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Validated Stability Indicating RP-HPLC Method for the Forced Degradation Study of Empagliflozin.

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KEYWORDS
Empagliflozin, Stability Indicating Method, Forced Degradation Method, Rp-Hplc.

ABSTRACT:
To expand an easy to use, available, reliable, and retable RP-HPLC method for determining the formulation and pure form of empagliflozin. Using an isocratic C18 reverse column (250 x 4.6 mm, particle length: 5 µm) with an ammonium acetate and acetonitrile (62:38 v/v) cell section, the method operates at a drift fee of 1ml/minute at a detection wavelength of 265 nm. Empagliflozin within the gift of deterioration merchandise as fast, economic, and dependable products. Forced degradation examine for acid, alkali, oxidation, picture stability, dry warmth, and impartial degradation. Empagliflozin and had measured retention times of 5.3 minutes each. The percentage recoveries of Empagliflozin have been 98.24% respectively. It became discovered that the assay's relative general deviation for empagliflozin was much less than 2%. The correlation coefficient for empagliflozin became determined to be 0.998. The limits of detection and quantification for empagliflozin had been 4.14 ng/ml and 12.55 ng/ml, respectively. For the motive of estimating empagliflozin, a completely unique, quick, sensitive, and solid RP HPLC method changed into evolved and validation changed into performed according with ICH guidelines.

1. Introduction

The USFDA has approved empagliflozin (EMP) to lower adult heart failure hospitalization and cardiovascular risk. It is used in addition to diet and exercise to help persons with type 2 diabetes mellitus improve their glycaemic control[1]. Excessive thirst and dry mouth, frequent and abundant urination, low energy, intense fatigue, blurred eyesight, weight loss, increased hunger, and dark patches of skin in skin folds are all signs of type 2 diabetes It is not advised to limit use in individuals with type 1 diabetes. It might put these patients at higher risk of developing diabetes. The recommended dosage and method of administration for EMP is 10 mg once day in the morning, with or without meals [2]. Resistance to the effects of insulin and an abnormality in insulin secretion are the two main characteristics of type 2 diabetes mellitus (T2DM). For those who have this kind of diabetes mellitus (DM), insulin is not necessary for survival. Hyperglycaemia refers to elevated blood glucose levels. Insulin plays a role in the blood's continued conversion of glucose to energy[3]. The inhibitors of the sodium-glucose reabsorption cotransporter 2 (SGLT2), also known as florins, function through an individual method. When a person does not have diabetes, glucose flows through the renal glomeruli filter but is completely reabsorbed in the renal tubules via SGLT2 processes.

The proximal tube contains the primary transport mechanism for glucose reabsorption, known as SGLT2. When using SGLT2 inhibitors, do not completely prevent glucose reabsorption. Eighty to ninety percent of the 160–180 mg of glucose that are filtered into the urine daily are reabsorption by the SGLT2 system. We looked into suggestions and guidelines for using SGLT2 in nations where a lot of people have type 2 diabetes. In China, SGLT2 has been suggested as a supplemental treatment for individuals whose monotherapy fails to produce glycaemic control[5].Type 2 diabetes trends at the national and worldwide levels for all age groups were compiled from 1990 to 2017. With 77 million diabetes cases worldwide, India comes in second place to China. A review of the literature found that there aren't many analytical techniques for analysing the two medications

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either by itself or in combination employing UV spectrophotometry, HPLC, and ultra pressure liquid chromatography[7]. Studies using the EMP as a stand-alone drug test (HPLC) are scarce.

The HPLC analysis was applied in a number of investigations using various medication combinations within [8]. Spectrophotometry and chromatographic techniques are among the methods available for determining EMP in pharmacological dosage forms and biological matrices, either independently or in combination. In addition, the established HPLC method offers numerous benefits over the routine HPLC methods that have been reported for EMP, including simpler mobile phases, higher resolution, fewer retention times, and improved sensitivity[9]. Numerous degrading circumstances have been employed in the previously documented techniques, demonstrating the vulnerability of EMP to oxidative media, alkaline, thermal exposure, and photolysis. It has recently been identified[30] that EMP acid degrades all through simple and impartial hydrolysis, oxidation, and publicity to excessive temperatures; its degradation beneath photolytic exposure has not been evaluated. Therefore, the cutting-edge work's objective turned into to create and compare a balance-indicating RP-HPLC method for comparing empagliflozin's pharmaceutical dose form[10].

