



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article

December 2017 Vol.:11, Issue:1


© All rights are reserved by Ahmed M Abdelzaher et al.

Stability-Indicating Bivariate and Multi-Variate Methods for Determination of Empagliflozin in Pure Form and Pharmaceutical Preparation



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203



Fathy M. Salama, Khalid A. M. Attia, Ahmed A. Abouserie, Ragab A. Mabrouk, Ahmed M Abdelzaher*

*Pharmaceutical Analytical Chemistry Department,
Faculty of Pharmacy, Al-Azhar University, 11751 Nasr
City, Cairo, Egypt.*

Submission: 28 November 2017
Accepted: 5 December 2017
Published: 30 December 2017

Keywords: Empagliflozin, Bivariate method, PCR, PLS and CLS.

ABSTRACT

Three rapid, simple, accurate and precise spectrophotometric methods were used for the determination of Empagliflozin in the presence of its oxidative degradation product. The methods under study are Bivariate method, PCR, PLS and CLS. All the methods were validated according to the ICH guidelines and the obtained accuracy, precision and repeatability were found to be within the acceptable limits. The selectivity of the proposed methods was tested using laboratory prepared mixtures and assessed by applying the standard addition technique. Hence, the proposed method can be used for the routine analysis of Empagliflozin in quality-control laboratories.



HUMAN JOURNALS

www.ijppr.humanjournals.com

INTRODUCTION:

Empagliflozin (IUPAC name: 1,5-anhydro-1-(4-chloro-3-(tetrahydrofuran-3-yloxy)D-Glucitol) (Fig 1) is an anti-diabetic agent, a sodium-glucose cotransporter inhibitor, Blocking SGLT-2 reduces blood glucose by blocking glucose reabsorption in the kidney and thereby excreting glucose via the urine act as an anti-diabetic agent for treatment of type-2 diabetes [1]. Few methods have been reported for the estimation of empagliflozin either alone or in other combinations. These methods include spectrophotometry [2-4], UPLC with UV detection [5, 6] and liquid chromatography/mass spectroscopy [7].

Spectrophotometry is a well-established platform for pharmaceutical analysis with many advantages including time saving, cost-effectiveness and the ability for resolving overlap of binary and multi-components without pretreatment of the sample [8-12]. In the present work, different methods manipulating ratio spectra methods namely bivariate [13-14], Chemometric PCR, PLS and CLS [15-16] were applied for the determination of empagliflozin in the presence of its oxidative degradation product.

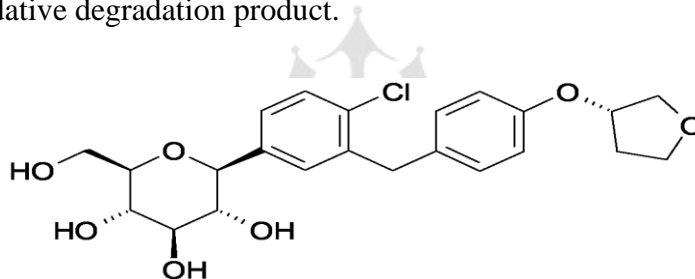


Fig. 1 Chemical structure of empagliflozin

MATERIALS AND METHODS:

Materials and reagents:

- Empagliflozin (certified to contain 99.25%) was kindly supplied by Al Andalous for Pharmaceutical Industries, Obour city, Egypt.
- Pharmaceutical Preparation: Jardiance 10 mg tablets batch no. 051, manufactured by Boehringer Ingelheim Pharmaceutical Company
- Methanol; Analytical grade, El-NASR Pharmaceutical Chemicals Co., Egypt.
- Hydrogen peroxide 10 %, 20 % & 30 % aqueous solution.

Instrument:

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1800 connected to a compatible IBM and an HP1020 laser jet printer. The spectral band was 2 nm and scanning speed is 2800 nm/min with 0.5 nm interval.

Software:

The bundled software, UV-Probe personal spectroscopy software version 2.43 (SHIMADZU) was used. PCR, PLS and CLS were done with our own written code in Matlab 8.2.0.701 (R2013b). The *t*-test and *F*-test were performed using Microsoft Excel. One way ANOVA test was performed using Graph Pad Prism version 5 (Graph Pad, San Diego, CA).

