doi.org/10.36721/PJPS.2021.34.3.SUP.1081-1087.1

Empagliflozin: HPLC based analytical method development and application to pharmaceutical raw material and dosage form

Anas M. Hanif[1], Rabia Bushra[2]*, Nahlah Elkudssiah Ismail[3], Rahila Bano[2], Saima Abedin[1], Shazia Alam[4], Maqsood Ahmed Khan[4] and Hafiz Muhammad Arif[5]

[1]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
[2]Department of Pharmaceutics, Faculty of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[3]Clinical Pharmaceutics Research Unit, Faculty of Pharmacy, MAHSA University, Selangor, Malaysia
[4]Department of Pharmaceutics, Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan
[5]Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

Abstract: The current investigation is based on efficient method development for the quantification of empagliflozin in raw and pharmaceutical dosage forms, as no pharmacopoeial method for the drug is available so far. The developed analytical method was validated as per ICH guidelines. C18 column with mobile phase (pH 4.8) consisted of 0.1% trifluoroacetic acid solution and acetonitrile (70:30 v/v) was used for drug analysis. The calibration plot showed good linear regression (r^2>0.999) over the concentration of 0.025-30 µg mL^{-1}. The LOD and LOQ were found to be 0.020 µg mL^{-1} and 0.061 µg mL^{-1}, respectively. The percentage recovery was estimated between 98.0 to 100.13%. Accuracy and precision data were found to be less than 2%, indicating the suitability of method for routine analysis in pharmaceutical industries. Moreover, the drug solution was found to be stable in refrigerator and ambient room temperature with mean % accuracy of >98%. Empagliflozin contents were also tested in both the raw API and marketed tablet brands using this newly developed method. The mean assay of raw empagliflozin and tablet brands were ranged from 99.29%±1.12 to 100.95%±1.69 and 97.18%±1.59 to 98.92%±1.00 respectively. Based on these findings, the present investigated approach is suitable for quantification of empagliflozin in raw and pharmaceutical dosage forms.

Keywords: Bulk dosage form, empagliflozin, HPLC, method development, validation.

INTRODUCTION

Method development and its validation is a ground and mandatory step in pharmaceutical analysis to assure the results of the developed method shall be reliable, accurate, suitable and rugged for routine application. In this instance, various analytical parameters are assessed on routine basis to judge the quality of the dosage form. In the light of current scenario, drug regulatory governing bodies play significant role and also have laid emphasizes on drug manufacturing companies to provide complete information regarding validation protocol of analytical methods. The prevalence of the type II diabetes mellitus has been widespread thus becoming the 7th leading cause of death globally (Nowakowska *et al.,* 2019). Anti-diabetic medications are preferentially indicated to alleviate the condition of hyperglycaemia (Chaudhury *et al.,* 2017). Several classic hypoglycaemic agents such as metformin, meglitinides, thiazolidinediones and sulfonylurea have demonstrated widespread and well-established therapeutic profile in the management of type 2 diabetes mellitus (Levine 2017; Hedrington and Davis 2015). Empagliflozin is chemically nominated as (1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofu-ran-3-yl-oxy) benzyl]-benzene (fig. 1). Physically yellowish to whitish crystalline solid with non-hygroscopic property. It is the latest class of selective and competitive inhibitor of sodium glucose transporter (SGLT2), displaying the prominent glucosuric effects (Gangadharan Komala and Mather, 2014; Anderson *et al.*, 2017; Acharya and Deedwania, 2019; Nair and Wilding, 2010).

Previous studies demonstrated few scientific methods for the quantification of empagliflozin in pharmaceutical preparations. Jyothirmai *et al.* (2016) developed economical UV-visible spectrophotometric methods for the analysis of empagliflozin in pharmaceutical formulations. Ayoub (2016) developed and validated spectrophotometric and chemometric based analytical technique for determination of empagliflozin and metformin simultaneously. High performance liquid chromatography (HPLC) is considered to be an efficient way to separate, identify, quantify and purify the various analytes in any given mixture. Therefore, nowadays HPLC-UV technique is documented to be one of the reliable and precise analytical procedures utilized to estimate different pharmaceutical active principles in one go (Kumar and Kumar, 2012). Padmaja and co-workers (2018) had reported the reverse-phase (RP) HPLC method for the quantification of empagliflozin in human plasma. Groups of researchers presented the validated stability indicating method employing RP-HPLC based technique for empagliflozin (Godasu and Sreenivas, 2017; Mounika *et al.,* 2019; Padmaja and Veerabhadram, 2016; Shyamala

