ORIGINAL ARTICLE

Stability-Indicating RP-HPLC Method Development for the Determination of Empagliflozin

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Abstract

Aim: This study aimed to develop and validate a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative analysis of empagliflozin in pharmaceutical formulations. **Materials and Methods:** The method was developed using an Agilent C18 column (5µm; 4.6 x 250 mm) and a mobile phase consisting of a mixture of methanol/ 0.05% pH 3.3 acetic acid (75:25) at a flow rate of 1 ml/min with UV detection at 224 nm. The developed RP-HPLC method demonstrated linearity with a correlation coefficient (r) of >0.999. Forced degradation studies revealed the method's ability to separate and quantify empagliflozin from its degradation products. The actual percentage degradation ranged from 2.85% to 7.43%. Precision was established where the mean interday and intraday RSD were 0.36% and 0.17%, respectively. **Result and Discussion:** The method's robustness was confirmed, which had negligible effects on the assay results (<0.2% RSD). The method was applied to commercially available tablets, yielding an average (SD) empagliflozin recovery of 100.44% (0.18). **Conclusion:** This RP-HPLC method is suitable for routine analysis of empagliflozin in pharmaceutical formulations and can be employed for stability studies and quality control purposes, ensuring the drug's quality and efficacy throughout its shelf life.

Key words: Empagliflozin, method development, method validation, pharmaceutical analysis, reverse-phase high-performance liquid chromatography, stability-indicating assay

INTRODUCTION

Empagliflozin is a C-glycosyl compound consisting of a beta-glucosyl residue having a (4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy]benzyl}phenyl group at the anomeric centre.[1] It is a selective inhibitor of sodium–glucose cotransporter 2, which reduces hyperglycemia in patients with type 2 diabetes by diminishing renal glucose reabsorption and subsequently enhancing urinary glucose excretion.[2,3] It is approved by the USFDA to improve glycemic control and reduce the risk of cardiovascular death in patients with type 2 diabetes mellitus.[4] The IUPAC name of empagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl] methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol. It has a molecular weight of 450.9 g/mol, water solubility of 0.28 mg/mL, and a partition coefficient of 2. The chemical structure of empagliflozin is depicted in Figure 1.[5]A stability-indicating method refers to a validated quantitative analytical procedure employed for assessing the stability of a drug substance. This method can analyse pharmaceutical products and active pharmaceutical ingredients (APIs), particularly in the context of forced degradation studies.[6] The ICH Q3B guidelines mandate the provision of evidence for the validation of analytical methods when the method is suitable for the detection and quantification of degradation products and impurities.[7] Multiple analytical techniques are reported to have been implemented for stability-indicating assays, with the most common ones being high-performance liquid chromatography (HPLC), gas chromatography, high-performance thin-layer chromatography, capillary electrophoresis, and supercritical fluid chromatography.[8]

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A few studies have reported stability-indicating method development for empagliflozin by application of micellar electrokinetic chromatography,[9] liquid chromatography,[10] reversed phase (RP)-ultra-HPLC,[11] and RP-HPLC using diode array detector.[12] HPLC is a major analytical technique used worldwide. More than 90% of the total APIs are analyzed by HPLC according to pharmacopeial specifications. This underscores the simplicity and efficiency of the technique.[13] While Pathak[12] have reported an ICH-validated method for the estimation of empagliflozin, the literature focusing on HPLC techniques is lacking and is in a nascent stage. Hence, this study aims to develop a stability-indicating RP-HPLC method for the determination of empagliflozin and to assess its suitability for monitoring the stability and purity of empagliflozin in various pharmaceutical formulations and under different stress conditions. This study also evaluates the method's linearity, accuracy, precision, specificity, and robustness as per ICH guidelines for analytical method validation.

MATERIALS AND METHODS

Chemicals and reagents

Empagliflozin was received as a gift sample from Jiyan Chemicals and Pharmaceuticals, India. The commercially available empagliflozin 25 mg tablet formulation, Gibtulio (Lupin Pharma) 25 mg tablet was used for the assay. The tablets were procured from the open market in India. HPLC-grade methanol (Honeywell), water (Honeywell) and acetic acid (Fisher Scientific) were procured from Chemtek Scientific Pvt. Ltd., Mumbai, India. The chemicals, mobile phase, standards, diluents and samples were filtered through a 0.22 µm MF-Millipore™ Membrane Filter.

