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DIFFERENT METHODS FOR THE ANALYSIS OF EMPAGLIFLOZIN IN THE PRESENCE OF ITS OXIDATIVE DEGRADATION PRODUCT

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ABSTRACT

Three rapid, simple, accurate and precise spectrophotometric methods were used for the determination of Empagliflozin in the presence of its oxidativec degradation product. The methods under study are ratio derivative, ratio difference and mean centering. 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. So, they can be used for the routine analysis of Empagliflozin in quality-control laboratories.

KEYWORDS: Empagliflozin; Stability-indicating; Ratio derivative; Ratio difference; Mean centering.

1. INTRODUCTION

Empagliflozin (IUPAC name: 1,5-anhydro-1-(4-chloro -3-tetrahydrofuran-3-yloxy)D-Glucitol) (Figure 1) is an anti-diabetic agent, a sodium-glucose co-transporter inhibitor, Blocking SGLT-2 reduces blood glucose by blocking glucose reabsorption in the kidney and there by excreting glucosevia the urine act as a 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 three methods manipulating ratio spectra methods namely ratio derivative[13], ratio difference[14], mean centering[14] were applied for the determination of empagliflozin in the presence of its oxidative degradation product.

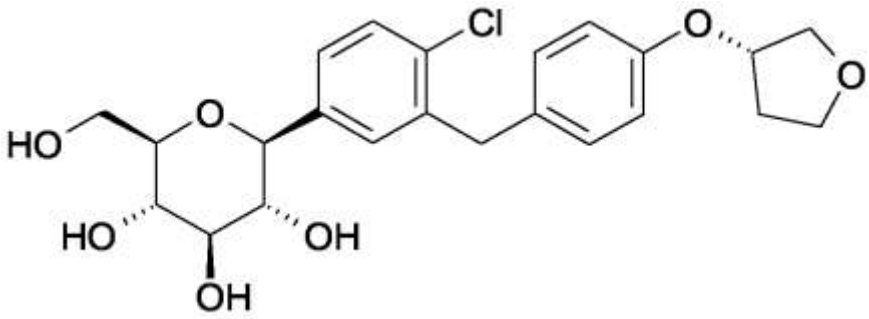

Fig. 1: Chemical structure of empagliflozin.

2. Experimental

2.1. Materials and reagents

a) Empagliflozin (certified to contain 99.25%) was kindly supplied by Al Andalous for Pharmaceutical Industries, Obour city, Egypt.

b) Pharmaceutical Preparation: " Jardiance 10 mg tablets batch no. 051, manufactured by Boehringer Ingelheim Pharmaceutical Company

c) Methanol; Analytical grade, El-NASR Pharmaceutical Chemicals Co., Egypt.

d) Hydrogen peroxide 10%, 20% & 30% aqueous solution.

2.2. Instruments

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.

2.3. Software

The bundled software, UV-Probe personal spectroscopy software version 2.43 (SHIMADZU) was used. Mean centering was 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).

2.4. Standard solutions

2.4.1. Preparation of Empagliflozin standard solution

A. Empagliflozin standard solutions; 100 µg /ml in methanol.

2.4.2. Preparation of the degradation product:

Ten mg of pure Empagliflozin powder were transferred to a 250-mL conical flask to which 10 mL of 30% H_2O_2 were added. The solution was heated under reflux for 5 hours. After cooling, the solution was evaporated to dryness at 120 rpm and 90°C to remove the remaining H_2O_2, 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.

3. Procedure

3.1. Linearity and construction of calibration curves

3.1.1. Ratio derivative method

Aliquots from Empagliflozin working standard solution were accurately measured, transferred into a set of 10-ml volumetric flasks and completed to volume with methanol to give (2-18 µg/ml). The zero order absorption spectrum of each solution was recorded versus methanol as a blank, divided by the spectrum of Empagliflozin degradation product (16 µg /ml) used as a divisor concentration. The first derivative corresponding to each ratio spectrum was recorded, using $\Delta\lambda$ = 8 nm and scaling factor = 10. The amplitude values were measured at 224 nm and plotted against the corresponding concentrations in µg/ml of Empagliflozin.

3.1.2. Ratio difference method

The ratio spectra obtained as before. Then the amplitudes difference of the ratio spectra at 231 and 212 nm ($\Delta P_{231\text{-}212}$) were plotted against the corresponding concentrations in µg/ml of Empagliflozin.

