

Mean Centering Method for determination of Empagliflozin and Metformin

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ABSTRACT

Introduction of computer-assisted analysis to pharmaceutical products is considered a significant approach. A new method was developed for simultaneous determination of empagliflozin and metformin manipulating their ratio spectra with application on recently approved pharmaceutical combination, Synjardy[®] tablets. Spiking technique was used to increase the concentration of empagliflozin after extraction from tablets to allow its simultaneous determination with metformin without prior separation. Validation parameters according to

ICH guidelines were acceptable over the concentration range of 2-12 µg/mL for both drugs. Mean centering was performed using Minitab[®] program. Using computer assisted programs after spiking accompanied with direct UV measurement of the drugs was satisfactory for accurate assay without complex instrumentations. The optimized method was proved to be suitable for industrial QC labs.

Keywords: Spiking; Chemometry; Computer-assisted analysis; Mean Centering; Empagliflozin; Metformin.

1. Introduction:

Synjardy[®] is composed of empagliflozin from the gliflozin class that was recently approved for the treatment of type 2 diabetes as a sodium glucose co-transporter 2 inhibitor enhancing urinary glucose excretion [1] and metformin which is the drug of choice in mixed therapy for type 2 diabetes. Only one chromatographic method [2] and one spectrophotometric method [3] were developed for its pharmaceutical analysis. The aim of the present work is to present a new simple computer-assisted mean centering method [4] for the analysis of empagliflozin and metformin taking in consideration the spiking criteria. Spiking technique was used to increase the concentration of empagliflozin after extraction from tablets allowing its determination despite its low contribution.

Spectrophotometric assays for some recently approved anti-diabetic drugs (Table 1) showed more facilitated, simple and cost effective methods [5-11]. Furthermore, it showed the advantages of low cost solvents, shorter analysis time and simple instrumentation instead of complex details implemented in the chromatographic method development. Spectrophotometric analysis exhibited more economic and simple assays [5-11] either using direct UV determination or

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Table 1. Spectrophotometric methods for analysis of some recently approved anti-diabetic drugs in pharmaceutical formulations namely linagliptin and alogliptin.

Method	Detection wavelength	Linearity ($\mu\text{g/mL}$)	Application
Direct UV	277 nm	2-6	Assay of linagliptin in Glyxambi [®] tablet ⁽⁵⁾
Direct UV	294 nm	5-25	Assay of linagliptin in Tradjenta [®] tablets ⁽⁶⁾
Direct UV	241 nm	10-35	Assay of linagliptin in Tradjenta [®] tablets ⁽⁷⁾
Direct UV	276 nm	5-35	Assay of alogliptin in Nesina [®] tablets ⁽⁸⁾
Simultaneous equation	224 nm	0.5-18	Assay of alogliptin in Kazano [®] tablets ⁽⁹⁾
Absorption ratio	251 nm	0.5-18	Assay of alogliptin in Kazano [®] tablets ⁽⁹⁾
First derivative	222 nm	2-16	Assay of alogliptin in Nesina [®] tablets ⁽¹⁰⁾

by manipulation of the obtained spectra with acceptable limit of detection (LOD) and limit of quantification (LOQ) values that ensured satisfying sensitivity. Mostly used solvents were distilled/deionized water and methanol. The applications of cost effective spectrophotometric methods have renovated the concept of analysis in a highly accurate and precise way.

2. Experimental

2.1. Instrumentation

JASCO[®] double-beam UV spectrophotometer (S/N C367961148, Japan) supported with Spectra Manager[®] software were used.

2.2. Reagents, reference standard samples and working solutions

Empagliflozin certified to contain 99.70 %, metformin certified to contain 99.80 % and Synjardy[®] tablets nominally containing 12.5 mg of empagliflozin and 500 mg of metformin per tablet were supplied from Boehringer Ingelheim (Germany). Working solutions (20 $\mu\text{g/mL}$) were prepared in methanol.