Standard solutions:

Preparation of Empagliflozin standard solution:

Empagliflozin standard solutions: 100 µg /ml in methanol.

Preparation of the degradation product:

Ten mg of pure Empagliflozin powder was transferred to a 250-mL conical flask to which 10 mL of 30 % H₂O₂ was added. The solution was heated under reflux for 5 hours. After cooling, the solution was evaporated to dryness at 120 rpm and 90⁰C to remove the remaining H₂O₂, the dried residue was extracted with methanol, filtered into 100-mL volumetric flask then the volume was adjusted to the mark by the same solvent to obtain a stock solution labeled to contain degradate derived from 100 µg/mL of empagliflozin.

Procedures:

Construction of the calibration curve (General procedure):

a. Bivariate method:

Aliquots of Empagliflozin standard working solution and its degradate equivalent to (2-16) µg were transferred separately into two sets of 10 ml volumetric flasks, then diluted to volume with methanol. Calibration curves at different wavelengths 210, 215, 220, 225, 230, 235, and 240 nm were constructed and the regression equation at each wavelength was calculated. From both sets of regression equations, the sensitivity matrices K was calculated

[13-14]; table (1) from which the optimum pair of wavelengths to carry out the determination (210 and 230 nm) were investigated. Calibration curves were constructed by plotting the absorbance versus the corresponding drug or degradate concentration in $\mu\text{g ml}^{-1}$.

b. Chemometric PCR, PLS and CLS methods.

Aliquots of Empagliflozin standard working solution and its degradate equivalent to 10 000 μg were transferred separately into two sets of 100 ml volumetric flasks, then diluted to volume with methanol. Multilevel multifactor design was used for the construction of the calibration and validation sets [15]. A five-level, five-factor calibration design was used. The concentrations details are given in table (2).

Chemometric PCR, PLS and CLS methods: Building the calibration models: A calibration set of seventeen different laboratory-prepared mixtures of Empagliflozin standard working solution and its oxidative degradate was prepared by transferring different aliquots from the working standard solution of Empagliflozin (100 $\mu\text{g /mL}$) and the working solutions of oxidative degradate (100 $\mu\text{g/mL}$) into 10-mL volumetric flasks and the volumes were completed with methanol. The absorption spectra of the prepared mixtures were recorded in the range of 220–330 nm against methanol as blank. The recorded spectra were then transferred to Matlab® version R2013b (8.2.0.701), together with PLS-Toolbox 2.1 for subsequent data analysis for calculating the calibration models (PCR, PLS, CLS) were constructed. Assay of external validation set. The absorption spectra of the validation set that consisted of twelve laboratory-prepared mixtures containing different ratios of Empagliflozin and its oxidative degradate were recorded in the range of 220–330 nm. The concentrations of Empagliflozin and its oxidative degradate were calculated using the optimized PCR, PLS and CLS calibration models.

Analysis of pharmaceutical preparation:

Ten tablets were weighed and finely powdered after removing the film coated by scratching and washing with methanol, Appropriate weight of powder equivalent to 10 mg Empagliflozin was accurately transferred to 100-ml volumetric flask and the volume was made up to 75 ml with methanol. The solution was shaken vigorously for 15 min then sonicated for 30 min. The volume was completed to 100 ml with solvent then filtered through Whatman filter paper no. 41.

Necessary dilutions of the filtrate were made with methanol to obtain different concentration of Empagliflozin samples as stated under linearity. To assess the accuracy of the proposed methods, standard addition technique was applied.

RESULTS AND DISCUSSION:

Spectral characteristics and optimization of the methods:

The zero-order absorption spectra of Empagliflozin and its oxidative degradate, as shown in **Fig. 2**, show severe overlap, which does not permit direct determination Empagliflozin in the presence of its degradate. To overcome this interference many manipulations have been done allowing the determination of Empagliflozin in the presence of its oxidative degradation product.

a- Bivariate method:

