9075595157

Conflict of interest: The authors declare that they have no known conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.
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[1] Department of Pharmaceutical Quality Assurance, Amrutvahini College of Pharmacy, Sangamner, Maharashtra, India.
[2] Department of Pharmaceutical Chemistry, Amrutvahini College of Pharmacy, Sangamner, Maharashtra, India.
[3] Department of Pharmaceutical Quality Assurance, Indira College of Pharmacy, Pune, Maharashtra, India.

ABSTRACT:

Empagliflozin is an oral antidiabetic drug is a selective sodium-glucose transport protein 2 (SGPT2) inhibitor. Stability indicating HPLC assay method was developed and validated for the quantitative determination of empagliflozin in bulk and formulation. An isocratic, RP-HPLC method was developed using YMC C18 5µ (150×4.6) mm column and the mobile phase C and mobile phase D in the ratio of 50:50, where mobile phase C is Ammonium acetate and mobile phase D is ACN: water (80:20). The detection was carried out at a wavelength 224 nm. The Linearity in the method was measured in the concentration range of 100–300 ppm, and the R^2 was found to be 0.9991. LOD and LOQ were found to be 3.19 µg/mL and 9.67 µg/mL, respectively. The forced degradation study for drug was done and degradants could be clearly seen. According to ICH and USFDA guidelines, the developed method is validated. The developed HPLC method could be precise, accurate, simple, and rapid for estimating the amount of empagliflozin present in bulk drugs and tablet dosage form. The proposed method can be used for routine analysis in bulk and pharmaceutical formulation due to its high recovery and low relative standard deviation.

Keywords: Empagliflozin, Forced-degradation, Validation, RP-HPLC

1. INTRODUCTION:

Empagliflozin is a sodium glucose cotransporter-2 (SGLT2) inhibitor. It is recommended as a supplement to diet and exercise to help adults with type 2 diabetes better control their blood sugar levels. Chemically it is known as (1S)-1,5-anhydro-1-(4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy] benzyl} phenyl)-D-glucitol, also known as D-Glucitol,1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3-furanyl] oxy] phenyl] methyl] phenyl] -(1S). The chemical structure of Empagliflozin was given in Fig.1.

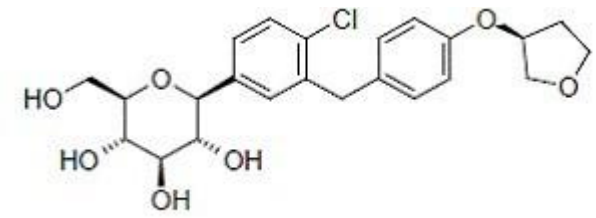

Fig. 1: Structure of Empagliflozin

It is a white to yellowish, non-hygroscopic, crystalline solid that is only very slightly soluble in water. It is also slightly soluble in acetonitrile and ethanol, sparingly soluble in methanol, and practically insoluble in toluene.

It is probably the most recent class of medication used to treat T2DM because it is an inhibitor of sodium-glucose co-transporter 2 (SGLT2). Due to the fact that SGLT2 are glucose-lowering drugs, they exhibit an insulin-independent mechanism, demonstrating their use in combination with other anti-diabetic medications for the treatment of T2DM. Furthermore, it aids in weight loss, lowers blood pressure, and reduces hyperglycemia [1,2].

Empagliflozin was measured separately using a few techniques, including UV [3] spectroscopy, HPLC [4, 5], and UPLC [6].

Additionally, one method is combined with another drug on HPLC. However, there was no RP-HPLC method for determining dapagliflozin in its API that indicated stability. In order to

determine Empagliflozin in API and its dosage form, a stability indicating RP-HPLC method was developed and validated in the current work.

2. MATERIAL AND METHODS:

2.1 Reagents and chemicals: Empagliflozin was supplied as a gift sample from Chromeln lab, Pune, India. empagliflozin tablet formulation (25 mg) was used of brand empaone 25 marketed by MSN Laboratories Pvt Ltd., Acetonitrile, Ammonium acetate, formic acid of HPLC grade were obtained from Chromeln lab, Pune, India.

2.2 Instrumentation: The chromatographic analysis was performed on Waters Alliance 2695 HPLC system equipped with PDA detector 2996. The output signals were monitored and processed using empower 2 software. The analytical column was YMC C18 5µ (150×4.6) mm.