*Corresponding author: e-mail: rabia_pharmacist@hotmail.com

et al., 2016; Susmita *et al.,* 2019; Swarupa *et al.,* 2016; Vinay Kumar and Seshagiri Rao, 2018). Nevertheless, the fact that up till now references concerning the analytical method development and validation of empagliflozin by trifluoroacetic acid as the mobile phase using normal phase HPLC has not been tackled yet. In this respect, the current manuscript defined the key steps based on analytical life cycle of method from the early development phase till its validation. The proposed study recapitulated to develop a simple, fast, reliable, sensitive, accurate and robust method to quantify empagliflozin in raw and pharmaceutical dosage form along with validation protocol executed by International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (ICH, 2005).

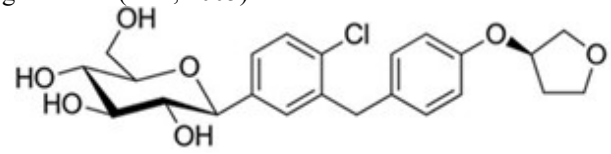

Fig. 1: Structural formula of empagliflozin

MATERIAL AND METHODS

Pharmaceutically active ingredient (empagliflozin) was kindly obtained as a gift from Sami Pharmaceutical (Pvt) Ltd. Karachi Acetonitrile, methanol and trifluoroacetic acid (HPLC grade) was purchased from Sigma-Aldrich, (USA). Chemicals and solvents, (analytical grade) were purchased from Merck (Germany). Milli-Q, Millipore system was used for purification of water (Poland). All solvents and solutions were filtered through a millipore 0.45 µm membrane filter assembly and degassed ultrasonically before use.

HPLC assay

Chromatographic conditions

The chromatography pump (model LC-20ATVP, Shimadzu, Japan) was equipped with a UV-detector (model SPD-M20A), and rheodyne (7725i injector) having 20 µL loop volume was connected to a microcomputer system. The analysis was carried out using Thermo Hypersil GOLD C_{18} column (250 × 4.6 mm, 5 μm pore size). LC solution software CBM-2 was used for chromatogram generation and data analysis. The HPLC analysis was conducted at room temperature (25±1ºC) using isocratic condition. The mobile phase (pH 4.8) consisted of 0.1% solution of trifluoroacetic acid and acetonitrile (70:30 v/v). The volume of injection was 20 µL with solvent flow of 0.8 mL min^{-1}. Mobile phase, stock and working solutions were sonicated for 30 min prior to use. The sample (empagliflozin) detection was carried out at 224 nm.

Preparation of stock solutions

Stock solution of empagliflozin was prepared by dissolving the accurately weighed amount in 0.05M potassium dihydrogen phosphate (co-solvent); volume was made up with methanol to obtain the final concentration of 0.2 mg mL^{-1}. This stock was prepared by series of dilution with mobile phase to obtain 0.01-30 µg mL^{-1} for calibration curve and linearity.

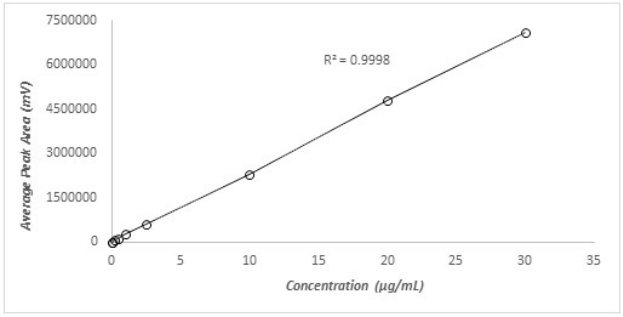

Fig. 2: Linearity of empagliflozin

Preparation of working solution

The assay method was performed on two commercial brands purchased from local pharmacies. All specifications such as batch number, manufacturing date and shelf life were noted. Average of twenty tablets were weighed and finely crushed. The equivalent content of each tablet (10 mg) was transferred into 50 mL volumetric flask and make up to the volume with mobile phase. From the prepared solution, 5 mL was added in 100 mL volumetric flask to obtain the final concentration of 0.01 mg mL^{-1}.

