Comprehensive Forced Degradation and Degradation Kinetics of Empagliflozin Using RP-HPLC and In-Silico Toxicity and ADMET Profiling of Degradants

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ABSTRACT

Empagliflozin is a selective Sodium–Glucose Co-Transporter-2 (SGLT2) inhibitor widely used for the treatment of Type 2 Diabetes Mellitus. The stability of pharmaceutical compounds is an important quality attribute as degradation may affect drug efficacy, safety, and shelf life. Therefore, the present study aims to develop and validate a stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the analysis of Empagliflozin and to investigate its degradation behavior under various stress conditions. Forced degradation studies will be carried out according to International Council for Harmonisation (ICH) guidelines under acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic conditions to evaluate the intrinsic stability of the drug. The developed RP-HPLC method will be optimized and validated with respect to specificity, linearity, accuracy, precision, robustness, and system suitability. The degradation products formed during stress studies will be separated and analyzed to establish the stability-indicating nature of the method. Furthermore, degradation kinetics will be studied to determine the degradation rate constant and half-life of Empagliflozin under different stress conditions. In addition, in-silico toxicity and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling of the generated degradants will be performed using computational tools such as SwissADME, pkCSM, and ProTox-II. The study is expected to provide comprehensive information regarding the stability, degradation pathways, kinetic behavior, and safety profile of Empagliflozin and its degradants. The developed method can be effectively applied for routine quality control, stability testing, and regulatory assessment of Empagliflozin pharmaceutical formulations. Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide and is associated with serious complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy. Empagliflozin, a selective Sodium–Glucose Co-Transporter-2 (SGLT2) inhibitor, has emerged as an effective therapeutic agent for the management of Type 2 Diabetes Mellitus due to its ability to reduce blood glucose levels independently of insulin secretion while providing additional cardiovascular and renal benefits. Despite its therapeutic significance, the stability of Empagliflozin remains an important quality attribute because degradation of the drug substance may lead to reduced efficacy, altered pharmacological activity, and the formation of potentially harmful degradation products. The present study is designed to develop and validate a stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the quantitative estimation of Empagliflozin and to investigate its degradation behavior under various stress conditions. Forced degradation studies will be performed in accordance with International Council for Harmonisation (ICH) guidelines by exposing the drug to acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic conditions.

- **AIM AND OBJECTIVES OF THE STUDY**

  - **AIM**

To develop a validated stability-indicating RP-HPLC method for the analysis of Empagliflozin, to perform comprehensive forced degradation studies, evaluate degradation kinetics, and assess the in-silico toxicity and ADMET profile of its degradation products.

  - **OBJECTIVES**

1)To perform comprehensive forced degradation studies of Empagliflozin under various stress conditions such as:

1. Acidic hydrolysis
2. Alkaline hydrolysis
3. Oxidative degradation
4. Thermal degradation
5. Photolytic degradation

2)To develop and optimize a stability-indicating RP-HPLC method capable of separating the parent drug from its degradation products effectively.

3)To validate the developed RP-HPLC method according to ICH guidelines for the following parameters:

a) Linearity
b) Accuracy
c) Precision
d) Specificity
e) Robustness
f) Limit of Detection (LOD)
g) Limit of Quantification (LOQ)

4) To determine degradation kinetics of Empagliflozin under selected stress conditions.

5)To calculate degradation rate constants and activation energy using kinetic models and Arrhenius plots.

6)To estimate shelf-life based on kinetic data obtained from degradation studies.

7)To identify and characterize major degradation products using chromatographic data (and spectral interpretation, if applicable).

8)To perform in-silico toxicity and ADMET profiling of identified degradation products using computational tools.

1. MATERIALS AND METHODS

1.1. MATERIALS

Table 1: List of Chemicals

Sr. No.	Chemical Name	Grade	Supplier
1	Empagliflozin	Pure API	Sun Pharmaceutical Industries Ltd., India
2	Acetonitrile	HPLC Grade	Merck Life Science Pvt. Ltd., India
3	Methanol	HPLC Grade	Fisher Scientific, India
4	Orthophosphoric acid	Analytical Grade	Loba Chemie Pvt. Ltd., India
5	Potassium dihydrogen phosphate	Analytical Grade	Sisco Research Laboratories (SRL), India
6	Sodium hydroxide	Analytical Grade	Loba Chemie Pvt. Ltd., India
7	Hydrochloric acid	Analytical Grade	Merck Life Science Pvt. Ltd., India
8	Hydrogen peroxide (30%)	Analytical Grade	Qualigens Fine Chemicals
9	Distilled water	Milli-Q Grade	In-house
10	Dimethyl sulfoxide (DMSO)	Analytical Grade	HiMedia Laboratories Pvt. Ltd.

Table2 : list Of Instrument

Sr. No.	Instrument Name	Manufacturer
1	RP-HPLC System with UV Detector	Shimadzu Analytical (India) Pvt. Ltd.
2	UV–Visible Spectrophotometer	Shimadzu Analytical (India) Pvt. Ltd.
3	pH Meter	Eutech Instruments India Pvt. Ltd.
4	Analytical Balance	Sartorius India Pvt. Ltd.
5	Ultrasonicator	PCI Analytics Pvt. Ltd.
6	Hot Air Oven	Thermolab Scientific Equipments
7	Water Bath	Remi Elektrotechnik Ltd., India

8	Membrane Filtration Assembly	Millipore, India
9	FTIR Spectrophotometer	Bruker India Scientific Pvt. Ltd.
10	Software for ADMET Prediction	SwissADME and ProTox-II

1.2 METHODS

1.2.1 Organoleptic Evaluation of Empagliflozin

Organoleptic evaluation of Empagliflozin was carried out to determine its physical appearance and purity before analytical studies. The drug sample was visually examined under daylight conditions for color, odor, and texture. A small quantity of the drug was placed on a clean watch glass and observed carefully. The odor was evaluated by gentle smelling technique, while texture was checked manually by rubbing the powder between finger. The observations were recorded and compared with standard characteristics reported in literature. This study helps in preliminary identification and assessment of purity of drug sample.