In the present study, stability indicating bivariate calibration spectrophotometric method was developed aiming to selective quantitation of Empagliflozin in its bulk powder, in pharmaceutical formulation or in presence of its degradation product. The principle of bivariate calibration is the measurement of two components (A and B) at two selected wavelengths (λ_1 and λ_2) to obtain two equations [23-24]

$$A_{AB1} = m_{A1}C_A C_B + e_{AB1}$$

$$A_{AB2} = m_{A2}C_A C_B + e_{AB2}$$

The resolution of each equation set allows the evaluation of C_A and C_B values:

$$C_A = (A_{AB1} - e_{AB1} - m_{B1}C_B) / m_{A1}$$

$$C_B = [m_{A2}(A_{AB1} - e_{AB1}) + m_{A1}(e_{AB2} - A_{AB2})] / m_{A2}m_{B1} - m_{A1}m_{B2}$$

Where C_A , C_B are the concentration of component A and component B; m_{A1} , m_{A2} are the slope values of A at λ_1 , λ_2 ; m_{B1} , m_{B2} are the slope values of B at λ_1 , λ_2 ; A_{AB1} , A_{AB2} are the absorbance of the binary mixture at λ_1 , λ_2 ; e_{AB1} , e_{AB2} are the sum of the intercepts of A and B at λ_1 and λ_2 , respectively.

In order to apply the bivariate method in the resolution of Empagliflozin and its degradate, the absorbance of the two component at seven different selected wavelengths was recorded in

the region of overlapping; 210, 215, 220, 225, 230, 235 and 240 nm. The calibration curve equations and their respective linear regression coefficients were obtained directly with the aim of ensuring that there was a linear relationship between the absorbance and the corresponding concentration.

According to Kaiser Method^[14], the slope values of the linear regression equations for both intact drug and its degradate at the selected wavelengths were used to calculate the sensitivity matrices **K** to find out the optimum pair of wavelength at which the binary mixtures were recorded, table (3).

$$K = \begin{bmatrix} m_{A_1} & m_{B_1} \\ m_{A_2} & m_{B_2} \end{bmatrix}$$

For the bivariate determination of Empagliflozin and its degradate, 210 and 230 nm were found to give the maximum value of K and thus can be used for the analysis; table (3).

b. Chemometric methods PCR, PLS and CLS.

Due to the overlapped spectra of the drug and degradate, Fig (2), the previous chemometric methods have been used to analyze this mixture. Various criteria have been developed to select the optimum number. Thirteen samples (odd numbers) were chosen and used for calibration and twelve (even numbers) were used for external validation. The appropriate selection of the number of factors to be used for building the model is crucial for achieving correct quantitation in PCR, PLS and CLS calibrations. The optimum number of latent variables was selected according to Haaland and Thomas criteria^[26]. The selected model was that with the smallest number of factors such that root mean square error of calibration for that model was not significantly greater than root mean square error of calibration from model with an additional factor. The optimum number of latent variables described by the constructed models was found to be two factors for both PCR, PLS and CLS as shown in Fig. (3 and 4) respectively.

In order to assess the predictive ability of each of the developed models, it was applied on an external validation set for determination of the two components. The recoveries, mean recoveries and RMSEP are summarized in table (4). The validation of the developed PCR, PLS and CLS models was assessed using several diagnostic tools. The predicted concentrations of the validation samples were plotted against the true concentration values.

This was used to determine whether the model accounted for the concentration variation in the validation set. All plots had a slope of nearly one and an intercept close to zero.

Validation of the methods:

Validation of the proposed methods was assessed as per the ICH guidelines [17] of accuracy, precision, repeatability, interday precision, linearity. Good results obtained as illustrated in **Table 1**.

Linearity and range:

For bivariate method: Calibration graphs were constructed for the determination of Empagliflozin by the proposed procedure, where Beer's law was obeyed in the range of 2 -16 $\mu\text{g ml}^{-1}$. The linear regression of the graphs was as follows.

$$A_{210} = 0.0576C - 0.0049 \quad (r=0.9999)$$

$$A_{230} = 0.0451 C - 0.0014 \quad (r=0.9998)$$

Where A is the absorbance, C is the drug concentration in $\mu\text{g ml}^{-1}$.

Limits of detection and quantitation:

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH guidelines [27] from the following equations:

$$\text{LOD} = 3.3 S_a / \text{slope}$$

$$\text{LOQ} = 10 S_a / \text{slope}$$

Where S_a is the standard deviation of y-intercepts of regression lines.

LOD and LOQ values of Empagliflozin for each method were listed in table (1).The small values of LOD and LOQ indicate good sensitivity.