Instrumentation

Agilent 1100 Series HPLC equipped with Agilent Diode Array Detector (with UV detection) and Agilent 1100 Series G1310A Isocractic Pump were employed for method development and validation studies. Agilent OpenLab ChemStation was used as a chromatography data system for data curation. Other analytical instruments used were as follows: Analytical balance (ATX-224; Shimadzu Japan), Sonicator (Branson CPX), pH meter (pH meter SevenDirect SD20), and Vacuum filter pump (Millipore, XI 5522050).

Chromatographic conditions

Agilent C18 (4.6 × 250 mm) column, of 5 µm particle size packing was used as a stationary phase. Around 40 trials were conducted to study the separation of empagliflozin using acetonitrile, water, methanol, and various buffers. The optimized mobile phase obtained consisted of methanol/0.05% pH 3.3 acetic acid in proportion of 75:25. The chromatographic parameters were as follows: detection wavelength: 224 nm, flow rate: 1 ml/min, Temperature: Ambient, and Sample size: 20 µl. Additional details regarding the HPLC system are detailed in Table 1.

Preparation of standard and sample solutions

HPLC grade methanol was used as a diluent for stock solution preparation. The standard sample preparation involved creating a series of standard solutions with concentrations ranging from 5 µg/ml to 30 µg/ml of empagliflozin by diluting a stock solution (500 µgm/ml empagliflozin) with methanol. The tablet solution preparation involves taking 11 mg of Gibtulio (Lupin Pharma) powder (20 tablets were crushed in mortar and pestle) and dissolving it in 10 ml of methanol to obtain a concentration of 500 µg/ml of empagliflozin. For the tablet assay, a 25 µg/ml solution is used, where 0.5 ml is taken from Stock II and made up to a total volume of 10 ml with a mobile phase. This solution is used for the assay.

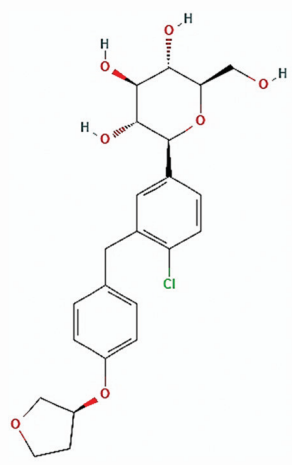

Figure 1: Chemical structure of empagliflozin (adapted from National Center for Biotechnology Information. PubChem Compound Summary for CID 11949646, Empagliflozin. https://pubchem.ncbi.nlm.nih.gov/compound/Empagliflozin. Accessed Nov. 13, 2023.)

Table 1: HPLC conditions

Parameter	Value
Pump unit	G1310A Iso.Pump
Maximum pressure	400 bar
Discharge rate	0.001–5 ml
Pressure limit range	400 bar
Pressure display accuracy	5%
No of mobile phase	4
Mixing ratio range	0–100%
Pump Unit	HP-1100 Reciprocating pump

Method validation[14]

The method development was carried out in accordance with ICH guidelines for Linearity, Accuracy, Precision, Repeatability, and Robustness. Linearity was evaluated by analyzing five serial dilutions (5–25 µg) to assess the analyte concentration in the dilutions. The correlation coefficient, y-intercept, and slope of the regression line were obtained using the linear regression method. Accuracy was assessed by 6 determinations over 3 concentration levels (4 µg/ml, 5 µg/ml, and 6 µg/ml). The sample solution of empagliflozin (10 µg/ml) was spiked at 80%, 100% and 120% by addition of standard empagliflozin solution. Precision was assessed over 3 concentration levels (5 µg/ml, 15 µg/ml, and 25 µg/ml) at different time intervals in inter- and intra-day settings. Repeatability was assessed by 2 determinations for a concentration of 15 µg/ml. Drug recovery and % RSD were calculated from the analyzed data for all parameters. Method robustness was determined by deliberate modifications in the system suitability parameters, i.e., changing volume of mobile phase, flow rate, and injection volume. A working solution of 25 µg/ml was used for all experimental runs of robustness parameters.