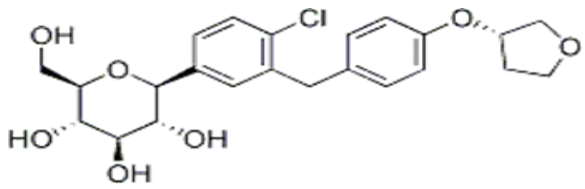

Fig I: Chemical structure of Empagliflozin

2. Materials & Methods

Materials: Chemicals and reagents

EMP standard drug was purchased from Sigma Pharmaceuticals. Jardiance tablets 10mg were purchased in local pharmacy. HPLC grade water (qualigens), Whatmann 0.2 µm PVDF syringe filter, acetonitrile HPLC grade (qualigens), and ammonium acetate HPLC grade (himedia). The chemicals and solvents that are HPLC grade were purchased from precision scientific in India.

Instruments: Statistical Equilibrium – Sartorius 220gms of weight capacity, quaternary pump-equipped Waters Alliance HPLC 2695 series, photo diode array detector, C18 reverse column 250×4.6mm particle size 5µm and autosampler integrated with empower software, Sonica ultrasonic cleaner mode l2200MH, LAB INDIA–pH meter model L1610, Micropipette[12].

Chromatographic condition for RP-HPLC: 5 mM ammonium acetate and 62:38% v/v acetonitrile, flowing at a rate of 1 ml/min, with a temperature of 25 ℃, an isocratic elution type, an injunction volume of 5µl, and a detection wavelength of 265 nm[11].

Table I Optimized chromatographic conditions

Detector	PDAdetector2998
Column	C18reversecolumn 250×4.6mmparticlesize5µm
Flowrate	1ml/min
Retention time	5.3±
Detection wavelength	265nm
Temperature	25℃
Mobile phase	5mM Ammonium acetate and Acetonitrile
Ratio	62:38%v/v
Runtime	10 min
Elution type	Isocratic

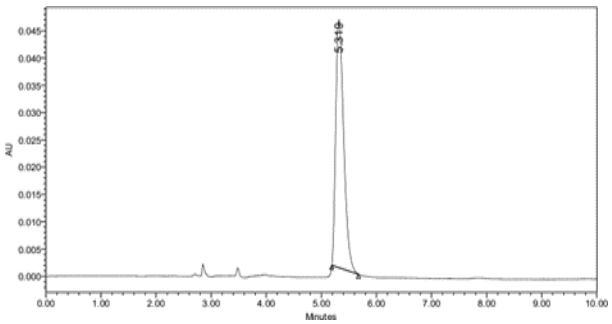

Fig II Optimized chromatogram of Empagliflozin

Standard stock solution preparation:
To make a dilution solution, 5 mg of EMP were accurately weighed, moved to a 25 ml volumetric flask, filled with diluents, and sonicated for 10 minutes.

Method validation

The following validation factors have been evaluated in accordance with the ICH guidelines: robustness, accuracy, precision, linearity, specificity, limits of detection (LOD), limits of quantification (LOQ), robustness, and system the suitability[18].

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Accuracy

To determine the accuracy of the method, three different concentrations were made at the LQC, MQC, and HQC% levels using the amount of recovery over the method's range.[14]

Precision

System precision (repeatability/intra-day precision): Chromatograms were recorded after a 10µl solution of a standard preparation was injected into the chromatographic system six times. %RSD was used to express the results of the EMP peak areas calculation[18].

Method Precision

The method Chromatograms were recorded after a 10µl sample preparation solution was added to the chromatographic system six times to ensure test technique precision. %RSD was used to express the findings of the EMP peak areas calculation[18].