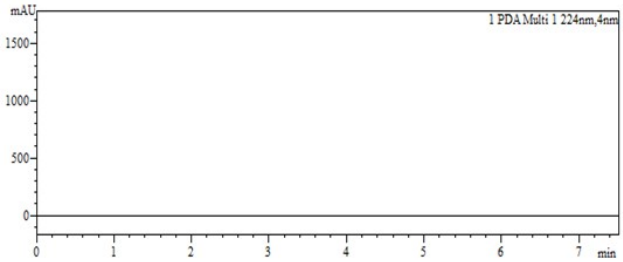

Fig. 3: Chromatogram of blank run (mobile phase)

Method validation

System suitability

The validation of the HPLC method was conducted as per ICH (2005) and Food and Drug Administration (FDA) (2001) guidelines. System suitability and specificity of standard solutions of empagliflozin at 20 µg mL^{-1} was determined. The acceptance criterion according to United States Pharmacopoeia (USP) (2014) of percent relative standard deviation (% RSD) for peak area and tailing factor was <2%, theoretical plates >2000 and retention time was greater than 2.0.

Linearity

Nine standard solutions from 0.025 to 30 µg mL^{-1} strength (0.025, 0.05, 0.25, 0.5, 1, 2.5, 10, 20 and 30 µg mL^{-1}) were analyzed for linearity. The calibration curve was constructed by plotting the peak area against concentration in µg mL^{-1}.

Table 1: System suitability parameters (n = 6)

Parameter	Findings	USP Limits
Peak Area (mean)	4801127	-
% RSD	0.004	<2.0
Retention time (min)	5.473	>2.0
% RSD	0.378	<2.0
Theoretical Plates	2321	>2000
USP tailing factor	1.13	<2.0
Resolution	N/A	

Table 2: Reproducibility (Inter and intraday precision and accuracy, n = 6)

Reproducibility	Added Concentration (µg mL^{-1})	Concentration found (µg mL^{-1}) (Mean ± SD)	Percent Mean Accuracy (%)	%CV for precision (<2%)
Precision Interday	0.05	0.049 ± 0.001	98.00	2.04
	15	14.94 ± 0.110	99.33	0.73
	30	29.98 ± 0.380	99.93	1.27
Precision Intraday	0.05	0.051 ± 0.001	100.04	1.96
	15	15.02 ± 0.043	100.13	0.28
	30	30.01 ± 0.390	100.03	1.29

Table 3: Robustness of an analytical method (n = 6)

Changes in chromatographic conditions using 5.0 µg mL^{-1}		Concentration obtained (µg mL^{-1}) (Mean ± SD)	CV (%)	Mean Accuracy (%)
Flow rate (mL min^{-1})	0.6	4.92 ± 0.06	1.24	98.4
	0.8	4.98 ± 0.08	1.26	99.6
	1.0	4.98 ± 0.14	2.78	99.6
Column Temperature (ºC)	33	5.05 ± 0.12	2.38	101.0
	30	4.98 ± 0.07	1.31	99.6
	27	4.99 ± 0.06	1.15	99.8
Detection wavelength (nm)	226	4.97 ± 0.09	1.82	99.4
	224	5.01 ± 0.12	2.45	100.2
	222	4.95 ± 0.06	1.34	99.0

Table 4: Short term stability study of empagliflozin

Concentration (µg mL^{-1})	Detected Mean Concentration (µg mL^{-1}) 0 hour (Mean ± SD) (n = 3)	% Mean Accuracy	Detected Mean Concentration (µg mL^{-1}) 24 hour (Mean ± SD) (n = 3)	% Mean Accuracy
Ambient Room temperature (25ºC)				
0.05	0.051 ± 0.23	100.02	0.0507 ± 0.003	100.00
15	15.003 ± 0.11	100.02	14.99 ± 0.11	99.93
30	30.01 ± 0.11	100.03	29.96 ± 0.35	99.86
Refrigerator (4ºC)				
0.05	0.049 ± 0.21	98.00	0.049 ± 0.004	98.00
15	15.02 ± 0.11	100.01	14.995 ± 0.18	99.96
30	30.021 ± 0.36	100.06	29.98 ± 0.12	99.93