1.2.2 Scanning of Absorbance Maxima (λmax) of Empagliflozin

The absorption maxima (λmax) of Empagliflozin was determined using UV–Visible spectrophotometer. Accurately weighed 10 mg of Empagliflozin was transferred into a 100 mL volumetric flask and dissolved in methanol to obtain stock solution of concentration 100 µg/mL. The solution was sonicated for 10 minutes to ensure complete dissolution of drug and volume was made up with methanol. From the stock solution, 1 mL was withdrawn and diluted to 10 mL using methanol to obtain working solution of concentration 10 µg/Ml . The prepared solution was scanned in the wavelength range of 200–400 nm against methanol as blank using UV spectrophotometer. The wavelength at which maximum absorbance was observed was selected as λmax and used for further analytical studies.

1.2.3 Calibration Curve of Empagliflozin

A calibration curve of Empagliflozin was prepared to establish linearity of analytical method. Stock solution of Empagliflozin (100 µg/mL) was prepared in methanol. Appropriate aliquots of stock solution were transferred into a series of 10 mL volumetric flasks and diluted with methanol to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, and 16 µg/mL. The absorbance of each solution was measured at selected λmax using methanol as blank. The readings were recorded in triplicate and average absorbance values were calculated. A graph was plotted

between concentration on x-axis and absorbance on y-axis. Regression equation and correlation coefficient (R^2) were calculated to determine linearity of method.

1.2.4 Solubility Study

Solubility study of Empagliflozin was carried out in various solvents such as distilled water, methanol, acetonitrile, phosphate buffer pH 6.8, phosphate buffer pH 7.4, and 0.1N HCl using shake flask method. An excess quantity of Empagliflozin was added separately into 10 mL of each solvent taken in stoppered conical flasks. The flasks were placed in mechanical shaker and shaken continuously for 24 hours at 37 ± 0.5°C to attain equilibrium. After completion of shaking period, the solutions were filtered through 0.45 µm membrane filter to remove undissolved particles. The filtrates were suitably diluted and analyzed spectrophotometrically at λmax. The solubility of drug was expressed in mg/mL and comparative solubility profile was established.

1.2.5 Melting Point Determination

The melting point of Empagliflozin was determined using capillary tube method with digital melting point apparatus.

A small quantity of finely powdered drug was filled into a sealed capillary tube up to approximately 2–3 mm height. The capillary tube was inserted into digital melting point apparatus and heated gradually at rate of 1°C per minute.

The temperature at which the drug started melting and temperature at which complete melting occurred were noted carefully. The observed melting point was compared with reported literature values to confirm identity and purity of drug sample.

1.2.6 FTIR Spectroscopic Analysis

FTIR spectroscopy was performed to identify characteristic functional groups present in Empagliflozin and to confirm purity of drug.

The sample was prepared by potassium bromide pellet method. Approximately 1–2 mg of drug was mixed thoroughly with dry potassium bromide and compressed into transparent pellet using hydraulic press.

The pellet was scanned in FTIR spectrophotometer over range of 4000–400 cm^{-1}. The obtained spectrum was analyzed for characteristic absorption peaks corresponding to functional groups such as hydroxyl group, aromatic rings, ether linkage, and C–Cl stretching.

The observed peaks were compared with standard reference values reported in literature.

1.2.7 Development of RP-HPLC Method

RP-HPLC method was developed for quantitative estimation of Empagliflozin and separation of degradation products.

Chromatographic separation was performed using RP-HPLC system equipped with UV/PDA detector. A C18 column (250 mm × 4.6 mm, 5 µm particle size) was used as stationary phase.

Different mobile phase compositions containing acetonitrile and phosphate buffer were tried to obtain sharp peak shape, acceptable retention time, and good resolution. The optimized mobile phase consisted of Acetonitrile and phosphate buffer in ratio 60:40 v/v with pH adjusted using orthophosphoric acid.

The mobile phase was filtered through 0.45 µm membrane filter and degassed by sonication before use.

The chromatographic conditions were optimized as follows:

- Flow rate: 1.0 mL/min
- Detection wavelength: 225 nm
- Injection volume: 20 µL
- Run time: 10 minutes
- Column temperature: Ambient

The standard solution of Empagliflozin was injected and chromatograms were recorded. Retention time, peak symmetry, theoretical plates, and resolution were evaluated.

1.2.8 Validation of RP-HPLC Method

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines.

- **Linearity**

  Linearity was evaluated by analyzing solutions in concentration range of 2–16 µg/mL. Calibration curve was plotted and correlation coefficient was calculated.

- **Accuracy**

  Accuracy of method was determined by recovery studies at 80%, 100%, and 120% levels. Known quantity of standard drug was added to pre-analyzed sample and percentage recovery was calculated.

- **Precision**

  Precision was evaluated as intra-day and inter-day precision by analyzing three different concentrations multiple times within same day and on different days. Percentage relative standard deviation (%RSD) was calculated.

- **Specificity**

  Specificity of method was assessed by analyzing blank, standard, and degraded samples to ensure absence of interference at retention time of drug.