Accuracy and precision:

According to the ICH guidelines^[17], three replicate determinations of three different concentrations of the studied drugs in pure form within their linearity ranges were performed in the same day (intra-day) and in three successive days (inter-day) for bivariate method.

Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) was calculated and results are listed in table (5). The small values of RSD% indicate high precision of the methods. Moreover, the good R% confirms excellent accuracy.

Specificity for Bivariate method:

The specificity of the proposed methods was assured by applying the laboratory prepared mixtures of the studied drug and its degradate. The results are listed in table (6).

Pharmaceutical Applications:

In order to compare the ability of the proposed methods for the determination of Empagliflozin in pharmaceutical preparation, the results obtained by applying each of the proposed method and the reported method [2] were subjected to statistical analysis **Table 7**. The calculated t and F values were less than the theoretical ones indicating that there were no significant differences between the proposed and the official methods.

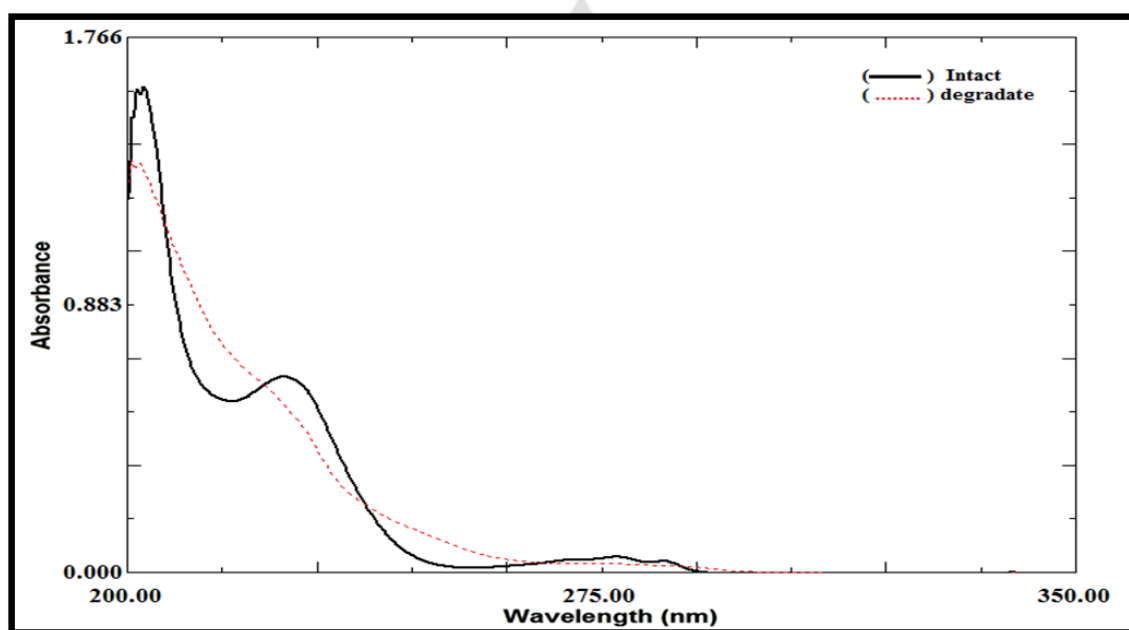


Fig. (1) Zero-order absorption Spectra of Intact Empagliflozin (12 µg ml⁻¹) (—) and its Degradation Product (12 µg ml⁻¹) (.....) in Methanol.