Linearity

To create a solution, the ammonium acetate and acetonitrile (20:80) mobile phase was appropriately diluted with the empagliflozin stock solution. At eight different empagliflozin concentration levels, the linearity was evaluated. Area versus concentration was plotted to get the curves[18].

LOD and LOQ

By figuring out the Limit of Quantification and Limit of Detection in terms of EMP, the values decided. The LOD and LOQ have been estimated with the aid of injecting a series of diluted solution. The following components became used to get the usual deviation: LOD = 3.3(Sy/S), where (S) is standard deviation of the reaction curve's slope and (Sy) is calibration curve's slope. Additionally, LOQ = 10σ / S, where S is slope of calibration curve and σ is standard deviation of the reaction. How the low concentration was determined[15].

Robustness

With planned alteration of the chromatographic parameters, consisting of the mobile phase composition, go with the flow charge, mobile phase pH change, and column temperature, the cautioned method's robustness become evaluated in accordance with ICH Guidelines beneath robustness settings, which blanketed temperature - (20 °C) and temperature + (30 °C), samples injected in replica below several conditions: float min - (0.8 ml/min), go with the flow + (1.1 ml/min), mobile phase. The resulting chromatograms have been used to evaluate system the suitability features. %RSD become now not past the limit[18].

System suitability

It was possible to determine the system's the suitability requirements, including peak tailing, resolution, and USP plate count, by making standard EMP solutions and injecting them three times. For the region of three standard injection results, the percentage RSD should be limited to 2%. 37±0.5°C12 was maintained for mL of fresh pH 7.4 PBS. At 286 nm, the example was examined by UV spectrophotometric analysis[23].

Forced degradation studies

Stress degradation studies to be performed under more stringent condition than those suggested for accelerated experimental testing conditions, according to ICH Q1A guidelines. All of the stressed samples were twice injected into HPLC system under optimal chromatographic conditions after being suitably diluted to the necessary concentration with diluents. The chromatograms were then recorded and assessed for peak purity. The % degradation of EMP was calculated[26].

A) Acid Degradation research:

5 mL of EMP stock answer and 1 mL of 2N hydrochloric acid have been introduced together and the aggregate changed into sonicated for half-hour at 60˚C. The answer changed into diluted to gain the answer. Then 10µl of the degraded solutions were injected into HPLC[26].

B) Alkali Degradation Studies:

5mL of EMP stock answer and 1 mL of 2N sodium hydroxide have been brought collectively and the mixture turned into sonicated for 30 minutes at 60˚C. The resulting solution became diluted so as to obtain the solution. Then 10µl of the degraded answer changed into injected into HPLC[16].

C) Oxidation:

1ml of the stock EMP solution become blended with 1ml of 20% hydrogen peroxide (H2O2). The answers have been kept at 60˚C for half an hour. The very last answer

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changed into diluted to obtain the answer. Then 10µl of the degraded answer changed into injected into HPLC[16].

D) Photo Stability research:

The photochemical balance of the drug predicted as properly with the aid of subjecting EMP strategy to UV light and keeping the beaker in the UV chamber for seven days, or 200 Watt-hours/m2, in the photostability chamber. The ensuing answer changed into diluted to create solutions. Then 10µl of the degraded solutions had been injected into HPLC[17].

E) Dry Heat Degradation Studies:

The standard drugs solution of EMP has been saved in an oven set at a hundred and 5˚C for 6 hours so one can have a look at dry warmth degradation. The answer for the HPLC analysis became diluted with the resulting solution. Then 10µl of the degraded answer changed into injected into HPLC[22].

F) Neutral Degradation Studies:

Stress trying out in impartial environments has been studied by refluxing the drug in water for 6 hours at 60°C. The resulting answer turn out to be distilled into it. Next, 10 µl of the the deteriorate solution was introduced to HPLC[27].

3. Results

Method validation

Linearity

Eight linear concentrations of (4.62-49.51ng/ml), were injected. The linearity equations obtained for Empagliflozin were y = 1735.x + 13983, and the average areas have previously given. For each drug, a correlation coefficient of 0.998 was found. The linearity shown in Figure III. And linearity data are shown in table II.