Table 5: Pharmaceutical assay of empagliflozin in raw and brand forms

Type of Material	Samples	Mean % Assay (n = 3)	Standard Deviation
Raw empagliflozin	Supplier A	99.29	1.12
	Supplier B	99.87	0.56
	Supplier C	100.95	1.69
Commercial empagliflozin tablets (10 mg)	Brand X	98.44	0.72
	Brand Y	98.92	1.00
	Brand Z	97.18	1.59

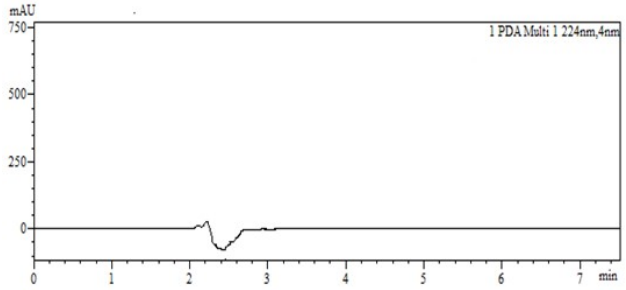

Fig. 4: Chromatogram of placebo sample

Specificity

It is analyzed by observing any interfering peak near the API by eluting the drug solution along with the blank (mobile phase) and the placebo mixture. Placebo mixture was prepared by adding the common tablet formulation ingredients including talc, magnesium stearate, microcrystalline cellulose, starch and lactose in concentration of 1 mg mL^{-1}.

Precision and Accuracy

The accuracy and precision of the method was established by performing recovery run tests using three different concentration levels with six replicate analysis (n = 6). Results of accuracy and precision were calculated and expressed as % accuracy and % coefficient variation of analytes respectively. Method is said to be precise and accurate when the percent coefficient of variation (CV) and % mean accuracy are found to be <2% and 90.00-110.00% respectively.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ values were calculated from calibration plot using the SD (σ) and slope (S). The expressions are given below;

$LOD = 3.3\ \sigma/S$ (Equation 1)

$LOQ = 10\ \sigma/S$ (Equation 2)

Robustness

It was determined by making change in temperature of column (30ºC ± 3ºC), rate of flow (± 0.2 mL min^{-1}) and λ (± 2 nm).

Stability studies

Short term stability study was performed on standard and working drug solution using three different concentration ranges (0.05, 15 and 30 µg mL^{-1}), exposed at ambient temperature (25°C) and refrigeration (4°C) for one day.

Application of the Analytical Technique

The currently developed method was applied to test the contents of the empagliflozin in the bulk procured from three different vendors (A, B and C) and commercially available three tablet brands (X, Y and Z) (10 mg). Tablets were crushed and contents were dissolved in mobile phase and analyzed after appropriate dilutions.

Data Processing

Mean, standard deviations, percent relative standard deviations (%RSD), coefficient of variation (CV), coefficient of regression, one way analysis of variation (ANOVA) with 95% confidence interval were computed where found necessary using SPSS (version 21) software.

RESULTS

Method was validated for each and every parameter as recommended by ICH guidelines of method development and validation. The data of various validation parameters are summarised in table 1-3. The linearity of the underlying analytical procedure is provided in fig. 2 keeping the drug concentration and mean peak area as X and Y variables. The coefficient of regression for linearity was found to be 0.999 with intercept and standard error values of 19085.15 and 28350.16 respectively. The ANOVA (95% CI) was applied to the calibration data, indicating the gradual decrease of peak area with respect to the drug concentration from 0.025 to 30 µg mL^{-1} ($p=0.519$). Figs. 3, 4 and 5 illustrate the chromatogram of blank, placebo and drug samples, reflecting the drug specificity during analysis. Results show that the proposed method is accurate, précised and robust as no variation was observed upon minor changes in the proposed protocol of the procedure. Moreover, drug was found to be stable in solvent at ambient and refrigerated conditions, details are given in table 4. Pharmaceutical assay of empagliflozin in three samples of raw material and commercial tablet brands is given in table 5.