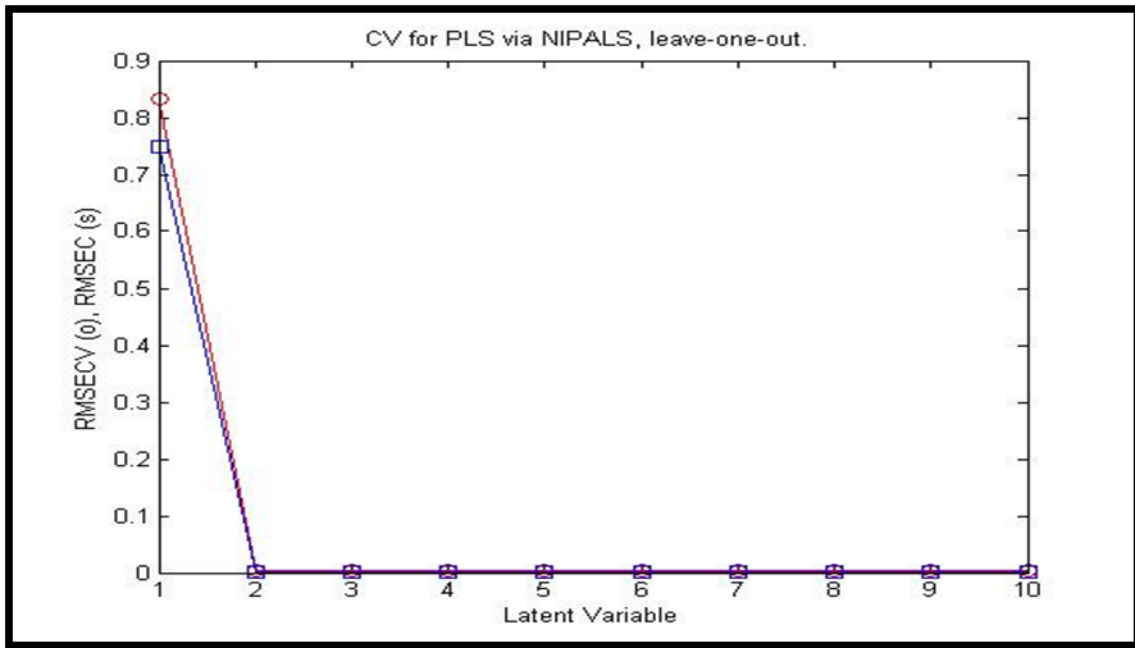


Fig. (2) Root mean square error of calibration (RMSEC) plot of the cross-validation results of the training set as a function of the number of principal components used to construct the PLS calibration

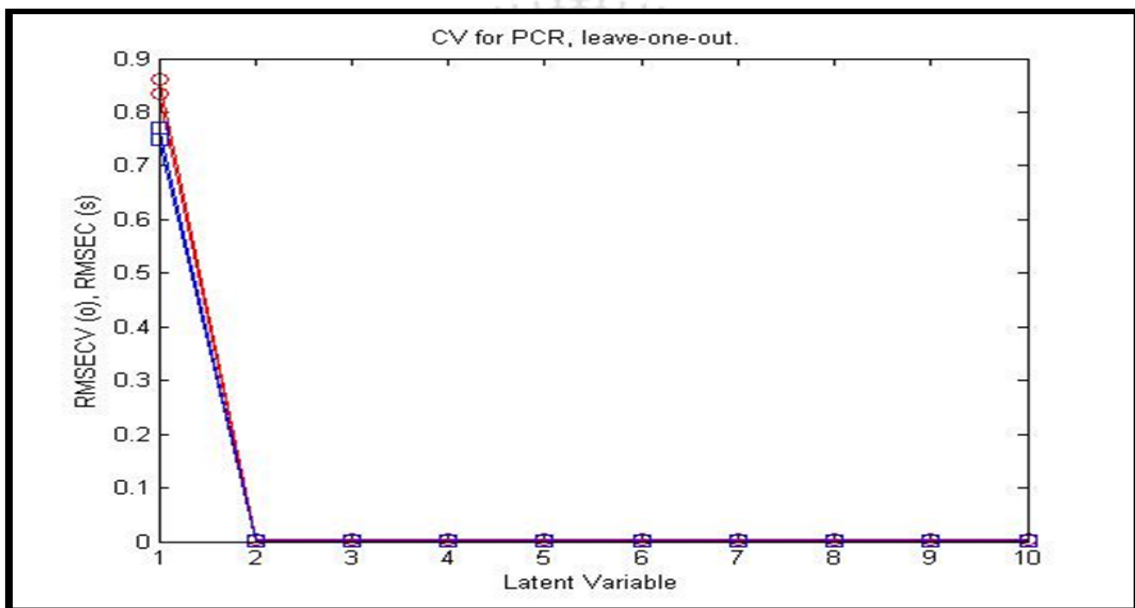


Fig. (3) Root mean square error of calibration (RMSEC) plot of the cross-validation results of the training set as a function of the number of principal components used to construct the PCR calibration

Table (1): Spectral data for determination of the studied drugs by the proposed methods:

Parameters	Bivariate	
	Wavelength (nm)	210
Linearity range (μgml^{-1})	2 — 16	2 — 16
LOD (μgml^{-1})	0.219	0.295
LOQ (μgml^{-1})	0.730	0.984
Regression equation*		
Slope (<i>b</i>)	0.0576	0.0451
Intercept (<i>a</i>)	0.0049	0.0014
Correlation coefficient (<i>r</i>)	0.9999	0.9998

* $y = a + bx$ where *y* is the response and *x* is the concentration.