Table II : Linearity for Empagliflozin

S no	Conc (ng/mL)	Peak area
1	4.62	21351
2	7.11	25049
3	10.16	31049
4	16.94	44961
5	21.72	53057
6	29.35	65307
7	38.61	81667
8	49.51	98345

Precision:

The areas obtained had been as previously defined, and six injections had been crafted from a single volumetric flask containing the running working standard solution. The average area, standard deviation, and percentage RSD were calculated for a single drug; empagliflozin yielded values of 0.5%, 0.5%, and 0.5%, respectively. This method changed into used to skip the system precision due to the fact the precision limit turned into less than "2". Table V suggests the precision information[19].

Table V. System precision of Empagliflozin

Sl.No	Concentration(mcg/ml)	Area of Empagliflozin
1.	38.61	81667
2.	38.61	82677
3.	38.61	81536
4.	38.61	81761
5.	38.61	81717
6.	38.61	81571
	Mean	81821.5
	S.D	427.689
	%RSD	0.52

Accuracy

The three levels of the Accuracy sample were prepared using the conventional addition method. For every accuracy level, three injections were given and a mean percentage of recovery of 98.24% was obtained for Empagliflozin respectively[20]. Accuracy data are shown in table VI.

Table VI. Accuracy table of Empagliflozin

QC	Extracted Analyte Area (mcg/mL)	Aqueous Analyte Area (mcg/mL)	% Recovery	Mean %Recovery
LQC	1100	1102	100.182	98.24%
	1172	1128	96.2457	
	1113	1121	100.719	
MQC	3275	3163	96.5802	
	3285	3185	96.9559	
	3267	3235	99.0205	
HQC	6991	6876	98.355	
	6987	6783	97.0803	
	6857	6795	99.0958	

Limit of Detection and Limit of Quantification

A single volumetric flask retaining the working standard solution was used for three injections, and the areas that were acquired were recorded above. The average areas were previously mentioned, and the linearity equations[29] for empagliflozin were found to be y = 1735.3.x 13983. For the single drugs, the correlation coefficient was found to be 4.143013 ng/ml.

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A single volumetric flask retaining the working standard solution was used for three injections, and the areas that were obtained were as previously mentioned. Since we just discussed the average areas, we can find that the linearity equation for empagliflozin is y = 1735.3.x 13983. For all of the drugs, the correlation coefficient was 12.55458 ng/ml. LOD and LOQ data are shown in table III.

Table III. Sensitivity table of Empagliflozin

Molecule	LOD(ng/ml)	LOQ(ng/ml)
Empagliflozin	4.143013ng/ml	12.55458ng/ml

Robustness

Robustness conditions included flow min (1 ml/min), mobile phase ratio (63B:62A), temperature (±30°C), and temperature plus (±40°C) have been maintained when samples have been injected in mirror 21. With restricted to no effect, every device suitability parameter handed. %RSD did now not exceed the most amount. Data on robustness is shown in Table IV.

Table IV. Robustness data for Empagliflozin

LOD for Empagliflozin S.no	Condition	%RSD of Empagliflozin
1	Flowrate(-)0.8ml/min	0.3
2	Flow rate (+)1.2ml/min	1.6
3	Mobile phase (-)60B:40ACN	0.5
4	Mobile phase (+)62B:38ACN	0.8
5	Temperature (-)30°C	0.3
6	Temperature(+)40°C	1.5

Degradation Studies

The formulation became used for degradation research, and the injected degraded samples had been analysed. After the injected samples' assay had been calculated, each sample passed the degradation limit[22].

It was found that the major analyte peaks in all forced degradation samples were separated from the degradant peaks, and that the peak purity of empagliflozin passed peak purity in all of the tests. The peak purity angle from the previously mentioned studies was smaller than the peak purity threshold. Degradation Studies data are shown in table VII.