3.1.3. Mean Centering (MC)

The ratio spectra obtained as before in the range of 205 – 290 nm were mean centered. The calibration curve was constructed by relating the values of the amplitudes at 279 nm to the corresponding concentrations of Empagliflozin.

3.2. Application to laboratory prepared mixtures

Accurate aliquots of Empagliflozin and its oxidative degradation product were transferred from their working solutions into a series of 10-ml volumetric flasks to prepare mixtures containing different ratios of both. The volumes were completed with methanol. The spectra of the prepared series from 200 to 400 nm were recorded and stored. The concentrations of Empagliflozin were calculated as described under linearity for each of the proposed methods.

3.3. Application to pharmaceutical preparation

Ten tablets were weighed and finely powdered after removeing 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.

4. RESULTS AND DISCUSSION

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.

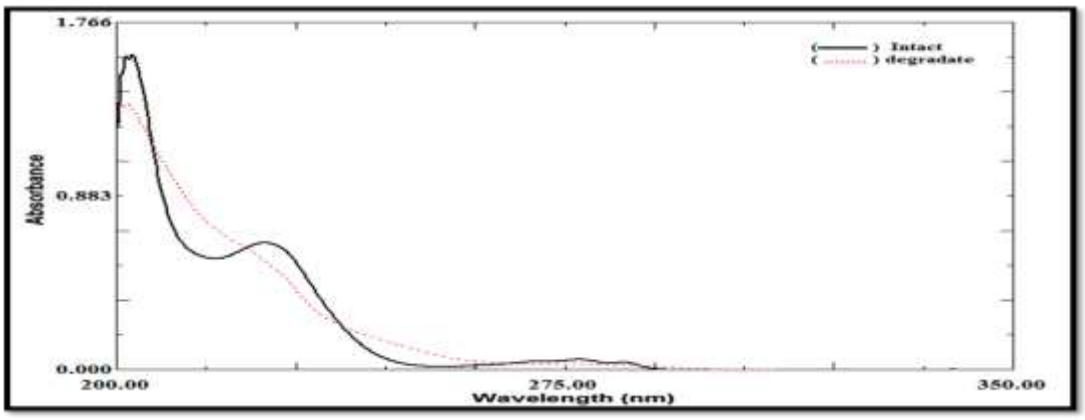

Fig. 2: Zero-order absorption spectra of intact empagliflozine (12 µg ml^{-1}) and its degradation product (12 µg ml^{-1}) in methanol.

4.1. Ratio derivative method

In this method, different divisors were examined and the divisor giving the best results was chosen (16 µg/ml). The absorption spectra of the drug were divided by the chosen divisor to get the ratio spectra, as shown in **Fig. 3**. The peak amplitudes of the first derivative of the ratio spectra at 224 nm using Δλ = 8 nm and scaling factor = 10 are proportional to the concentrations of the drug without interference from its degradate (divisor), as shown in **Fig. 4**.

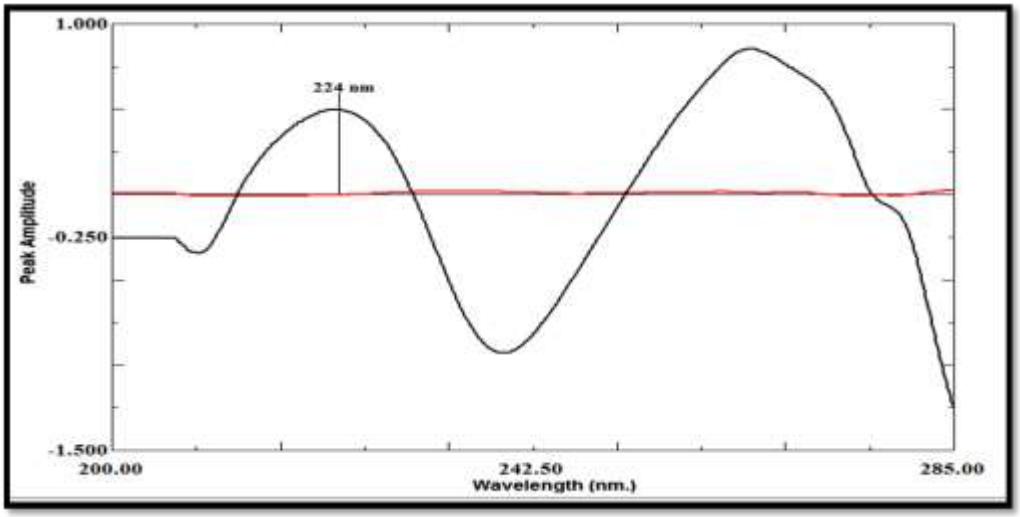

Fig. 3: The ratio spectra of empagliflozine (18 µg/ml and its oxidative degradate using (16 µg/ml) of degradate as a divisor.