2.3. Sample preparation

The coats of ten Synjardy[®] tablets were carefully removed and then the tablets were powdered and mixed. An accurately weighed amount equivalent to 2.5 mg of empagliflozin and 100 mg of metformin was made up to 100 mL with methanol, sonicated to dissolve and filtered. One mL of the extract was transferred to a 100 mL volumetric flask, spiked with 10 mL of empagliflozin working solution and finally completed to volume with methanol.

2.4. Procedure

2.4.1. Preliminary investigation

Zero-order absorption spectra of empagliflozin (8 $\mu\text{g/mL}$) and metformin (8 $\mu\text{g/mL}$) were recorded separately against methanol as a blank showing the maximum absorption (λ_{max}) at 225 nm and 237 nm, respectively (Figure 1).

2.4.2. Linearity

Accurately measured aliquots of working solutions equivalent to 20-120 μg of each drug were transferred separately into a series of 10 mL volumetric flasks, completed to volume with methanol. The previously scanned spectra of empagliflozin and metformin were divided separately by (10 $\mu\text{g/mL}$ of metformin) and (12 $\mu\text{g/mL}$ of empagliflozin, respectively). The obtained ratio spectra were mean centered and then the mean centered values of empagliflozin and metformin were measured at 221.8 nm and 249.2 nm, respectively. Calibration curves were attained by plotting the mean centered values against concentration.

2.4.3. Assay of laboratory prepared mixtures

Three different ratios (1:3, 1:1 and 3:1) of the laboratory prepared mixtures were prepared using concentrations equivalent to (3, 6, 9 $\mu\text{g/mL}$) and (9, 6, 3 $\mu\text{g/mL}$) of empagliflozin and metformin, respectively. The zero order spectra of each mixture were recorded using methanol as blank. Then obtained spectra were divided by (10 $\mu\text{g/mL}$ of metformin) and (12 $\mu\text{g/mL}$ of empagliflozin) separately in order to get the ratio spectra that will be manipulated by the mean centering method to calculate the corresponding concentration of each drug. The ratio spectra divided by metformin were mean centered using Minitab[®] program.