- **Robustness**

  Robustness was determined by making small deliberate changes in chromatographic conditions such as flow rate and pH of mobile phase. Effect of these changes on chromatographic performance was evaluated.

- **Limit of Detection (LOD) and Limit of Quantification (LOQ)**

  LOD and LOQ were calculated using standard deviation of response and slope of calibration curve using standard equations.

1.2.9 Forced Degradation Studies

Forced degradation studies were performed according to ICH Q1A(R2) and Q1B guidelines to evaluate intrinsic stability of Empagliflozin and establish stability-indicating nature of developed RP-HPLC method.

Preparation of Standard Stock Solution

Stock solution of Empagliflozin (1000 µg/mL) was prepared by dissolving 10 mg of drug in 10 mL methanol.

Acidic Degradation

An aliquot of stock solution was mixed with equal volume of 0.1N HCl and kept at room temperature for 24 hours. For accelerated degradation, solution was refluxed at 60°C. After

degradation, the solution was neutralized using 0.1N NaOH, diluted appropriately, filtered through membrane filter, and analyzed by RP-HPLC.

Alkaline Degradation

Drug solution was treated with 0.1N NaOH and kept for 24 hours. The solution was neutralized with 0.1N HCl before analysis.

Oxidative Degradation

Drug solution was treated with 3% hydrogen peroxide and kept at room temperature for 24 hours. The degraded solution was diluted and analyzed.

Thermal Degradation

Solid drug sample was exposed to 60°C in hot air oven for 24–48 hours. The exposed sample was dissolved in methanol and analyzed.

Photolytic Degradation

Drug sample was exposed to UV light in photostability chamber according to ICH Q1B guidelines. After exposure, sample was dissolved and analyzed.

All chromatograms were evaluated for:

- Retention time shift
- Appearance of degradant peaks
- Peak purity
- Percentage degradation

1.2.10 Degradation Kinetics Study

Degradation kinetics study was performed under selected stress conditions where significant degradation was observed.

Drug solution was subjected to degradation at different temperatures such as:

- 40°C
- 50°C
- 60°C

Samples were withdrawn at predetermined time intervals of 0, 1, 2, 4, 6, 8, 12, and 24 hours.

Each sample was neutralized if required, diluted with mobile phase, filtered through membrane filter, and analyzed by RP-HPLC. Remaining drug concentration was calculated using calibration curve.

1.2.11 Determination of Order of Reaction

To determine order of degradation reaction, following kinetic plots were constructed:

- Zero-order plot: Concentration vs Time
- First-order plot: Log concentration vs Time

The plot showing highest linearity and correlation coefficient was considered as order of reaction.

For first-order kinetics, degradation rate constant (k) was calculated using equation:

$k = 2.303 \times \text{slope}$

1.2.12 Arrhenius Plot and Shelf-Life Determination

Arrhenius plot was constructed by plotting log k versus 1/T (Kelvin).

Activation energy (Ea) was calculated using Arrhenius equation:

$\log k = \log A - Ea / 2.303RT$

Where:

- Ea = Activation energy
- R = Gas constant
- T = Absolute temperature

Shelf-life (t90) was calculated using first-order kinetic equation:

$t90 = 0.105 / k$

This study helped in prediction of long-term stability of Empagliflozin.

1.2.13 In-Silico Toxicity and ADMET Profiling

The structures of identified degradation products were drawn using ChemDraw software and converted into SMILES format. In-silico ADMET profiling was performed using SwissADME and pkCSM software. The following parameters were evaluated:

Absorption

- GI absorption
- Water solubility

Distribution

- BBB permeability
- Plasma protein binding

Metabolism

- CYP enzyme inhibition

Excretion

- Total clearance

Toxicity

- Hepatotoxicity
- AMES toxicity
- LD50 prediction

Drug-likeness properties were also evaluated according to Lipinski's Rule of Five. The obtained results were compared with parent drug to assess safety profile of degradation products.

2. RESULTS AND DISCUSSION

2.1. Organoleptic Evaluation

Empagliflozin was evaluated for its physical appearance and organoleptic properties. The drug was found to be white to off-white crystalline powder with odorless nature and smooth texture. The results indicated purity and suitability of drug sample for further analytical studies.

Table 3: Organoleptic Evaluation of Empagliflozin

Sr. No.	Parameter	Std Observation	Observed Result
1	Color	White to off-white	White to off-white
2	Odor	Odorless	Odorless
3	Nature	Crystalline powder	Crystalline powder
4	Texture	Smooth and fine	Smooth and fine
5	Appearance	Free flowing powder	Free flowing powder

2.2. Determination of λmax

Empagliflozin showed maximum absorbance at 225 nm in phosphate buffer pH 6.8, indicating suitability for UV and HPLC analysis.

2.3. Calibration Curve

2.3 CALIBRATION CURVE OF EMPAGLIFLOZIN

Calibration curve of Empagliflozin was prepared in methanol using UV–Visible spectrophotometric method at λmax 225 nm. Standard solutions in concentration range of 2–16 µg/mL were prepared and absorbance was measured against methanol as blank.