DISCUSSION

Method validation

Due to recent advancement in analytical technologies, the primary need is to develop an effective, reliable and selective quantification method for active pharmaceutical ingredients. This reliability and accuracy in analytical techniques is extremely desirable as it affects the quality characterization of many pharmaceuticals. Various analytical methods have been used for drug estimation however, HPLC is still popular and being utilized successfully for the quantification of many chemicals by researchers (Kapupara *et al.,* 2018; Koppala *et al.,* 2017). Therefore, in the present study, a simple, accurate and sensitive HPLC method has been developed and validated as per ICH recommendations for empagliflozin in the bulk powder.

At present, no pharmacopoeial method is available for the determination of empagliflozin while numerous brands of empagliflozin are available in the commercial market, manufactured by national/local pharmaceutical

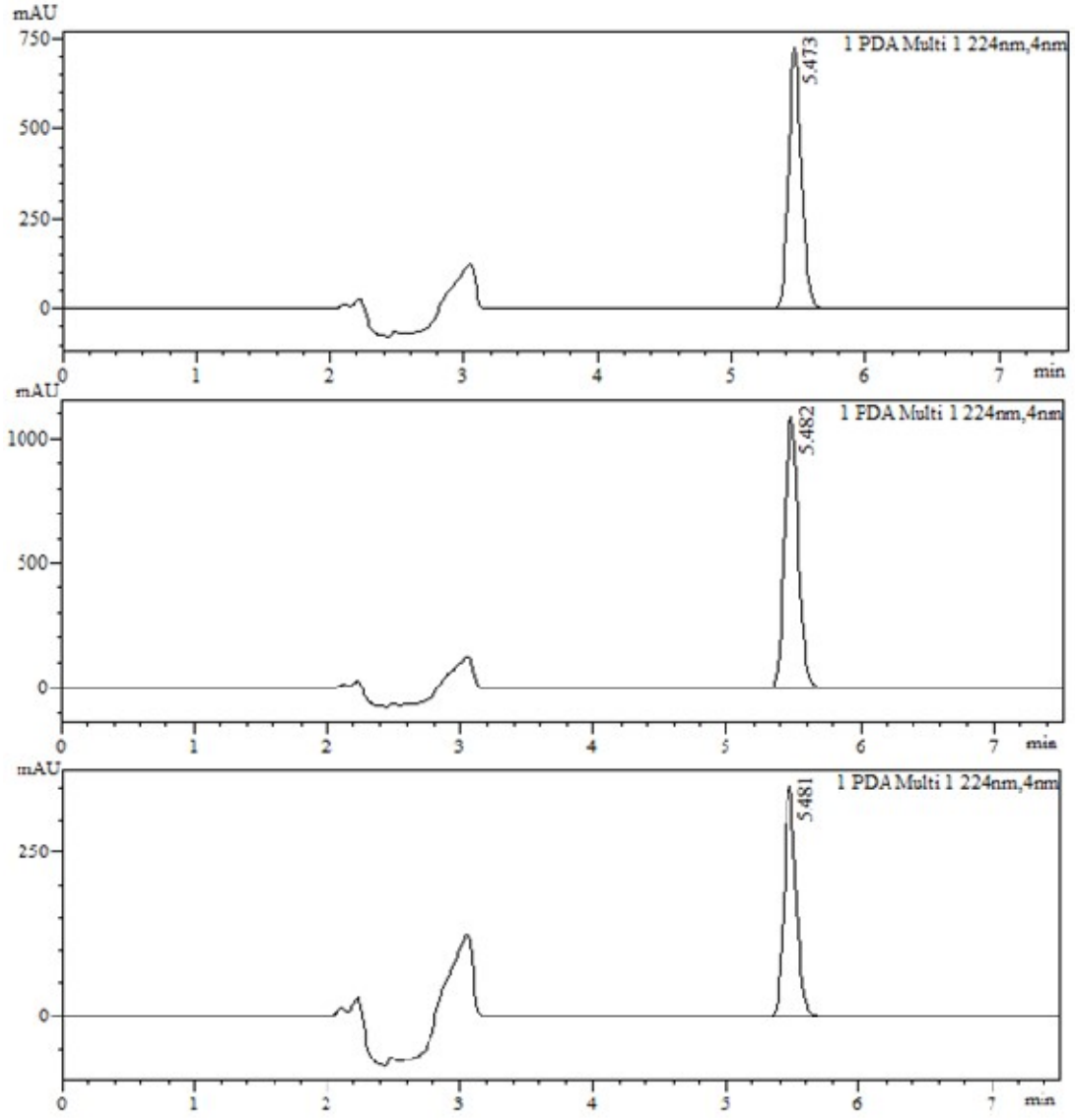

Fig. 5: Scans of empagliflozin

companies. The same developed method was also applied to the pharmaceutical assay of commercially available empagliflozin tablets (10 mg). After several trials during method development, the composition (70:30 v/v) ratio of trifluoroacetic acid with acetonitrile was found to be suitable for mobile phase. Empagliflozin has estimated previously using orthophosphoric acid in combination with acetonitrile (Susmita *et al*., 2019; Jaiswal *et al.,* 2017; Ali and Kumar, 2017), acetonitrile with water (Manoel *et al.,* 2020) and methanol with phosphoric buffer of pH 3 (Godasu and Sreenivas, 2017). The chromatographic separation was achieved using C18 column giving sharp, symmetric peak with retention time of 5.470 ± 0.378 minutes. The UV detection of eluent was recorded at λ 224 nm. Further to monitor the system suitability performance the number of theoretical plates and tailing factor were observed and temperature was maintained around 30ºC. The obtained results for system suitability were in close agreement with the previous studies conducted for various medicinal agents (Gedawy *et al*., 2020; Dayyih *et al*., 2021). Calibration data of the empagliflozin showed good linearity within concentration range of 0.025 µg mL^{-1} to 30 µg mL^{-1}. Moreover upon statistical analysis, insignificant variation was observed among the peak area, slope, and intercept to the corresponding drug concentrations. Alquadeib in 2019 had proposed a new analytical method for the estimation of the diclofenac sodium in a pharmaceutical dosage form. Author was further declared the reproducibility of the procedure by comparing and fitting the ANOVA to the regression plots. Probability value showed the insignificant differences in the slopes and intercepts among the calibration curves (Alquadeib, 2019). The regression equation of the plot y =236893x + 6136.1 with