The limit of detection (LOD) and limit of quantification (LOQ) were determined based on the slope of the calibration plot and the standard deviation of the response to the blank sample. The formulas used LOD and LOQ were:

LOD = 3.3 × (Standard Deviation of Blank Responses)/(Slope of the Calibration Plot)

LOQ = 10 × (Standard Deviation of Blank Responses)/(Slope of the Calibration Plot)

Forced degradation studies

The degradation sample preparation methods involved using a mobile phase with a 90:10 composition. These methods include acid degradation, basic degradation, neutral degradation, oxidation with 3% H_2O_2, and photo degradation. Excluding photolytic degradation, each method followed a standard procedure for preparing samples. 0.2 ml of standard empagliflozin solution was introduced in a 10ml volumetric flask. This further included the addition of specific reagents (5 ml) and time intervals for sampling and analysis. The reagents used for each test were as follows: acid (0.1 N HCl), basic (0.1 N NaOH), neutral (water), and oxidation (3% hydrogen peroxide). For photodegradation, the API is exposed to sunlight for 24 h, and a 20 µg/ml solution is prepared for injection.

RESULTS

The wavelength maxima of empagliflozin were found to be 224 nm (20 mcg sample in methanol) on a UV Vis-Spectrophotomer. The spectrum is depicted in Figure 2. The lambda max of empagliflozin has also been previously reported. Kamal *et al*., reported the wavelength maxima of empagliflozin between 239.8 and 282.6 nm using dual-wavelength method which was directly proportional to empagliflozin concentration.[15] Furthermore, Patil *et al*., reported the wavelength maxima of empagliflozin to be 224 nm as well in a study conducted for simultaneous estimation of empagliflozin and metformin hydrochloride in bulk drugs.[16] Multiple trials were conducted to investigate the maximum retention of empagliflozin in a suitable mobile phase. The final trial demonstrated an optimized mobile phase of methanol/0.05% pH 3.3 acetic acid in proportion of 75:25. The mobile phase trial results are illustrated in Figure 3.

Method validation studies

The results for accuracy, precision, repeatability, and robustness are illustrated in Table 2. The chromatograms for determinations of accuracy, precision, repeatability, and robustness are depicted. A linear relationship was established between increasing serial dilutions of empagliflozin standard solution and absorbance where the regression coefficient was found to be 0.999. The slope and intercept were found to