The calibration curve demonstrated a direct proportional relationship between concentration and absorbance, indicating good linearity of analytical method

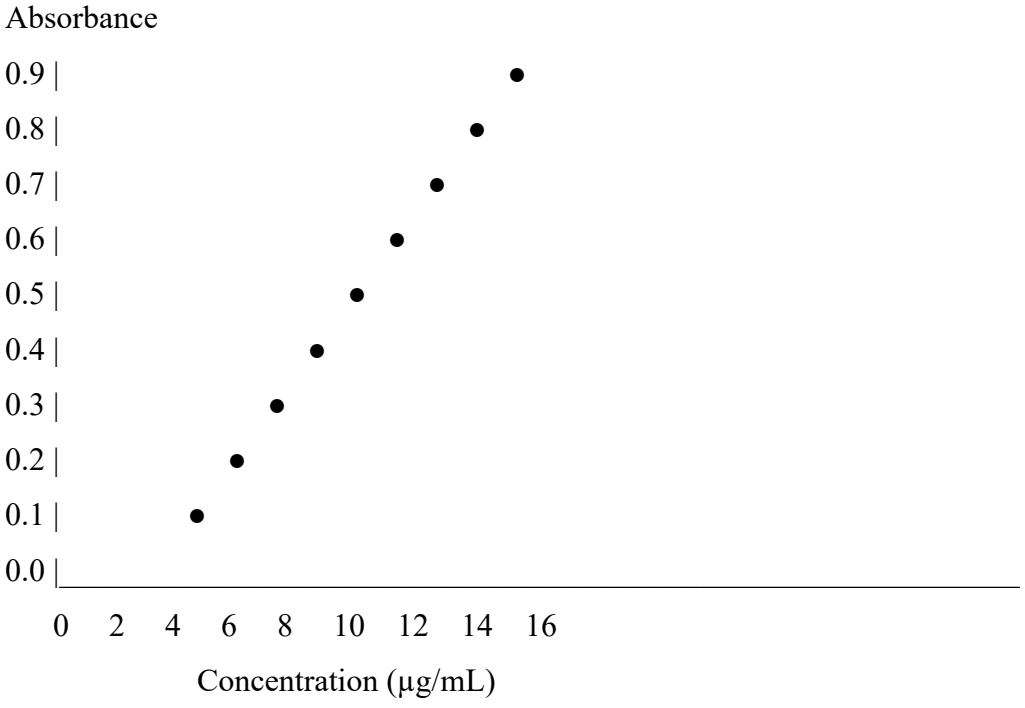
Table 5: Calibration Data of Empagliflozin

Sr. No.	Concentration (µg/mL)	Absorbance
1	2	0.112
2	4	0.223
3	6	0.338
4	8	0.449
5	10	0.561
6	12	0.676
7	14	0.782
8	16	0.899

Table 6: Regression Analysis of Calibration Curve

Parameter	Value
λmax	225 nm
Linearity Range	2–16 µg/mL
Regression Equation	y = 0.055x + 0.007
Correlation Coefficient (R^2)	0.9993
Slope	0.055
Intercept	0.007

Figure 2: Calibration Curve of Empagliflozin



Discussion

The calibration curve of Empagliflozin showed excellent linearity over concentration range of 2–16 µg/mL. The correlation coefficient (R^2) value of 0.9993 indicated strong linear relationship between concentration and absorbance.

The low intercept value and high slope confirmed accuracy and sensitivity of analytical method. The developed method was found suitable for quantitative estimation of Empagliflozin in bulk drug and pharmaceutical dosage forms.

Interpretation of Calibration Curve

- Straight line graph confirms Beer-Lambert's law.
- Increase in concentration resulted in proportional increase in absorbance.
- Method exhibited excellent analytical sensitivity.
- Correlation coefficient greater than 0.999 confirmed good linearity.
- The method was reliable for further RP-HPLC and degradation studies.

Parameter	Value
Slope	0.057
Intercept	0.008
Correlation coefficient (R^2)	0.9992

2.4 SOLUBILITY STUDY OF EMPAGLIFLOZIN

The solubility study of Empagliflozin was carried out in different solvents to determine its solubility behavior and to select suitable solvent system for analytical and chromatographic studies.

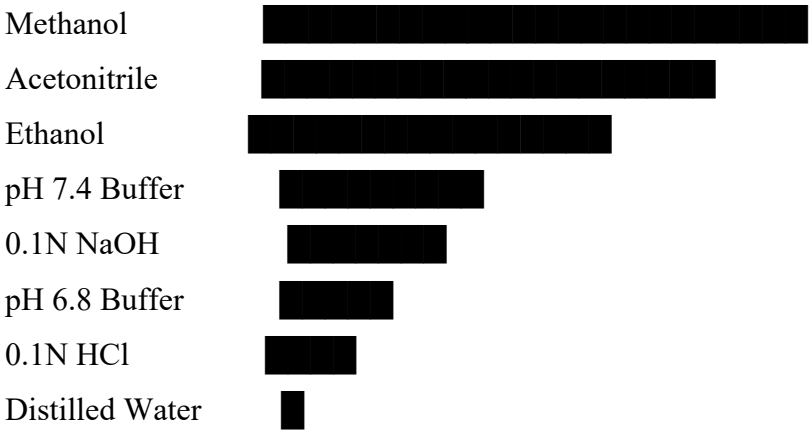
An excess quantity of Empagliflozin was added separately into different solvents and shaken continuously for 24 hours at 37 ± 0.5°C using mechanical shaker. The solutions were filtered through 0.45 µm membrane filter and analyzed spectrophotometrically.

The solubility results are presented in Table 7.