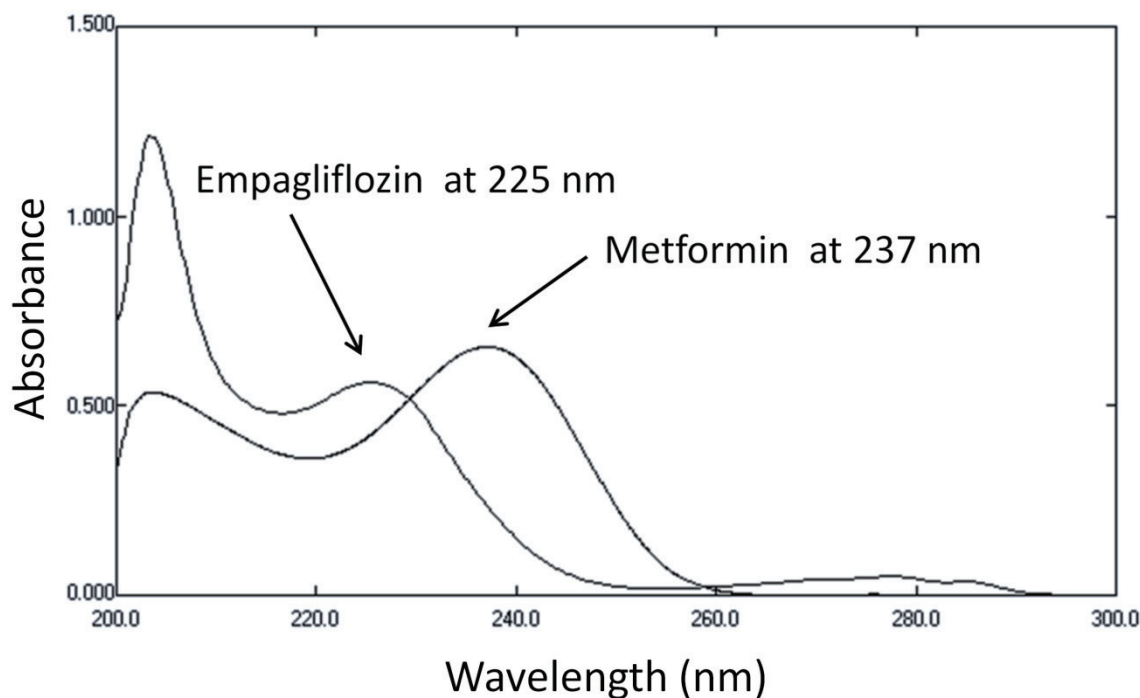


Figure 1. Overlay of the absorption spectra of EG (8 µg/mL) and MT (8 µg/mL), using methanol as a blank.

The mean centered values were measured at 221.8 nm for empagliflozin determination, while the mean centered values of the ratio spectra divided by empagliflozin were used for determination of metformin by measuring the mean centered values at 249.2 nm.

2.4.4. Accuracy, repeatability and intermediate precision

The laboratory prepared mixtures prepared under 2.4.3 were analyzed using the proposed method three times within the same day and on three successive days. The mean of the percent recoveries (R %) and the percent relative standard deviation (RSD %) were then calculated for each method.

2.4.5. Assay of Synjardy[®] tablets

The absorbance spectrum of the tablet extract prepared under section 2.3 was recorded using methanol as blank. The obtained spectra were divided by (10 µg/mL metformin) and (12 µg/mL empagliflozin) separately in order to record the ratio spectra that will be manipulated by the mean centering method to calculate the corresponding concentration of each drug as discussed under (section 2.4.3).

2.4.6. Forced degradation of empagliflozin

Forced degradation study of empagliflozin, as a recently approved drug, included solid state (thermal and photo-degradation) and solution state (acid, alkali and H₂O₂) stress conditions were performed. To a series of test tubes, each one contains 2.5 mL of working solution, 2.5 mL of 0.3% H₂O₂, 1N NaOH and 1N HCl were added separately and heated at 60 °C for 0.5 h, then the acid and alkali mixtures were neutralized and each mixture was completed to 10 mL with methanol. The dry powder of empagliflozin was placed in oven at 55 °C for 6 h & placed under UV lamp for 6 h and then dissolved in methanol to give a proposed concentration of 5 µg/mL.

3. Results and discussion

The absorption spectra of each drug were divided by the spectrum of a selected divisor (the other drug) to get the ratio spectra, then mean centering method [4] was applicable as empagliflozin and metformin are non-interactive drugs and each of them obey Beer's law according to the following equation ($V_a = A_{EG} C_{EG} + A_{MT} C_{MT}$). Where, V_a is the vector of absorbance, ($A_{EG} - A_{MT}$) are the molar absorptivities, ($C_{EG} - C_{MT}$) are the concentrations of empagliflozin and metformin,

respectively. After division over A_{MT} , the produced ratio spectra will be mean centered; C_{MT} will be zero value enabling the determination of C_{EG} without interference from metformin and the same concept regarding A_{EG} .

The obtained absorption spectra of both empagliflozin and metformin were divided by the spectrum of 10 $\mu\text{g/mL}$ metformin and 12 $\mu\text{g/mL}$ empagliflozin, respectively and then mean centered using Minitab* program (Figure 2). Empagliflozin and metformin were determined at 221.8 nm and 249.2 nm, respectively in a concentration range of 2-12 $\mu\text{g/mL}$.

All the validation parameters were found to be compatible with ICH guidelines [12]. The validity of the calibration curves over the concentration range (2-12 $\mu\text{g/mL}$) was validated by the obtained low values of LOD - LOQ parameters, and acceptable values of STEYX, S_b & S_a as shown in (Table 2). Where; LOD is the limit of detection

which represents drug concentration at STEYX/S ratio of 3.3, LOQ is the limit of quantification at which STEYX/S is 10, STEYX is the residual standard deviation of the regression line, S_b is the standard deviation of the slope and S_a is the standard deviation of the intercept. Also the developed method was adopted successfully for determination of the investigated drugs in laboratory prepared mixtures (Figure 2).