2.3 Chromatographic Conditions: Initially, A: B (60:40) and C:D (60:40) using Thermo C18 5µ (150mm x 4.6mm) separately. Among which C:D mobile phase showed well resolved elution of drug. In order to get proper well resolved sharp peak various proportions for mobile phase with changing the column to YMC C18 5µ (150mm x 4.6mm) were tried. Finally selected method was C:D in the ratio 50:50 using YMC C18 5µ (150mm x 4.6mm). Injection volume was 10 µl, flow rate was 1.0 ml/min and the eluent was detected at 224 nm (detected by uv shown in fig.2) at column temperature 30°C. These conditions showed sharp peak of empagliflozin with retention time of 3.41 min.

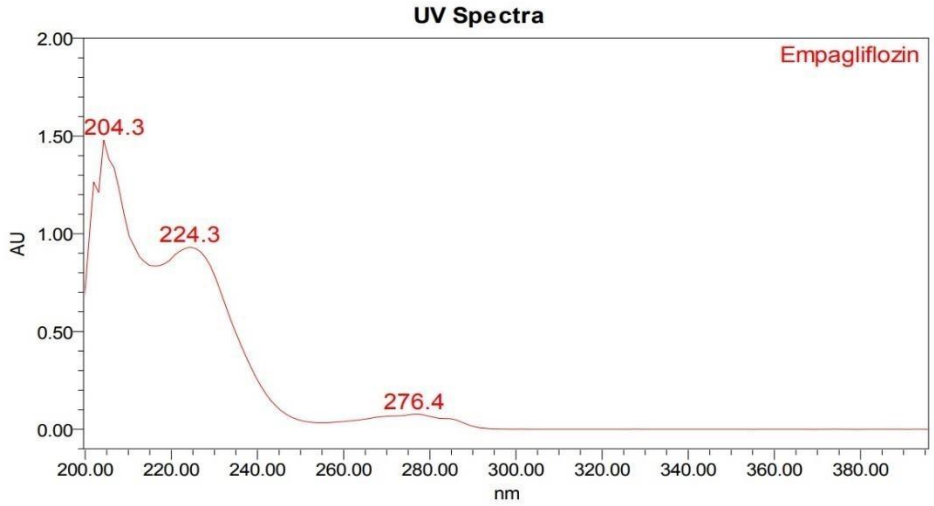

Fig. 2: UV spectrum of empagliflozin

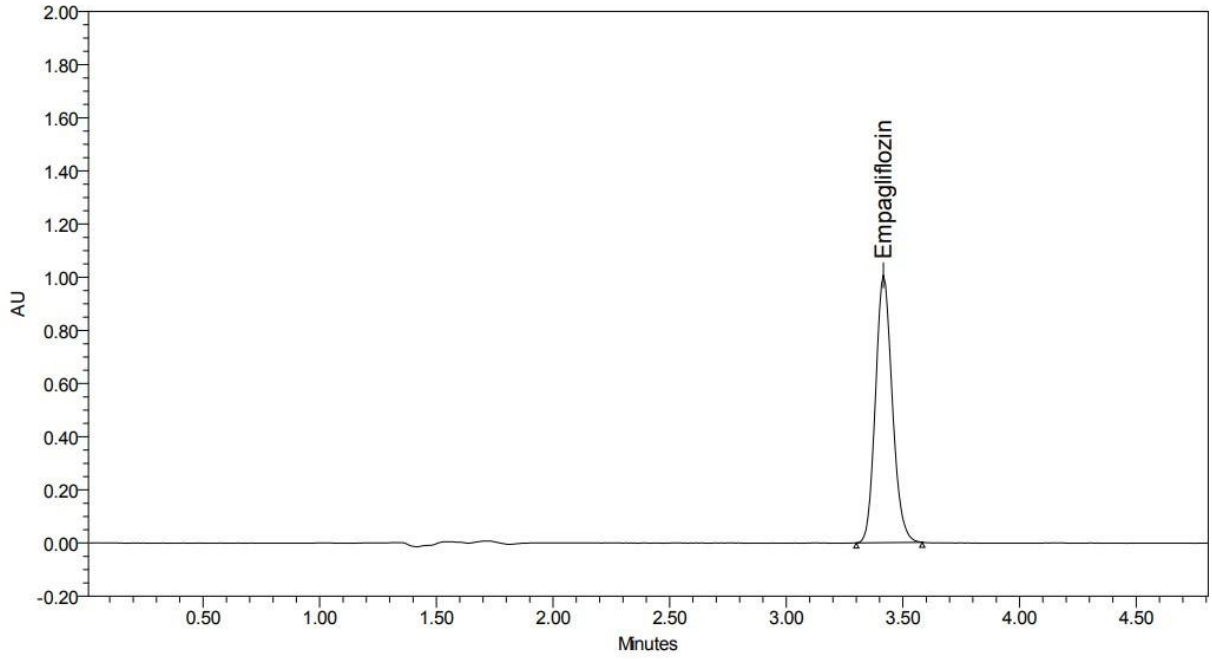

Fig. 3: Typical chromatogram of Empagliflozin

TABLE 1 OPTIMIZED CHROMATOGRAPHIC CONDITIONS OF EMPAGLIFLOZIN

Parameters	**Conditions**
Column	YMC C18 5µ
Mobile phase	C:D (50:50)
Flow rate	1.0 ml/min
Injection volume	5 µl
Wavelength	224 nm
Column temperature	30°c
Run time	10 min
Retention time	3.417 min
Theoretical plates	10673.36
Tailing factor	1.14

2.4 Preparation Of Diluent: Prepare mixture of acetonitrile and water in the ratio 50:50 used this solution as a diluent to prepare standard stock solution and samples.

2.5 Preparation of standard stock solution: Accurately Weigh 25 mg Empagliflozin and transfer it into volumetric flask. add 25 ml of diluent.

2.6 Analytical method validation:

2.6.1 Linearity: From a stock solution Aliquots 1, 1.5, 2, 2.5 and 3 ml were taken in 10 ml of volumetric flask and diluted up to the mark with mobile phase to get final concentration in range of 100-300µg/ml. Calibration curve was constructed by plotting the peak area vs. the drug concentration.

2.6.2 Precision: Precision can be performed at two different levels intraday and interday precision. Intra-day precision was determined by analyzing, the three different concentrations 100, 150 and 200µg/mL of empagliflozin, for three times in the same day. Day to day

variability was assessed using above mentioned three concentrations analyzed on three consecutive days for inter-day precision.

2.6.3 Accuracy: Accuracy was done by recovery study at levels of 50%,100% and 150% using standard addition method. known amount of standard empagliflozin was added to the sample and subjected to the proposed HPLC method. The accuracy studies were carried out three times, and the % recovery and % RSD were calculated.