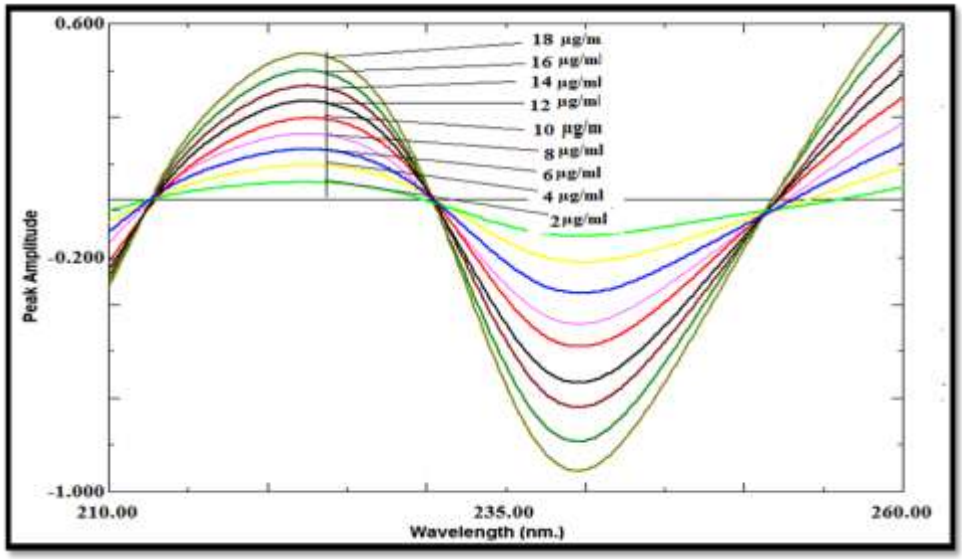

Fig. 4: The ratio spectra.

4.2. Ratio difference method

This method comprises two critical steps, first is the selection of the divisor and this has been discussed in section **4.1.** The second critical step is the choice of the wavelengths at which measurements are recorded. Each of the two chosen wavelengths should exhibit a good linearity and they should have different amplitudes in the ratio spectra. Several wavelengths pairs have been tested and 231 and 212 nm showed the best results.

4.3. Mean centering method

The ratio spectra were obtained by testing different concentrations of the divisors (but the concentrations 16 µg/ml of Empagliflozin degradate gave minimum noise in ratio spectra and maximum sensitivity. The ratio spectra were mean centered in the range (205–290 nm) for Empagliflozin as shown in **Fig. 5**. The concentrations of Empagliflozin were calculated by using the regression equation representing the linear relationship between (MC values) at 279 nm and the corresponding concentrations.