goodness of fit value of 0.9998 was achieved, demonstrating strong correlation. The values of limits of detection and quantification (LOD and LOQ) were calculated through analytical curves. The minimum detection and quantification limits were found to be 0.020 μg mL^{-1} and 0.061 μg mL^{-1}, respectively. Previously Shyamala and co-workers reported HPLC technique to estimate the empagliflozin in raw and dosage form using 0.1% orthophosphoric acid and acetonitrile. The computed values of LOD and LOQ were 0.068 μg mL^{-1} and 0.207 μg mL^{-1} correspondingly (Shyamala *et al.,* 2016). In our study, the lower values of detection were observed due to the appropriate selection and composition of mobile phase.

The recovery of the samples was investigated to evaluate the accuracy of the method. The drug assay results showed lower standard deviations and coefficient of variations thus indicating high procedure accuracy. Selectivity and specificity of an analyte is required for peak purity of sample. Drug solution was also run along with the placebo mixture containing common excipients that are documented to be utilized for the tablet formulations. The current method was suggestive for selectivity and specificity as no co-elution peak noticed during the retention time of drug sample. The developed method was successfully applied to quantify the drug in the marketed tablets, as no peak of any additive was observed during analytical run of empagliflozin. A study was recently conducted by Sharmin and team to optimize the analytical procedure for the quantitative estimation of aceclofenac in matrix tablets. The developed method was tested for the drug content in presence of various matrix components. Specificity was established further by peak responses and peak purity of the standard, sample, excipient and mobile phase (blank) solutions in assay concentration (Sharmin *et al*., 2020). The precision studies confirmed the nearness of agreement between successive measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The results indicated that the current method was highly accurate (101%) and precise (% relative standard deviation or coefficient of variation < 2%) for the determination of empagliflozin with better system suitability and specificity (table 1). The underlying method was found to be robust as no change in concentration was observed upon variation of flow rate, column temperature and wavelength. Past investigations also established the robustness of analytical techniques, usually by varying the composition of solvent, wavelength, flow rate and column temperature to a limited extent (Moid *et al*., 2018). A HPLC method was developed by Naseef and team (2018) to assess the content of antidiabetic agent (alogliptin benzoate) in mobile phase. Author validated the method according to the official guidelines of ICH and FDA, however, stability of drug was analyzed in refrigerator and ambient room temperature for 24 hours (Naseef *et al.,* 2018). Based on the outcomes of the present study, empagliflozin samples were found stable both in refrigerator (4°C) and also at ambient room temperature (25°C) for 0-24 hours (table 4).