Table VII. Degradation Data of Empagliflozin

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid Degradation	5.51	0.682	0.881
2	Alkali Degradation	4.37	0.643	0.848
3	Oxidation Degradation	2.77	0.649	0.858
4	Dry heat Degradation	2.63	0.491	0.678
5	Photo stability Degradation	1.48	0.534	0.706
6	Neutral Degradation	0.59	0.48	0.665

4. Discussion

To develop the method, multiple columns, buffers, pH, mobile phase ratios, etc. were changed. This study showed that an RP HPLC method for EMP in the pharmaceutical dosage form has been developed and validated. It is simple, accurate, sensitive, and stability-indicating[23]. To optimize chromatographic conditions used for EMP are given in Table I. Chromatogram shown in the Fig. II.

To determine the accuracy of the method[24], the samples' recovery was looked into. Lower standard deviations and coefficients of variation were seen in the drug assay results, indicating good procedure accuracy. The analyte's selectivity and specificity are necessary for the sample's peak purity. The current approach was suggestive for both specificity and selectivity because there was no co-elution peak observed during the drug sample's retention time[25]. All systems suitability parameter fell within the ICH guideline's allowed limit. The very low LOD and LOQ values indicate that the recommended method for the analysis of empagliflozin is more sensitive[28].

5. Conclusion:

For the objective of estimating EMP, a novel, quick, sensitive, and economically stable RP-HPLC technique has been developed, and Its validation was completed in accordance with ICH guidelines. Forced degradation studies had been accomplished in accordance with ICH guidelines, and the consequences confirmed that the method turned into appropriate for the stability of EMP under quite a few degradation situations, including acid, alkali, oxidation, dry warmth, mild balance, and impartial degradation. Finally, this method is sensitive, simple, precise, and capable of separating drugs from degradation products.

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References

1. Assessment of beneficial herb - drug interactions -A pharmacokinetic approach.
2. FDA approves treatment for wide range of patients with heart failure u.s. food and drug administration (FDA) (press release)
3. Kalra S. Sodium Glucose Co-Transporter-2 (SGLT2) Inhibitors: A Review of Their Basic and Clinical Pharmacology. Diabetes Therapy [Internet]. 2014 Nov 26;5(2):355–66. DOI: 10.1007/s13300-014-0089-4
4. Santer R. Molecular Analysis of the SGLT2 Gene in Patients with Renal Glucosuria. Journal of the American Society of Nephrology. 2003 Nov 1;14(11):2873–82. DOI: 10.1097/01.asn.0000092790.89332.d2
5. Xu D, Chandler O, Wee C, Ho C, Affandi JS, Yang D, et al. Sodium-Glucose Cotransporter-2 Inhibitor (SGLT2i) as a Primary Preventative Agent in the Healthy Individual: A Need of a Future Randomized Clinical Trial? Frontiers in Medicine. 2021 Aug 23;8. DOI: 10.3389/fmed.2021.712671
6. Khan MA, Hashim MJ, King J, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes – Global Burden of Disease and Forecasted Trends. Journal of Epidemiology and Global Health [Internet]. 2020 Mar 10;10(1):107–11. doi: 10.2991/jegh.k.191028.001
7. P. Geetha Swarupa, Prasad S, K. Suresh Babu, J. Vinoth Kumar. DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ESCITALOPRAM AND L-METHYLFOLATE IN BULK AND TABLET DOSAGE FORM. Rasayan journal of Chemistry. 2019 Jan 1;12(04):2338–47. DOI:10.31788/RJC.2019.1245438
8. Pathak S, Mishra P. Stability-indicating HPLC-DAD method for the determination of empagliflozin. Future Journal of Pharmaceutical Sciences. 2021 Aug 30;7(1). DOI: https://doi.org/10.1186/s43094-021-00329-w
9. Krishna Rao Vankalapati, Pallavi Alegete, Sathyanarayana Boodida. Stability-indicating HPLC method development and validation for simultaneous estimation of metformin, dapagliflozin, and saxagliptin in bulk drug and pharmaceutical dosage form. 2022 Apr 17;36(7). DOI: 10.1002/bmc.5384
10. R. G Chatwal, Anand K.S. High performance liquid chromatography. Instrumental methods of chemical analysis, 5th ed; Himalaya publishers: Mumbai, 2010; 2.570-2.629.
11. B. K Sharma, High performance liquid chromatography. Instrumental methods of chemical analysis, 24th ed; Goel publishers: Meerut, 2005; 295-300.
12. Dong MW. Modern HPLC for Practicing Scientists. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2006. DOI:10.1002/0471973106
13. Skoog, DM West, FJ Holler, Fundamentals of Analytical Chemistry, 7th edition, Saunders College Publishing, Philadelphia, 1992, P.1-3
14. K. A Corners. Textbook of Pharmaceutical Analysis, A Wiley- inter science Publication, 1st edition 1967, P.475-478.
15. ICH (2003) Stability testing of new drug substances and products Q1A (R2), IFPMA, Geneva, Switzerland. ICH (1996) Impurities in new drug products, IFPMA, Geneva, Switzerland
16. Kovaríková P, Klimes J, Dohnal J, Tisovská L. HPLC study of glimepiride under hydrolytic stress conditions. Journal of Pharmaceutical and Biomedical Analysis. 2004 Sep 1;36(1):205–9DOI: 10.1016/j.jpba.2004.05.005
17. Center for Biologics Evaluation and Research (US) (1996) Guidance for industry Q1B photo stability testing of new drug substances and products in U.S. Dept of Health and Human Services. Food and Drug Administration Center for Biologics Evaluation and Research, Rockville, MD, p.11-15
18. FDA (2003) Guidance for Industry, INDs for Phase II and III Studies Chemistry, Manufacturing, and Controls Information. Food and Drug Administration, p. 1-27.
19. Singh S, Bakshi M (2000) Guidance on conduct of stress tests to determine inherent stability of drugs. Pharma Tech 24: 1-14
20. Oyler AR, Segmuller BE, Sun Y, Polshyna AE, Dunphy R, Armstrong BL, et al. Forced degradation studies of rapamycin: Identification of autoxidation products. 2012 Feb1;59:194–200. DOI: 10.1016/j.jpba.2011.10.017