Influence of different variables was studied, including scanning speed, wavelength of measurements, divisor concentration and smoothing factor. Different concentrations of both drugs (6, 8, 10, 12, 14 and 16 $\mu\text{g/mL}$) were tried as divisors. Concentration of 12 $\mu\text{g/mL}$ empagliflozin and 10 $\mu\text{g/mL}$ metformin were selected as the best divisors with minimum noise. No significant difference was found using different smoothing factors, so it was excluded from the method.

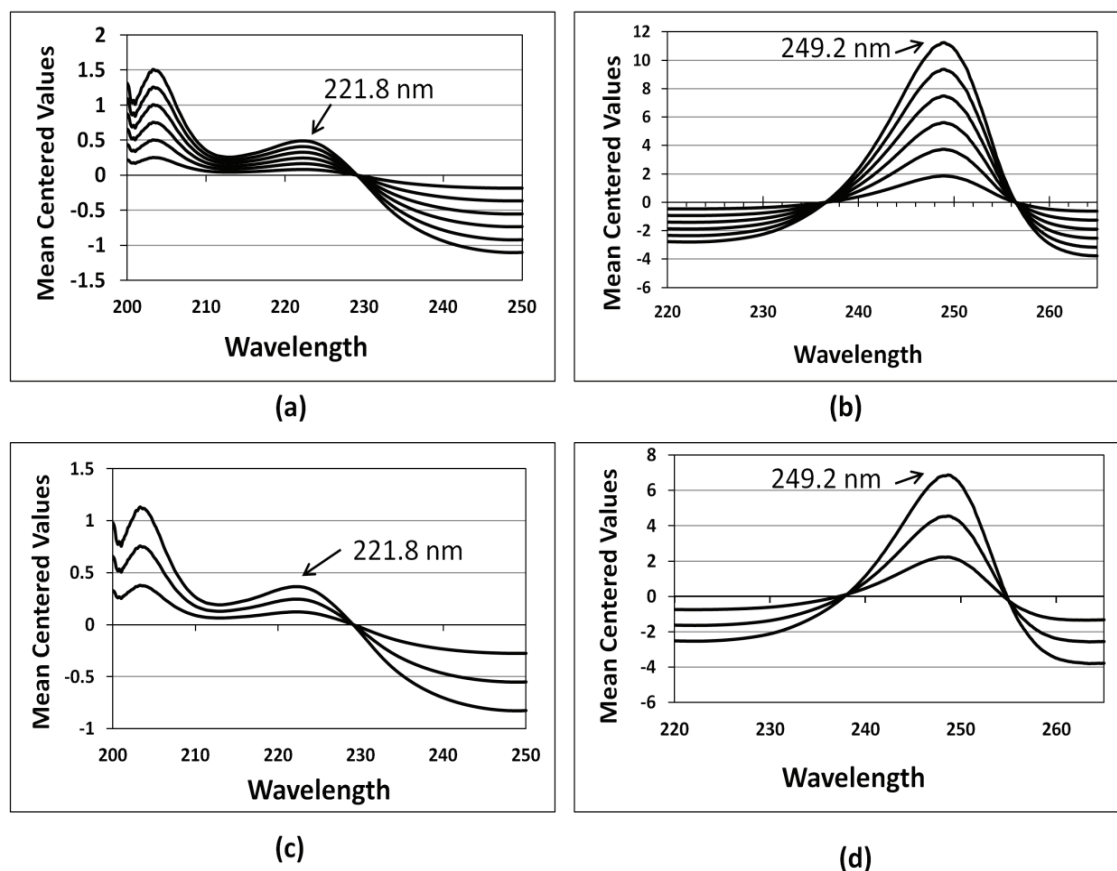


Figure 2. The mean centered values of the ratio spectra representing (2-12 $\mu\text{g/mL}$) EG divided by 10 $\mu\text{g/mL}$ MT (a), the ratio spectra of (2-12 $\mu\text{g/mL}$) MT divided by 12 $\mu\text{g/mL}$ EG (b) and the ratio spectra of the laboratory prepared mixtures divided separately by 10 $\mu\text{g/mL}$ MT (c), and 12 $\mu\text{g/mL}$ EG (d).