CONCLUSION

The proposed developed analytical method has been found to be accurate, reliable and cost-effective for the determination of empagliflozin in raw and the pharmaceutical dosage form. It is robust and specific therefore could be successfully utilized by regulatory bodies, pharmaceutical industries and research laboratories as no any official or pharmacopoeial method is available. This newly developed method further opens future prospects for bioanalytical method development.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Atif Iqbal of High-Q Pharmaceuticals for kind and valuable support in this study.

REFERENCES

Acharya T and Deedwania P (2019). Cardiovascular outcome trials of the newer anti-diabetic medications. *Prog. Cardiovasc. Dis*., **62**(4): 342-348.

Ali SI and Kumar P (2017). Stability indicating simultaneous estimation of metformin and empagliflozin in pharmaceutical tablet dosage form by RP-HPLC. *Asian J. of Res. in Chem.,* **10**(6): 783-788.

Alquadeib BT (2019). Development and validation of a new HPLC analytical method for the determination of diclofenac in tablets. *Saudi Pharm. J*., **27**(1): 66-70.

Anderson JE, Wright EE Jr and Shaefer CF Jr (2017). Empagliflozin: Role in treatment options for patients with type 2 diabetes mellitus. *Diabetes Ther*., **8**(1): 33-53.

Ayoub BM (2016). Development and validation of simple spectrophotometric and chemometric methods for simultaneous determination of empagliflozin and metformin: Applied to recently approved pharmaceutical formulation. *Spectrochim Acta A Mol Biomol Spectrosc.,* **168**: 118-122.

Chaudhury A, Duvoor C, Dendi VSR, Kraleti S, Chada A, Ravilla R, Marco A, Shekhawat NS, Montales MT, Kuriakose K, Sasapu A, Beebe A, Patil N, Musham CK, Lohani GP and Mirza W (2017). Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Front Endocrinol. (Lausanne),* **8** (6): 1-12.

Dayyih WA, Al Ani L, Al-Shdefat RI, Zakareia Z, Hamid SA and Shakya AK (2021). Development and validation of a stability-indicating HPLC method for empagliflozin and linagliptin in tablet dosage form. *Asian J. Chem.,* **33**(2): 484-488.

Food and Drug Administration (FDA) (2001). 66 FR 28526 - Guidance for Industry on Bioanalytical Method Validation; Availability. Federal Register 66 (100): 28526-28527**.**

Gangadharan Komala M and Mather A (2014). Empagliflozin for the treatment of Type 2 diabetes. *Expert Rev. Clin. Pharmacol*., **7**(3): 271-279.

Gedawy A, Al-Salami H and Dass CR (2020). Advanced and multifaceted stability profiling of the first-line antidiabetic drugs metformin, gliclazide and glipizide under various controlled stress conditions. *Saudi Pharm J*., **28**(3): 362-368.

Godasu SK and Sreenivas SA (2017). A new validated RP-HPLC method for the determination of metformin HCl and empagliflozin in its bulk and pharmaceutical dosage forms. *Int. J. Pharm. Sci. Res.*, **8**(5): 2223-2232.

Hedrington MS and Davis SN (2015). The role of empagliflozin in the management of type 2 diabetes by patient profile. *Ther. Clin. Risk Manage.,* **11**: 739-749.

International Conference on Harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use (2005). Validation of analytical procedures: Text and Methodology. ICH Q2 (R1) Harmonised Tripartite Guideline, pp.15-64.

Jaiswal SH, Katariya MV, Katariya VR, Karva GS and Koshe K (2017). Validated stability indicating HPLC method for determination of process related impurities in empagliflozin drug substances. *World J. Pharm. Res*., **6**(7): 1025-1037.

Jyothirmai N, Anil Kumar M and Nagaraju B (2016). Novel UV and visible spectrophotometric methods for the analysis of empagliflozin a type 2 diabetic drug in bulk and pharmaceutical formulations. *Journal de Afrikana*, **3**(1): 177-187.