2.6.4 Robustness: The robustness of the method was studied by making small deliberate changes in a few parameters. The flow rate (1±0.1 ml/min), mobile phase (50:50±5), and temperature (30±5°C) were varied. Robustness was assessed at a concentration of 200µg/ml.

2.6.5 LOD and LOQ: LOD is the lowest concentration in a sample that can be detected and LOQ is the lowest concentration of analyte in a sample that can be quantified. Empagliflozin concentrations 0.1,1,2,5,10,50 were used to calculate LOD and LOQ.

LOD and LOQ were calculated by the formulas

LOD = 3.3 SD/Slope

LOQ = 10 SD/Slope

2.6.6 System suitability parameters: The assurance of the quality performance of a chromatographic system depends on system suitability testing. System suitability testing was performed on previously prepared solutions for chromatographic conditions.

2.7 Force degradation study:

We can interpret the sample's acid, alkaline and oxidation degradation through these studies.

2.7.1 Acidic Degradation: Empagliflozin was subjected to forced degradation by acidic hydrolysis using 0.1N HCl maintained at 60°C for 24 h. The solution was filtered through 0.45µ syringe filter and injected under the chromatographic conditions and peak area was measured.

2.7.2 Alkaline Degradation: Empagliflozin was subjected to forced degradation by alkaline hydrolysis using 0.1N NaOH maintained at 60°C for 24 h. The solution was filtered through 0.45µ syringe filter and injected under the chromatographic conditions and peak area was measured.

2.7.3 Oxidative Degradation: Empagliflozin was subjected to forced degradation by hydrogen peroxide hydrolysis using 3% hydrogen peroxide maintained at 60°C for 24 h. The solution was filtered through 0.45µ syringe filter and injected under the chromatographic conditions and peak area was measured. The drug peak area and appearance of other/secondary peaks were taken into consideration when analysing the chromatogram. Any alteration in the size and visibility of secondary peaks will be regarded as degradation.

3. RESULT AND DISCUSSION:

3.1 Method development and optimization of chromatographic condition: Several mobile phases with various compositions and flow rates were tried to develop an accurate, precise, and specific stability-indicating RP-HPLC method for estimating empagliflozin using stressed samples. Chromatographic conditions were established and optimized after several compositions and combinations. We successfully estimated the empagliflozin with good peak symmetry and a stable baseline.