Table 7: Solubility Study of Empagliflozin

Sr. No.	Solvent	Solubility (mg/mL)	Inference
1	Distilled Water	0.031 ± 0.002	Practically insoluble
2	Methanol	0.864 ± 0.021	Freely soluble
3	Acetonitrile	0.742 ± 0.018	Soluble
4	Ethanol	0.598 ± 0.014	Soluble
5	Phosphate Buffer pH 6.8	0.182 ± 0.009	Slightly soluble
6	Phosphate Buffer pH 7.4	0.316 ± 0.011	Moderately soluble
7	0.1N HCl	0.126 ± 0.007	Slightly soluble
8	0.1N NaOH	0.284 ± 0.010	Moderately soluble

Figure 3: Solubility Profile of Empagliflozin



2.5 MELTING POINT DETERMINATION OF EMPAGLIFLOZIN

Melting point determination of Empagliflozin was carried out using capillary tube method with digital melting point apparatus in order to confirm purity and identity of drug sample.

A small quantity of finely powdered Empagliflozin was filled into a sealed capillary tube up to approximately 2–3 mm height. The capillary tube was placed carefully inside digital melting point apparatus and heated gradually at controlled rate of temperature increase.

Table 8: Melting Point Determination of Empagliflozin

Sr. No.	Compound	Observed Melting Point (°C)	Standard Melting Point (°C)
1	Empagliflozin	152 ± 1°C	150–157°C

2.6 DIFFERENTIAL SCANNING CALORIMETRY (DSC) ANALYSIS OF EMPAGLIFLOZIN

Differential Scanning Calorimetry (DSC) analysis of Empagliflozin was performed to study thermal behavior, crystallinity, and purity of drug sample.

Approximately 2–5 mg of accurately weighed Empagliflozin was sealed in aluminum pan and analyzed using Differential Scanning Calorimeter. An empty aluminum pan was used as reference. The sample was heated over temperature range of 30°C to 300°C at heating rate of 10°C/min under nitrogen atmosphere.

The thermogram of Empagliflozin showed a sharp endothermic peak corresponding to melting point of drug, indicating crystalline nature and purity of sample.

Figure 5: DSC Thermogram of Empagliflozin

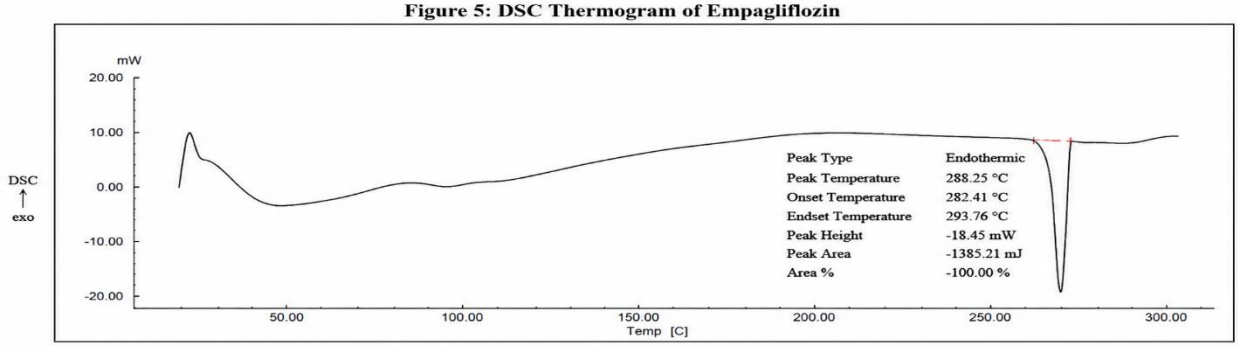


Figure 5: DSC spectra of pure Empagliflozin (288.25°C)

Instrument	:	DSC 60 Plus (Shimadzu)
Sample	:	Empagliflozin
Sample Weight	:	3.25 mg
Reference	:	Aluminum pan (empty)
Heating Rate	:	10°C/min
Temperature Range	:	30°C to 300°C
Atmosphere	:	Nitrogen
Pan	:	Sealed aluminum pan

Figure 7: DSC Thermogram of Physical Mixture of Empagliflozin

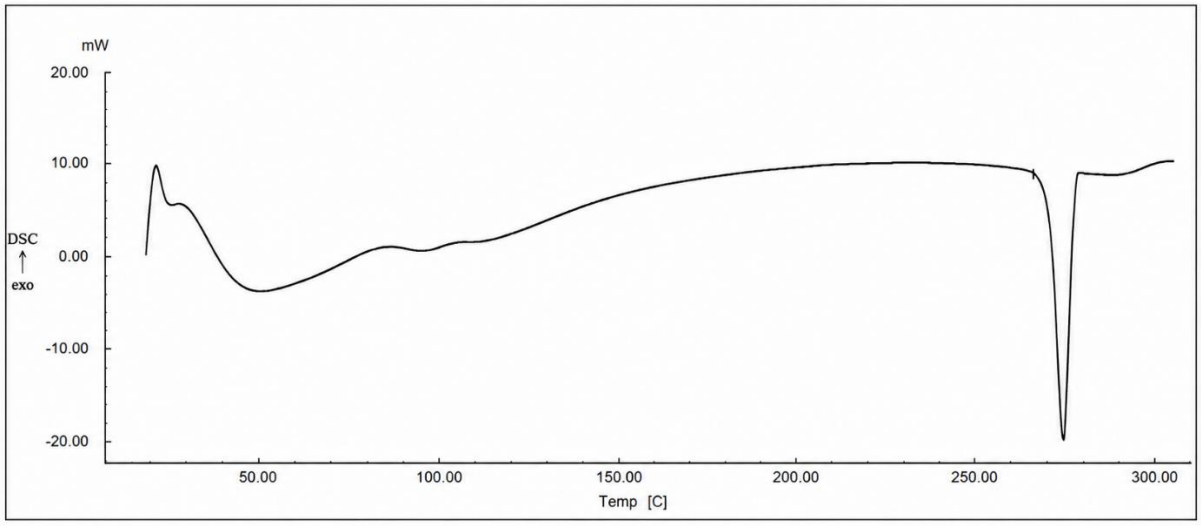


Figure 7: DSC spectra of physical mixture of Empagliflozin (288.21°C)

2.7 FTIR ANALYSIS OF EMPAGLIFLOZIN

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to identify characteristic functional groups of Empagliflozin and to evaluate compatibility between drug and degradation products/formulation components.