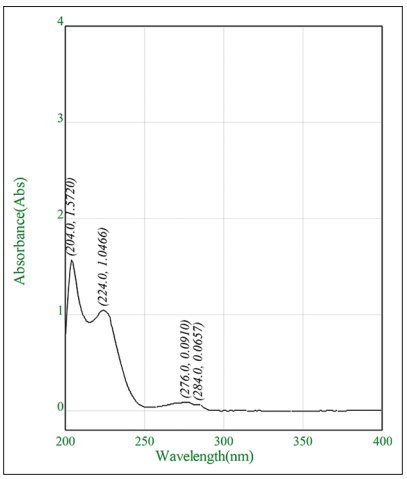

Figure 2: UV spectrum of empagliflozin

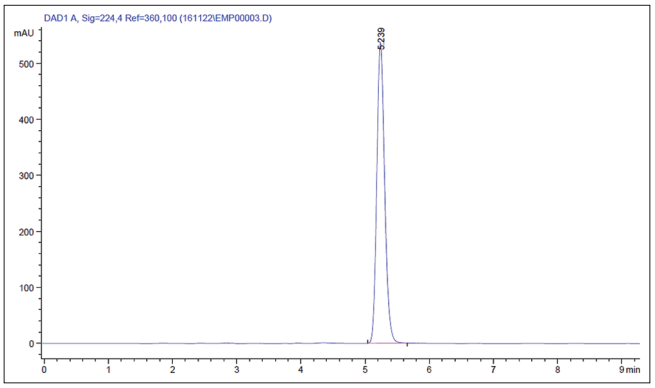

Figure 3: Optimized mobile phase and retention of empagliflozin

Table 2: Results of method validation studies

Linearity			
Concentration [x] (µg/ml)	**Mean peak area* [y] (AU)**	**SD**	**%RSD**
5	417.3	1.6	0.4
10	769.5	5.5	0.7
15	1150.4	5.3	0.5
20	1507.9	5.4	0.4
25	1876.6	10.5	0.6
Linear regression equation (y=mx+c): y=73.138x+47.289			
Correlation Coefficient (R2) = 0.999			
Accuracy Fixed sample concentration (5 µg/ml)			
% Spiked level	**Amount Spiked (µg/ml)**	**Mean % Recovery (SD)**	**%RSD**
80	4	100.7 (0.8)	0.79
100	5	100.7 (0.2)	0.2
120	6	99.4 (0.2)	0.19
Precision			
Concentration (µg/ml)	**Mean peak area* (AU)**	**Mean % Recovery (SD)**	**%RSD**
Intraday			
5	416.1	100.9 (2.4)	0.6
15	1162.7	101.7 (2.2)	0.2
25	1881.5	100.3 (5.8)	0.3
Inter-day			
5	415.2	100.6 (0.5)	0.1
15	1152.6	100.8 (2)	0.2
25	1882.8	100.4 (3.8)	0.2
Repeatability			
15 (2 h)	1151.2	100.6 (1.5)	0.1
15 (4 h)	1149.2	100.5 (0.6)	0
15 (6 h)	1153.2	100.8 (2.8)	0.2
Robustness			
Parameter	**Concentration (µg/ml)**	**Mean peak area AU (SD)**	**%RSD**
Flow rate (0.9 ml/min)	25	1842.5 (2.7)	0.14
Flow rate (1.1 ml/min)	25	1921 (1.6)	0.08
Mobile PHASE (74:26)	25	2295.5 (1.5)	0.06
Mobile Phase (76:24)	25	2130.8 (14.8)	0.69
Detection wavelength (223 nm)	25	1945.2 (1.5)	0.07
Detection wavelength (226 nm)	25	1896.1 (1.4)	0.07

be 73.13 and 47.28, respectively. These values were further used for calculation of concentration of the empagliflozin in various validation and degradation studies. The recovery of empagliflozin in spiked samples for accuracy studies ranged from 100.7 to 99.4% with an RSD range of 0.8–0.2. The drug recovery from intra- and inter-day precision studies ranged from 100.3 to 100.9%. The developed method showed repeatability in a range of 100.5–100.8%. The robustness study demonstrated the drug recovery upon changing analytical parameters by showing the results within approximately 100% recovery with minimal relative standard deviation (RSD). The LOD and LOQ were found to be 0.26 µg/ml and 0.78 µg/ml, respectively.

Assay of the commercially marketed formulation

The developed method was validated and applied to determine the concentration of empagliflozin in marketed drug formulation. The mean (SD) recovery of empagliflozin was 100.44% (0.18) and the % RSD was 0.18%. These results were within the prescribed ICH limits. The chromatograms for assay determinations of the commercially marketed formulation is depicted in Supplementary Figure S6.

Forced degradation studies

The stability-indicating study was carried out on a fixed concentration of 15 mcg/ml of empagliflozin. The results are noted in Table 3. The chromatograms are illustrated in Figure 4. The drug recovery after acidic and basic degradation were around 94% up to 2 h of exposure. While maximum degradation was noted with oxidative stress (7.43%), exposure to water resulted in minimal degradation of 2.85% after 2 h. Photolytic stress saw a rise of drug concentration by 16% when exposed to the sunlight for 24 h. A liquid chromatography method demonstrated <2% degradation in the stability indicating assay.[10]

DISCUSSION

The obtained results depict the successful development and validation of a stability-indicating RP-HPLC method for empagliflozin. The wavelength maxima results corroborate previous reports, emphasizing consistency in the analytical characterization of empagliflozin. This study is the first to conduct an analytical method based on acetic acid proportion. Previously, studies have used different solvents such as acetonitrile, and o-phosphoric acid.[17,18] Furthermore, Gurrala *et al*., reported application of acetonitrile: phosphate buffer pH 5 for simultaneous analysis of three antidiabetic drugs (metformin hydrochloride/empagliflozin/linagliptin) in a fixed-dose combination.[19]

ICH guidelines were employed to validate the developed method. The method validation process, essential for analytical research, has undergone significant evolution in the past five decades. Formerly overlooked across various scientific disciplines, it has now emerged as a universally acknowledged procedure to confirm the accuracy of analytical techniques. In alignment with the principles outlined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), stability-indicating methods are crucial for evaluating the stability of drug substances. This study developed a stability-indicating RP-HPLC method for empagliflozin, assessing its suitability in monitoring the stability and purity of the compound in solid pharmaceutical formulation and in under various stress conditions.[20] Similar results for other validated methods were observed in studies using RP-HPLC