3.2 Linearity: The empagliflozin standard curve was linear over the analyzed concentration range of 100-300 µg/ml. The least square regression method was used to determine the linearity, and the resultant value of R^2 was 0.99 (see Fig. 4).

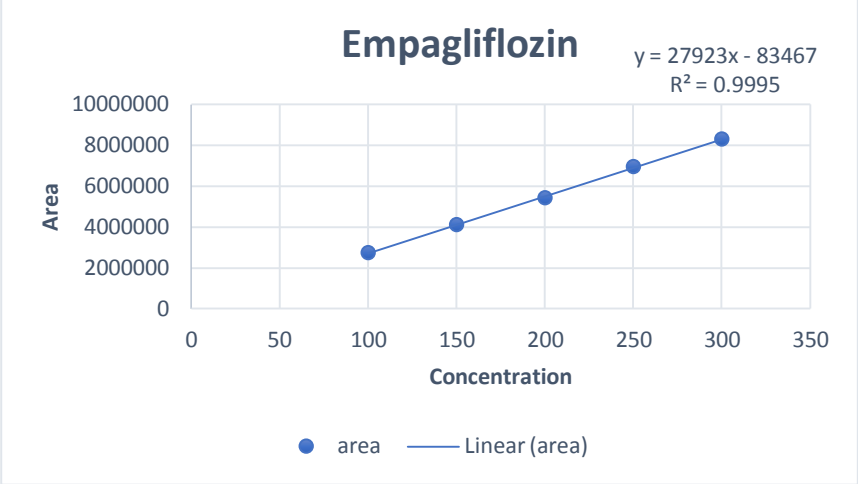

Fig. 4: Calibration curve of empagliflozin

3.3 Precision: Intra-day and inter day precision were studied using three conc 100,150 and 200 ppm. The developed method is found to be precise with SD and % RSD within the limits shown in table 2.

TABLE 2 INTRADAY AND INTERDAY PRECISION DATA OF EMPAGLIFLOZIN

Precision		**Concentration**	**% amount found (mean±SD)**	**%RSD**
Intra day	Morning	100	99.80±0.17	0.17
		150	99.18±0.77	0.52
		200	98.08±1.8	0.92
	Evening	100	98.81±0.36	0.37
		150	99.26±0.33	0.22
		200	99.30±1.2	0.6
Inter day	Day 1	100	99.02 ±0.45	0.45
		150	99.63 ±0.46	0.3
		200	98.51 ±0.36	0.18
	Day 2	100	99.63±0.13	0.13
		150	99.52±0.2	0.13
		200	99.13±0.93	0.46

3.4 Accuracy: the recovery studies show the accuracy of the proposed method. The outcomes are listed in table 3.

TABLE 3 RESULT OF ACCURACY

Levels	Conc.	API added	% recovery	%RSD
50	100	50	99.55	0.01
	150	75	99.11	0.61
	200	100	99.35	0.44
100	100	100	99.46	0.08
	150	150	99.86	0.70
	200	200	99.01	0.06
150	100	150	99.13	0.16
	150	225	99.53	0.53
	200	300	99.50	0.27

3.5 Robustness: The robustness was found to be unaffected by the deliberate changes in flow rate, temperature, and mobile phase. The results are shown in table 4.

TABLE 4 RESULT OF ROBUSTNESS

Parameter		SD	%RSD
Mobile phase	55:45	1.74	0.88
	50:50	0.11	0.05
	45:55	1.35	0.68
Temperature	25°c	1.27	0.64
	30°c	0.19	0.09
	35°c	0.4	0.2
Flow rate	0.9 ml/min	0.42	0.21
	1.0 ml/min	0.18	0.09
	1.1 ml/min	1.59	0.81

3.6 Limit of detection and limit of quantification: The LOD with signal to noise ratio of 3:1 and LOQ with S/N ratio of 10:1 was calculated for empagliflozin using equation

LOD=3.3×SD/slope

LOQ=10×SD/slope

Where SD=Standard deviation

The values for LOD and LOQ were found to be 3.19 µg/ml and 9.67 µg/ml respectively.

3.7 System suitability parameter: The number of theoretical plates, retention time and tailing factor were calculated. They were found to be within limits as listed in table 5.