21. ICH Q3C (R3) (2002) Impurities: Guidelines for Residual solvents (Step 5), International Conference on Harmonization.
22. Ranjit S, Rehman Z (2012) Current trends in forced degradation study for pharmaceutical product development. Journal of pharmaceutical and educational research, p. 54-63.
23. Riddhiben MP, Piyushbhai MP, Natubhai MP (2011) Stability indicating HPLC method development-a review. Int Res J Pharm 2(5): 79-87
24. Annapurna MM, Mohapatro C, Narendra A. Stability-indicating liquid chromatographic method for the determination of Letrozole in pharmaceutical formulations. Journal of Pharmaceutical Analysis. 2012 Aug;2(4):298–305. https://doi.org/10.1016/j.jpha.2012.01.010
25. Kats M (2005) Forced degradation studies: regulatory considerations and implementation. Bio Pharm Int 18(7): 1-7.
26. ALSANTE K, ANDO A, BROWN R, ENSING J, HATAJIK T, KONG W, et al. The role of degradant profiling in active pharmaceutical ingredients and drug products. Advanced Drug Delivery Reviews. 2007 Jan 10;59(1):29 37. DOI: 10.1016/j.addr.2006.10.006
27. Gupta A, Yadav JS, Rawat S (2011) Method development and hydrolytic degradation study of Doxofylline by RP HPLC and LC–MS/ MS. Asian J Pharm Anal 1(1): 14-18
28. Allwood MC, Plane JH. The wavelength-dependent degradation of vitamin A exposed to ultraviolet radiation. International Journal of Pharmaceutics. 1986 Jul;31(1-2):1–7. https://doi.org/10.1016/0378-5173(86)90206-1
29. ICH Q3A (R2) (2006) Impurities in New Drug Substances and Products (Step 4), International Conference on Harmonization, p. 1-15
30. A.V Kasture., Wadodkar S.G., Mahadik K.R., More H.N. Textbook of Pharmaceutical Analysis – II, Published by Nirali Prakashan, 13th edition, 2005