Table 3: Results of forced degradation studies

S. No.	Degradation	Area of standard	Area of degraded Sample	Degraded upto %	Actual % degradation
		After 1 h			
1	Acid degradation	1150.418	1125.1228	97.80	2.20
2	Basic degradation	1150.418	1116.41	97.04	2.96
3	H2O2 degradation	1150.418	1109.58	96.45	3.55
4	Neutral	1150.418	1125.8	97.86	2.14
		After 2 h			
1	Acid degradation	1150.418	1083.26	94.16	5.84
2	Basic degradation	1150.418	1082.64	94.11	5.89
3	H_2O_2 degradation	1150.418	1064.99	92.57	7.43
4	Neutral	1150.418	1117.64	97.15	2.85
		Photolytic degradation after 24 h			
24 h		1150.418	1331.2451	115.72	−15.72

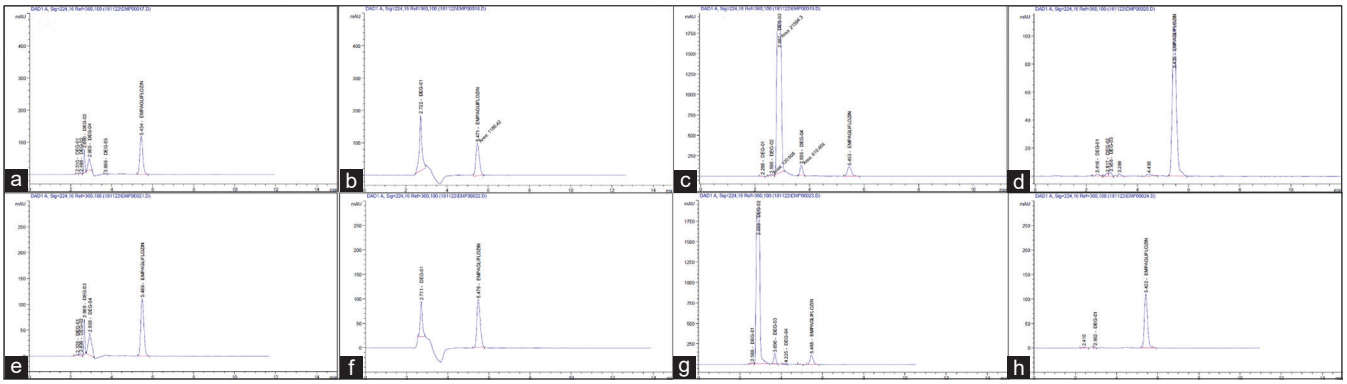

Figure 4: Chromatograms for stability-indicating forced degradation studies; (a) Acidic at 1 h, (b) Basic at 1 h, (c) Oxidative at 1 h, (d) Neutral at 1 h, (e) Acidic at 2 h, (f) Basic at 2 h, (g) Oxidative at 2 h, (h) Neutral at 2 h

on empagliflozin where all the %RSD parameters had a similar range as this study.[12,21]

Forced degradation studies are crucial stability-indicating methods, simulating various stress conditions to evaluate the drug's stability. This study systematically examines acid, basic, neutral, oxidative, and photolytic degradation, shedding light on the compound's susceptibility to different stressors. The method's selectivity for empagliflozin is a critical aspect highlighted in this study. No detectable inactive or degradative material peaks were observed, affirming the method's ability to specifically identify and quantify empagliflozin without interference from other substances. This ensures that the developed method is capable of detecting any expected change in the drug product assay during stability studies as well.

CONCLUSION

In summary, this study successfully developed and validated an analytical method for quantifying empagliflozin, using RP-HPLC. The study optimized a unique mobile phase using methanol and acetic acid. The method's validation, following ICH guidelines, confirmed its accuracy, precision, repeatability, and robustness. It exhibited a reliable linear relationship for determining empagliflozin concentrations in various studies. Our analysis of a marketed formulation also met ICH standards. Forced degradation studies indicated the method's stability-indicating nature, with minimal degradation in acidic and basic conditions. Oxidative stress and exposure to water caused relatively higher degradation, while photolytic stress unexpectedly increased the drug concentration after 24 h of sunlight exposure. In conclusion, our study provides a reliable analytical tool for empagliflozin quantification, ensuring the quality and credibility of pharmaceutical research and formulations involving this drug.

DECLARATIONS

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Conflict of interests

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability statement

Thedata is available from the corresponding author upon reasonable request.

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