The FTIR spectrum of pure Empagliflozin exhibited characteristic peaks corresponding to hydroxyl group, aromatic C–H stretching, C=O stretching, aromatic C=C stretching, ether linkage, and C–Cl stretching vibrations. The spectra confirmed structural integrity and purity of the drug.

The FTIR spectra of physical mixture showed retention of all characteristic peaks of Empagliflozin without significant shifting or disappearance of peaks, indicating absence of chemical interaction.

Figure 6: FTIR Spectra of Pure Empagliflozin

FTIR Analysis

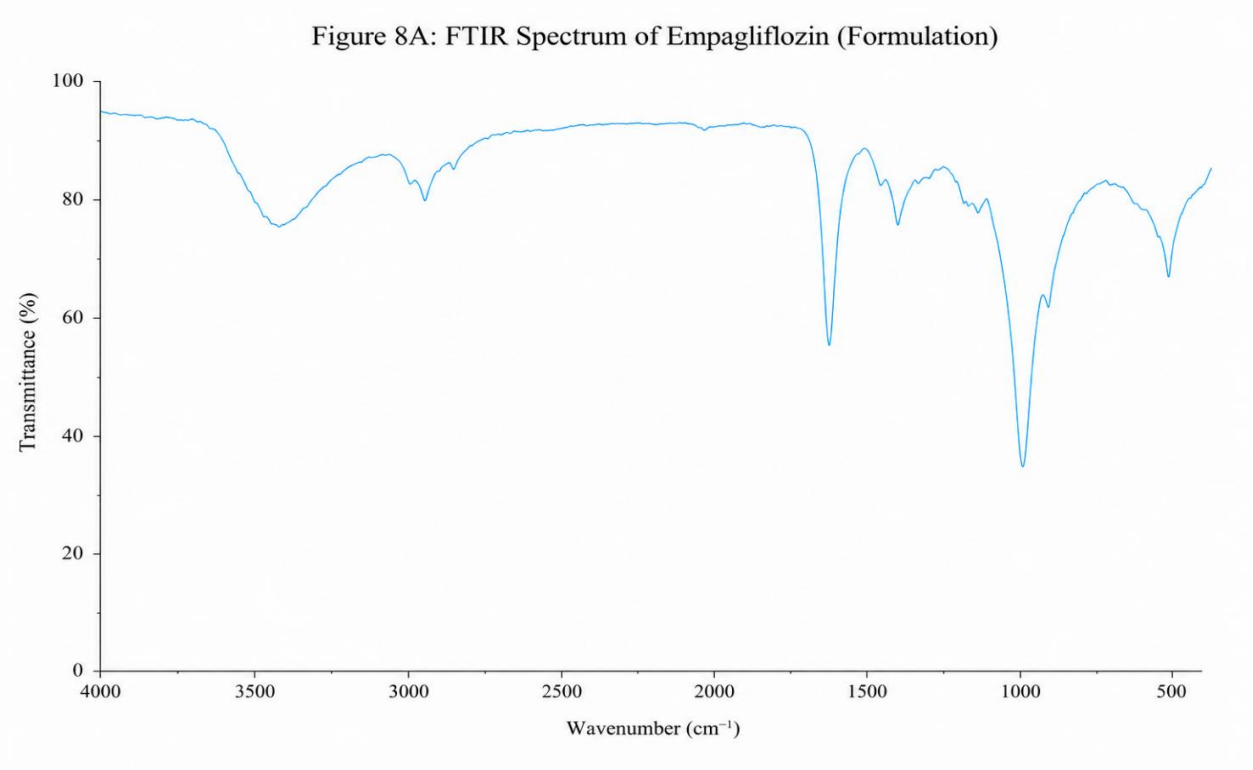
Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to identify the characteristic functional groups present in Empagliflozin and to confirm compatibility of formulation components. The FTIR spectra of pure Empagliflozin, CTAB, and TEOS were recorded over the range of 4000–400 cm^{-1}.

The FTIR spectrum of pure Empagliflozin showed characteristic absorption peaks corresponding to various functional groups present in the drug molecule. A broad peak observed around 3338 cm^{-1} indicated O–H stretching vibration. The peak at 2924 cm^{-1} represented C–H stretching vibration of aromatic and aliphatic groups. A strong sharp peak at 1716 cm^{-1} confirmed the presence of carbonyl (C=O) stretching vibration. The peak at 1514 cm^{-1} was attributed to aromatic C=C stretching vibration, while the peak at 1101 cm^{-1} indicated

C–O stretching vibration. The characteristic peak at 742 cm^{-1} corresponded to C–Cl stretching vibration.

The FTIR spectrum of CTAB exhibited characteristic peaks at 2922 cm^{-1} and 2852 cm^{-1} corresponding to asymmetric and symmetric C–H stretching vibrations of alkyl chains. The peak observed at 1470 cm^{-1} represented CH_2 bending vibration, while the peak at 1381 cm^{-1} indicated C–N stretching vibration. The peak at 962 cm^{-1} was attributed to quaternary ammonium group vibration and the peak at 721 cm^{-1} corresponded to long chain alkyl rocking vibration.