TABLE 5 RESULTS FOR SYSTEM SUITABILITY

System suitability parameter	**Standards**	**Proposed method**
Retention time	<2.5-10 min	3.41
Theoretical plates	More than 2000	10675.3
Tailing factor	Less than 2	1.14

3.8 Degradation studies: The chromatogram obtained from samples exposed to acidic, alkaline and oxidative degradation depicted well separated peaks of pure empagliflozin having RT 3.4 min and some additional peaks at different values. Acidic degradation shows 2 additional peaks. Basic and oxidative degradation shows 3 additional peaks for each. The % degradation of each is listed in table. Results of force degradation are shown in Fig 5-7.

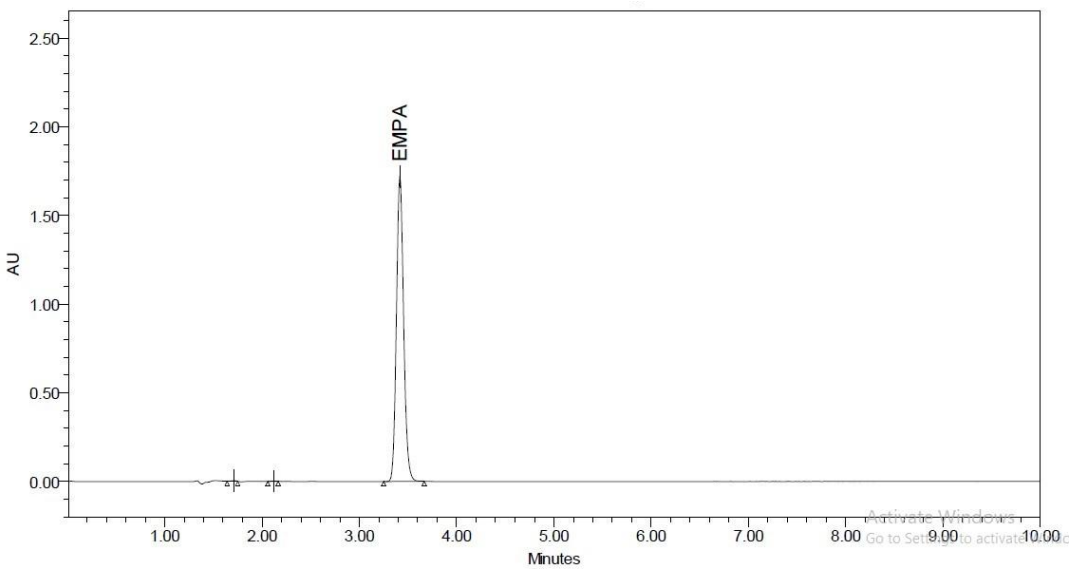

Fig. 5: Acidic stressed sample

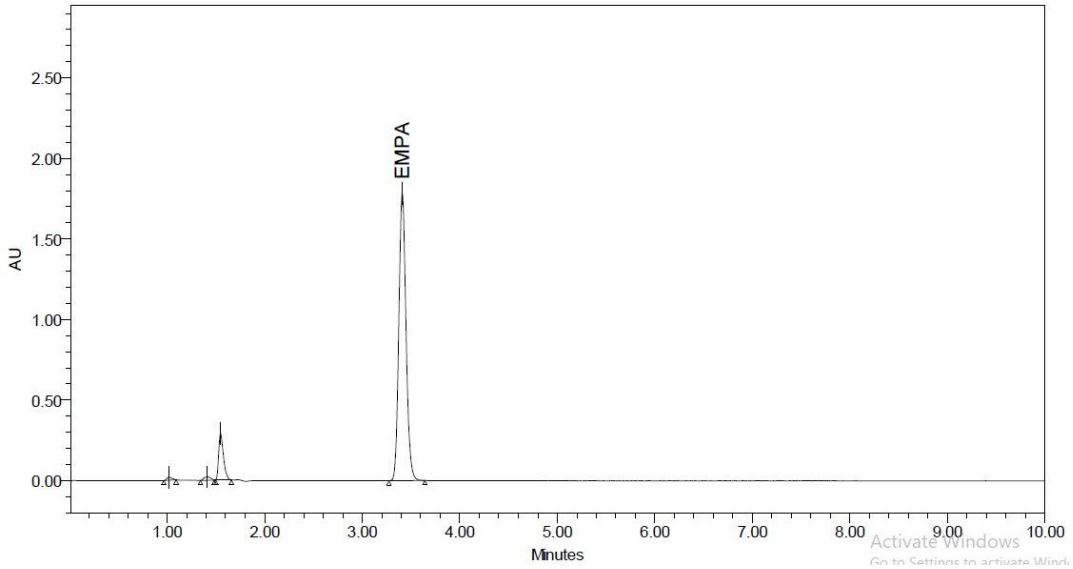

Fig. 6: Alkali stressed sample

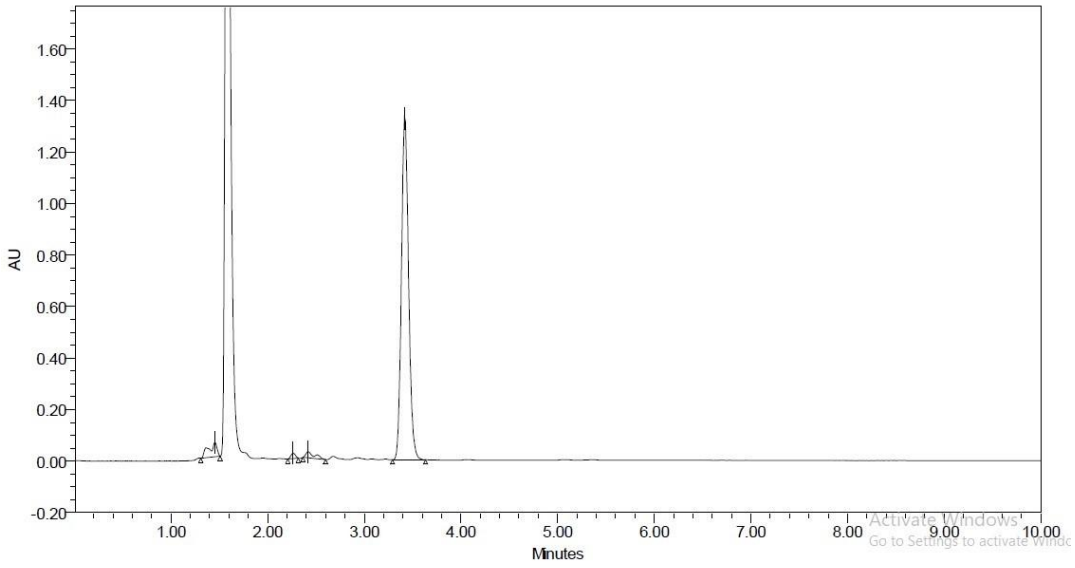

Fig. 7: Oxidative stressed sample

TABLE 6 FORCE DEGRADATION RESULTS

Degradation parameter	**% degradation**	**% Active drug after degradation**
Acidic degradation	0.21	99.79
Alkali degradation	11.12	88.88
Oxidative degradation	7.44	92.56

Discussion: A few methods were reported for the estimation of empagliflozin. The proposed method is discovered to be faster, accurate, precise, sensitive, and cost-effective. In the present method the empagliflozin was eluted at min with a run time of 3.4 min with a run time of 10 min. The present method was developed using mobile phase C and mobile phase D in the ratio of 50:50, where mobile phase C is Ammonium acetate and mobile phase D is ACN: water (80:20). Forced degradation study was performed on empagliflozin using accelerated stress conditions. In the force degradation experiment, we found that empagliflozin degradation was more prominent

in alkaline and oxidative environments. When exposed to an acidic environment, empagliflozin did not further degrade.

Validation was carried out in accordance with ICH guideline and the results was within limits. Linearity was obtained in over concentration range of 100-300 ug/ml with R^2 as 0.999.LOD and LOQ were found to be 3.19 µg/ml and 9.67 µg/ml respectively. The method was found to be precise concluded from intraday and inter day precision. The accuracy was between 97.83% to 99.93%.

4. CONCLUSION:

The current study developed a precise, accurate, robust and stability-indicating RP-HPLC method for the determination of empagliflozin in the presence of degradation products. A forced degradation study was used to study the stability of empagliflozin under various stress conditions. The method was successfully validated according to USFDA guidelines for all the parameters which were found within acceptance criteria. The estimation of Empagliflozin API using the suggested method is both safe and effective for routine analysis.

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Abbreviations: HPLC: High-performance liquid chromatography; ppm: parts per million; T2DM: type 2 diabetes mellitus; SD: standard deviation; RSD: relative standard deviation; LOD: limit of detection; LOQ: limit of quantification; RT: Retention time; OPA: ortho phosphoric acid.