Kapupara PP, Sorathiya V, Patel RP and Shah KV (2018). Development and validation of HPLC analytical method for chlorantraniliprole in bulk and in the formulation. *Folia Med.* (Plovdiv)., **60**(1): 133-140.

Koppala S, Ranga Reddy V and Anireddy JS (2017). User-friendly HPLC method development and validation for determination of enalapril maleate and its impurities in enalapril tablets. *J. Chromatogr. Sci*., **55**(10): 979-988.

Kumar SD and Kumar HDR (2012). Importance of RP-HPLC in analytical method development: A review. *Int. J. Pharm. Sci. Res*., **3**(12): 4626-4633.

Levine MJ (2017). Empagliflozin for type 2 diabetes mellitus: An overview of Phase 3 clinical trials. *Curr. Diabetes Rev*., **13**(4): 405-423.

Manoel JW, Primieri GB, Bueno LM, Wingert NR, Volpato NM, Garcia CV, Schapoval EES and Steppe M (2020). The application of quality by design in the development of the liquid chromatography method to determine empagliflozin in the presence of its organic impurities. *RSC Adv.,* 1**0**: 7313-7320.

Moid M, Afzal S, Rahim N, Ali T, Iffat W, Bashir L and Naz S (2018). High performance liquid chromatographic method validation for determination of rosuvastatin calcium in tablet dosage forms. *Pak. J. Pharm. Sci*., **31**(4): 1577-1582.

Mounika PS, Hemant TK, Srinivasa YR and Vara Prasad KR (2019). RP-HPLC method for quantification of empagliflozin in pharmaceutical formulation. *Asian J. Pharm. Tech.,* **9**(3): 208-211.

Nair S and Wilding JP (2010). Sodium glucose cotransporter 2 inhibitors as a new treatment for diabetes mellitus. *J. Clin. Endocrinol. Metab.,* **95**(1): 34-42.

Naseef H, Moqadi R and Qurt M (2018). Development and validation of an HPLC Method for determination of antidiabetic drug alogliptin benzoate in bulk and tablets. *J. Anal. Methods Chem.,* 1902510: 1-7.

Nowakowska M, Zghebi SS and Ashcroft DM (2019). The comorbidity burden of type 2 diabetes mellitus: Patterns, clusters and predictions from a large English primary care cohort. *BMC Med.,***17**(145): 1-10.

Padmaja N and Veerabhadram G (2016). Development and validation of a novel stability-indicating RP-HPLC method for the determination of empagliflozin in bulk and pharmaceutical dosage form. *Int. J. Pharm. Sci. Res*., **7**(11): 4523-4530.

Padmaja N, Desalegn T, Sharathbabu M and Veerabhadram G (2018). New validated RP-HPLC method for the estimation of empagliflozin in human plasma. *Int. J. Pharm. Sci. Res.,* **9**(11): 4885-4889.

Sharmin S, Sohrab MH, Moni F, Afroz F, Rony SR and Akhter S (2020). Simple RP-HPLC method for aceclofenac quantitative analysis in pharmaceutical tablets. *Pharmacia.,* **67**(4): 383-391.

Shyamala NK, Mounika J and Nandini B (2016). Validated stability-indicating RP-HPLC method for determination of empagliflozin. *Der. Pharm. Lett.,* **8**(2): 457-464.

Susmita AG, Rajitha G, Ramya Yadav Y and Uma P (2019). Analytical method development and validation of new stability-indicating reverse-phase high-performance liquid chromatography method for simultaneous estimation of metformin hydrochloride and empagliflozin in tablet dosage form. *Asian J. of Pharm and Clinical Res.,* **12**(1): 241-244.

Swarupa PG, Rao KL, Prasad KRS and Babu KS (2016). Development and validation of stability indicating reversed phase high pressure liquid chromatography method for simultaneous estimation of metformin and empagliflozin in bulk and tablet dosage form. *Asian J Pharm Clin Res.*, **9**(7): 126-135.

United States Pharmacopeial (USP) Convention (2014).

Vinay Kumar D and Seshagiri Rao JVLN (2018). A new validated stability indicating RP-HPLC method for simultaneous estimation of metformin hydrochloride and empagliflozin in tablet dosage forms. *Inter. Res. J. of Pharm and Med Sci*., **1**(5): 16-22.