Table 2. Results obtained by the proposed mean centering method

Item	Empagliflozin	Metformin
Wavelength of measurements	221.8 nm	249.2 nm
Linearity range	2-12 µg/mL	2-12 µg/mL
Regression equation	$M_v = 0.0404 C_{\mu\text{g/mL}} + 0.0024$	$M_v = 0.9338 C_{\mu\text{g/mL}} + 0.0391$
Correlation coefficient (r)	0.9998	0.9999
S_b	1.7×10^{-4}	0.004
S_a	1.6×10^{-3}	0.04
C.I. of the slope	$0.0404 \pm 7 \times 10^{-6}$	$0.9338 \pm 3.8 \times 10^{-3}$
C.I. of the intercept	$0.0024 \pm 3.97 \times 10^{-6}$	$0.0391 \pm 1.5 \times 10^{-3}$
STEYX	0.0014	0.034
LOD (µg/mL)	0.11	0.12
LOQ (µg/mL)	0.34	0.36
Accuracy (Mean ± SD)	99.88 % ± 0.62	100.09 % ± 0.55
Intraday RSD %	0.17-0.22	0.10-0.23
Interday RSD %	0.13-0.18	0.12-0.27
Drug in dosage form (Mean ± SD)	96.44 % ± 0.35	99.96 % ± 0.32

Where M_v is the mean centered value, C is the concentration of the drug, S_b is the standard error of slope, S_a is the standard error of intercept, C.I. is the confidence interval, STEYX is the residual standard deviation of the regression line, LOD is the limit of detection, LOQ is the limit of quantification, SD is the standard deviation, RSD % is the percent relative standard deviation.

Accuracy of the results was checked by calculating the percent recovery of different concentrations of each drug in laboratory prepared mixtures. The mean of the recovery and standard deviations were shown in (Table 2). Precision of the methods was checked using intraday and interday records of the same laboratory prepared mixtures. The RSD % of recoveries was calculated and found to be less than 1%.

To check the specificity of the method, each drug was determined in laboratory prepared mixture and in Synjardy[®] tablets in the presence of excipients including copovidone, corn starch, colloidal silicon dioxide, magnesium stearate, hypromellose, titanium dioxide, talc, polyethylene glycol 400 and ferric oxide. And the results are shown in (Table 2). The forced degradation study showed 24 % degradation in case of alkaline stress conditions, 5 % in case of acidic degradation and no degradation behavior was observed under the applied oxidative, thermal and photo-degradation conditions as a required specificity for the validated procedure. Statistical analysis (Table 3) showed no significant difference in comparison to a spectrophotometric method [3] at $P > 0.05$,

with $F = 0.001$, $P = 0.977$ for empagliflozin and $F = 0.335$, $P = 0.594$ for metformin.

Table 3. One way ANOVA results at $P < 0.05$ for the proposed method for determination of empagliflozin and metformin against reference method [3].

Groups	Empagliflozin		Metformin	
	Mean	S.D.	Mean	S.D.
*Reference method [3]	99.86	0.95	100.48	1.03
*Proposed method	99.88	0.62	100.09	0.55

*Studied groups showed no significant difference at $P > 0.05$, with $F = 0.001$, $P = 0.977$ for empagliflozin and $F = 0.335$, $P = 0.594$ for metformin.

Abbreviations: S.D. = standard deviation

4. Conclusion

The proposed analytical method proved to be accurate for determination of empagliflozin and metformin as an economic assay based on simple instruments and computer assisted programs. The methods were applied successfully on the pharmaceutical dosage form with acceptable validation results. Spiking technique was crucial in the developed method which allowed the direct measurement of the minor component in the mixture. The developed method should be of interest to the analysts in the area of drug control and can be used by QC laboratories for the analysis of recently approved Synjardy[®] tablets.

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