Table (2): Concentrations of Empagliflozin and its oxidative degradation in the calibration and validation set for PCR and PLS

No. of Mixture	Intact ($\mu\text{g/ml}$)	Degraded ($\mu\text{g/ml}$)
1	10	10
2	10	8
3	8	8
4	8	12
5	12	9
6	9	12
7	12	10
8	10	9
9	9	9
10	9	11
11	11	12
12	12	11
13	11	10
14	10	12
15	12	12
16	12	8
17	8	11
18	11	8
19	8	10
20	10	11
21	11	11
22	11	9
23	9	8
24	8	9
25	9	10

Table (3): Values of The Sensitivity Matrix Determinants Calculated according to Kaiser's Method ($K \times 10^6$) For The Mixture of Empagliflozin And its Oxidative Degradate by the Proposed Bivariate Method:

λ/λ	210	215	220	225	230	235	240
210	0	-77.92	-746.48	-1455.88	-1549.44	-794.12	-212.88
215		0	-534.12	-1099.84	-1184.48	-602.12	-154.51
220			0	-549.41	-718.21	-320.81	-20.15
225				0	-260.25	-41.58	116.54
230					0	98.44	162.54
235						0	69.54
240							0

Table (4): Percentage recoveries of Empagliflozin and oxidative degradate in the validation set using PCR and PLS models

Validation mixture	PCR		PLS	CLS	
	Intact	Degradate	Intact	Intact	Degradate
1	99.23	100.18	100.01	100.56	99.05
2	101.77	101.45	101.28	101.03	102.16
3	101.55	99.39	99.77	100.57	101.88
4	98.75	99.25	101.54	100.23	98.66
5	99.48	100.34	100.47	101.42	99.72
6	100.88	99.34	99.69	98.62	100.77
7	100.62	101.87	100.01	100.64	100.86
8	100.32	99.38	101.31	100.11	99.81
9	97.45	100.13	99.73	99.48	97.09
10	99.37	99.79	98.98	99.82	99.48
11	97.33	99.33	100.86	99.94	97.04
12	98.82	100.75	101.23	100.33	98.69
Mean+%RSD	99.61+1.469	100.1+0.875	100.40+0.821	100.16+0.815	99.60+1.646
RMSEP	0.0830	0.1402	0.0813	0.0814	1.578

Table (5): Intraday and interday accuracy and precision for the determination of the Empagliflozin by bivariate methods

Method	Conc µg.ml ⁻¹	Intraday		Interday	
		Accuracy (R%)	Precision (RSD%)	Accuracy (R%)	Precision (RSD%)
Bivariate	2	98.76	0.700	99.10	0.698
	7	99.79	0.834	99.75	1.233
	14	99.98	0.812	99.19	1.276

Table (6): Determination of the Empagliflozin and its degradate in their laboratory mixtures by bivariate method

	Intact in (µg ml ⁻¹)	Degradate in (µg ml ⁻¹)	Percent of degradate	Intact found in (µ ml ⁻¹)	Recovery % of intact
Bivariate method	4	12	75	4.02	100.50
	6	10	62.5	5.99	99.83
	8	8	50	8.06	100.75
	10	6	37.5	9.88	98.80
	12	4	25	12.04	100.33
	Mean ± SD%				

Table (7): Determination of Empagliflozin in Jardiance® 10 mg tablets by the proposed and reported methods:

	Bivariate	PLS	PCR	CLS	Reported method ^[2]
<i>N</i> *	5	5	5	5	5
\bar{X}	100.29	101.17	100.47	101.01	99.35
<i>SD</i>	1.141	0.753	0.724	0.237	1.109
<i>RSD</i> %	1.138	0.744	0.720	0.235	1.116
<i>t</i> **	0.435 (2.306)	1.373 (2.306)	0.951 (2.306)	1.014 (2.306)	---
<i>F</i> **	1.275 (6.388)	1.798 (6.388)	2.562 (6.388)	1.827 (6.388)	---

* No. of experimental.