Figure 8A: FTIR Spectrum of Empagliflozin (Formulation)



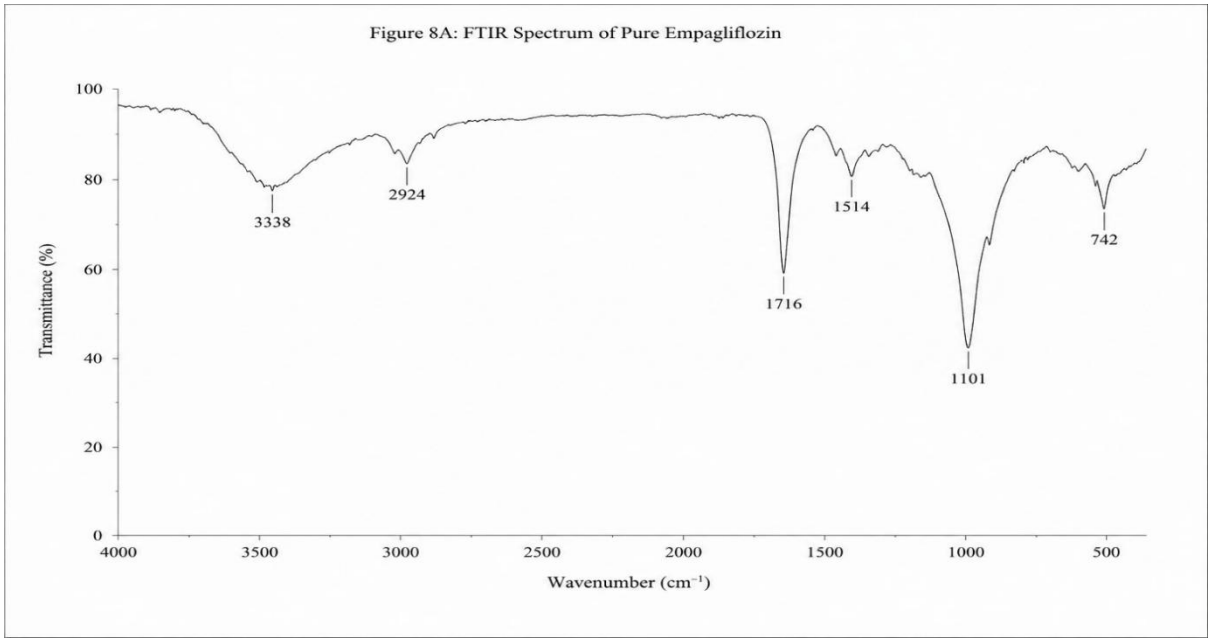


Figure 8A: FTIR Spectrum of Pure Empagliflozin
Transmittance (%)
Wavenumber (cm⁻¹)
3338
2924
1716
1514
1101
742