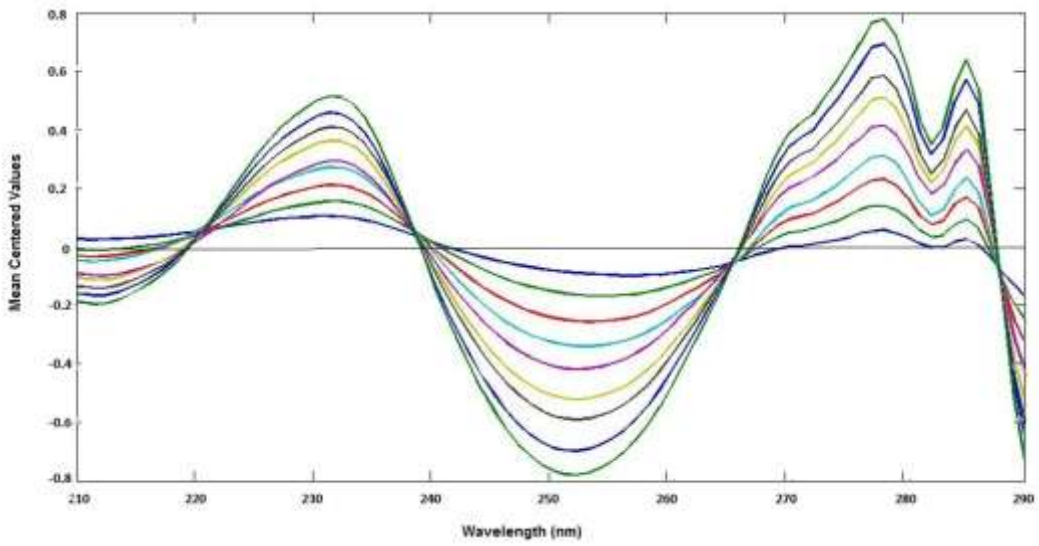

Fig. 5: Mean centering of the ratio spectra of empagliflozine at various concentrations (2-18 µg/ml) using 16 µg/ml of degradate as a divisor.

5. Validation of the methods

Validation of the proposed methods were assessed as per the ICH guidelines[15] of accuracy, precision, repeatability, interday precision, linearity. Good results obtained as illustrated in **Table 1**. **Table 2** shows the specificity; recovery percentages of the laboratory prepared mixture of the drug with its oxidative degradation product. The validity of the proposed

procedures is further assessed by applying the standard addition technique showing no interference from excipients. The results obtained were shown in **Table 3.**

Table 1: Assay validation sheet of the proposed methods.

Parameter	Ratio derivative	Ratio difference	Mean centering
Accuracy (mean ± RSD)[a]	99.94 ± 0.215	100.08± 0.576	99.80± 0.385
Precision			
Repeatability (RSD) [b]	1.115	1.190	1.060
Intermediate precision (RSD) [c]	1.315	1.270	1.226
Linearity and range	(2-18 µg/ml)		
Slope	0.030	0.039	0.043
Intercept	0.0010	0.0061	-0.0019
Regression coefficient (r^2)	0.9998	0.9998	0.9998
LOD (µg/ml)	0.1719	0.1608	0.1757
LOQ (µg/ml)	0.5210	0.4873	0.5327

[a]Average of three determinations for three concentrations (4, 9 and 14 µg/mL) for empagliflozin repeated three times.

[b]The intraday (n = 3), average of three concentrations (4, 9 and 14 µg/mL) for empagliflozin repeated three times within the day.

[c]The interday (n = 3), average of three concentrations (4, 9 and 14 µg/mL) for empagliflozin repeated three times in three days.

Table 2: Determination of empagliflozin in laboratory prepared mixtures with oxidative degradate by the proposed methods.

Intact (µg/ml)	Degradate (µg/ml)	Degradate %	Ratio derivative [a]	Ratio difference [a]	Mean centering [a]
16	2	11.11	98.77±0.234	98.22±0.412	99.79±0.397
12	6	33.33	99.62±0.356	99.12±0.386	100.89±0.308
9	9	50	98.87±0.369	98.89±0.332	98.69±0.542
6	12	66.67	100.50±0.478	101.55±0.542	99.88±0.434
2	16	888.89	101.51±0.865	98.60±0.486	98.17±0.687

[a]Average of three determinations ± SD.

Table 3. Application of standard addition technique to the analysis of Jardiance® 10 mg tablets by applying the proposed methods.

Pharmaceutical conc. µg/mL	Added standard µg/mL	Ratio derivative [a]	Ratio difference [a]	Mean centering [a]
6	2	100.94±0.569	99.78±0.478	100.30±0.568
	6	101.57±0.432	100.38±0.678	98.95±0.759
	10	99.81±0.869	99.71±0.597	99.79±0.741

[a] Average of three determinations ± SD.

6. Statistical analysis

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 4**. 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.

Table 4. Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of empagliflozin in Jardiance® 10 mg tablets.

Parameter	Ratio derivative	Ratio difference	Mean centering	Reported method[2]
Mean	98.68	99.47	98.95	99.35
S.D.	0.601	0.829	1.089	1.109
n	5	5	5	5
Variance	0.632	0.875	1.105	1.116
***t* test [a] (2.306)**	0.342	0.521	0.432	-----
***F* value [a] (6.388)**	1.012	1.185	1.138	------

[a] The values in the parenthesis are the corresponding theoretical values of *t* and *F* at (*P* = 0.05).

7. CONCLUSION

Three methods namely ratio derivative, ratio difference and mean centering were presented as powerful methods for the determination of Empagliflozin in the presence of its oxidative degradation product. The proposed methods were very simple with minimum manipulation steps, very sensitive, accurate and precise. They do not need any sophisticated apparatus and could be easily applied in quality control laboratories.

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