** The values in the parenthesis are tabulated values of t and F at ($p= 0.05$).

CONCLUSION:

The proposed methods are simple, rapid, accurate and precise and can be used for the determination of Empagliflozin in pure form and in pharmaceutical dosage form.

ACKNOWLEDGMENT:

I am deeply thankful to **ALLAH**, by the grace of whom this work was realized. I wish to express my indebtedness and gratitude to staff members Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt for their valuable supervision, continuous guidance, and encouragement throughout the whole work.

REFERENCES

1. Empagliflozin, <https://pubchem.ncbi.nlm.nih.gov/compound/Empagliflozin>.
2. S.D. Patil, S.K. Chaure, M.A.H. Rahman, P.U. Varpe, S. Kshirsagar, Development and Validation of Simple UV-Spectrophotometric Method for the Determination of Empagliflozin, *Asian Journal of Pharmaceutical Analysis* 7(1) (2017) 18-22.
3. B. Ayoub, Mean Centering Method for determination of Empagliflozin and Metformin, *Marmara Pharmaceutical Journal* 21(3) (2017).
4. B.M. Ayoub, Development and validation of simple spectrophotometric and chemometric methods for simultaneous determination of empagliflozin and metformin: Applied to recently approved pharmaceutical formulation, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 168 (2016) 118-122.
5. N. Padmaja, G. Veerabhadram, A Novel Stability Indicating Rp-Uplc-Dad Method for Determination of Metformin and Empagliflozin in Bulk and Tablet Dosage form, *Oriental Journal of Chemistry* 33(4) (2017) 1949-1958.
6. B.M. Ayoub, UPLC simultaneous determination of empagliflozin, linagliptin and metformin, *RSC Advances* 5(116) (2015) 95703-95709.
7. B.M. Ayoub, S. Mowaka, LC-MS/MS Determination of Empagliflozin and Metformin, *Journal of Chromatographic Science* (2017) 1-6.
8. K.A. Attia, N.M. El-Abasawi, A. El-Olemy, A. Serag, Different spectrophotometric methods applied for the analysis of simeprevir in the presence of its oxidative degradation product: A comparative study, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 190 (2018) 1-9.
9. K.A. Attia, M.W. Nassar, M.B. El-Zeiny, A. Serag, Stability indicating methods for the analysis of cefprozil in the presence of its alkaline induced degradation product, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 159 (2016) 1-6.
10. K.A. Attia, M.W. Nassar, M.B. El-Zeiny, A. Serag, Firefly algorithm versus genetic algorithm as powerful variable selection tools and their effect on different multivariate calibration models in spectroscopy: A comparative study, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 170 (2017) 117-123.
11. K.A. Attia, M.W. Nassar, M.B. El-Zeiny, A. Serag, Different spectrophotometric methods applied for the analysis of binary mixture of flucloxacillin and amoxicillin: A comparative study, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 161 (2016) 64-69.

12. K.A. Attia, M.W. Nassar, M.B. El-Zeiny, A. Serag, Zero order and signal processing spectrophotometric techniques applied for resolving interference of metronidazole with ciprofloxacin in their pharmaceutical dosage form, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 154 (2016) 232-236.
13. Mossart DL, Vandeginste BG, Deming SN, Michotte Y and Kaufman L. *Chemometrics: A textbook*, Elsevier, Amsterdam, 124, 1988.
14. Rashed NS, Abdallah OM, Farag RS and Awad SS. Validated Bivariate Calibration Spectrophotometric and High Performance Liquid Chromatographic Methods for Simultaneous Determination of Dantrolene Sodium and Paracetamol in Pharmaceutical Dosage form *Advances in Analytical Chemistry*. 2014, **4**(1): 1-8.
15. Brereton R, Multilevel multifactor designs for multivariate calibration, *Analyst* 122(1997)1521–1529.
16. Haaland D, Thomas E, Partial least-squares methods for spectral analyses.1. Relation to other quantitative calibration methods and the extraction of qualitative information, *Anal. Chem.* 60 (1988)1193–1202.
17. Q.B. International Conference on Harmonization (ICH), Federal Register 62, 1997