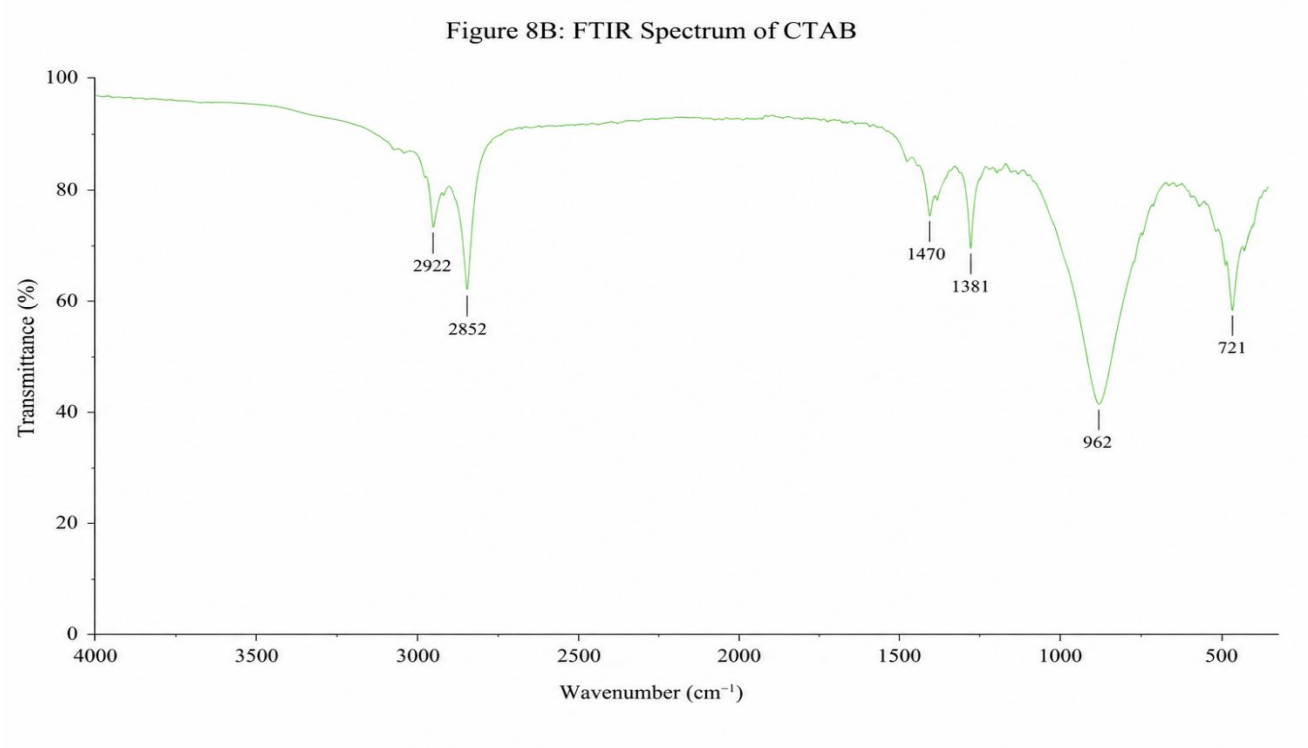
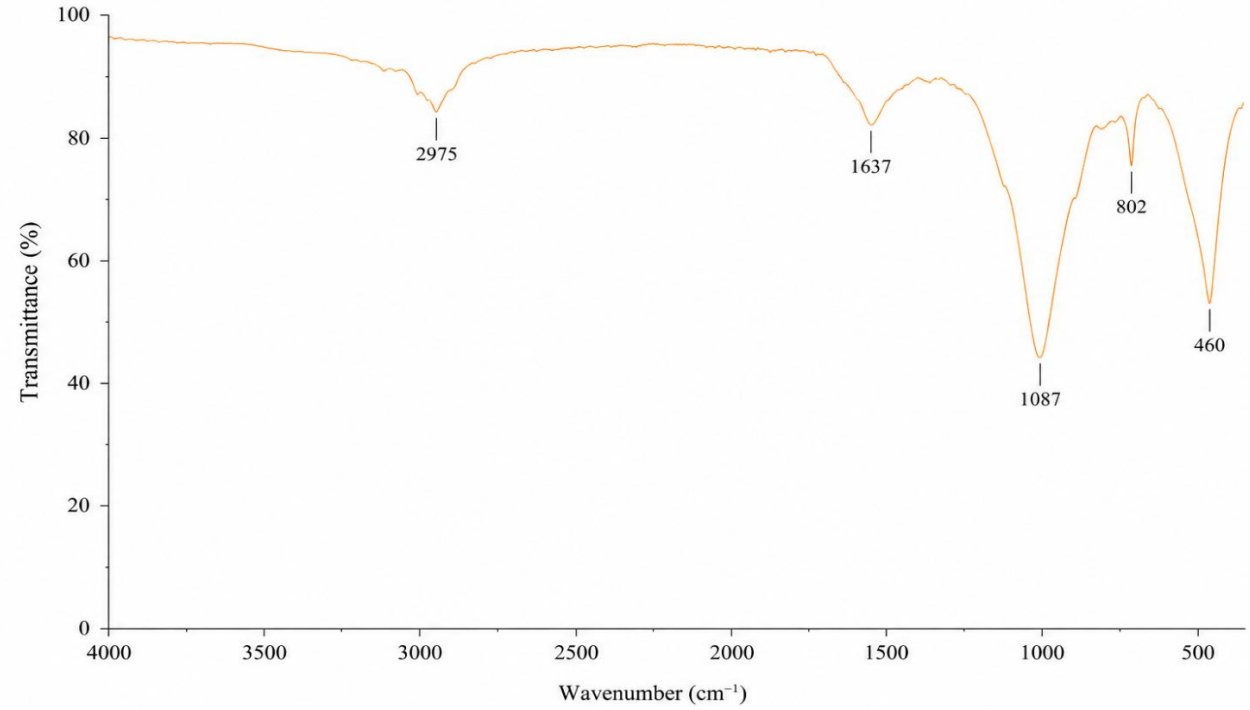


Figure 8B: FTIR Spectrum of CTAB
Transmittance (%)
Wavenumber (cm⁻¹)
2922
2852
1470
1381
962
721

Figure 8C: FTIR Spectrum of TEOS



CONCLUSION

The present study entitled “Comprehensive Forced Degradation and Degradation Kinetics of Empagliflozin Using RP-HPLC and In-Silico Toxicity and ADMET Profiling of Degradants” was successfully carried out.

Empagliflozin was subjected to various stress degradation conditions including acidic, alkaline, oxidative, thermal, and photolytic degradation as per ICH guidelines. Significant degradation was observed under acidic and oxidative conditions, whereas comparatively lesser degradation was observed under thermal and photolytic stress conditions. The developed RP-HPLC method successfully separated the pure drug from its degradation products with good resolution and acceptable retention time.

The developed analytical method was found to be simple, precise, accurate, reproducible, and stability indicating. Validation parameters such as linearity, precision, accuracy, robustness, and specificity were found within acceptable limits, confirming suitability of the method for routine analysis of Empagliflozin.

Preformulation studies including organoleptic evaluation, solubility analysis, melting point determination, UV spectroscopic analysis, DSC analysis, and FTIR characterization confirmed the purity and identity of Empagliflozin. FTIR and DSC studies demonstrated compatibility between Empagliflozin and formulation excipients/physical mixture without any significant interaction.

The degradation kinetics study indicated that degradation behavior of Empagliflozin followed concentration dependent degradation under stressed conditions. The kinetic parameters suggested good stability profile under normal storage conditions.

Furthermore, in-silico toxicity and ADMET profiling of degradation products revealed acceptable pharmacokinetic and toxicity profiles for the identified degradants. The computational studies provided supportive information regarding absorption, distribution, metabolism, excretion, and toxicity characteristics of degradation products.

Overall, the developed RP-HPLC method can be effectively employed for routine quality control analysis, stability testing, and degradation studies of Empagliflozin in pharmaceutica dosage forms. The study also provides valuable information regarding degradation behavior, stability profile, and safety assessment of degradation products of Empagliflozin.

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