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QUANTITATIVE ANALYSIS OF DRUGS WITH HIGHLY DIFFERENT CONCENTRATIONS OF PHARMACEUTICAL COMPONENTS USING SPECTRAL SUBTRACTION TECHNIQUES

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Two simple spectrophotometric methods were developed for determination of empagliflozin and metformin by manipulating their ratio spectra with application on a recently approved pharmaceutical combination, Synjardy® tablets. A spiking technique was used to increase the concentration of empagliflozin after extraction from the tablets to allow its simultaneous determination with metformin. Validation parameters according to ICH guidelines were acceptable over the concentration range of 2–12 μg/mL for both drugs using constant multiplication and spectrum subtraction methods. The optimized methods are suitable for QC labs.

Keywords: *spiking, constant multiplication, spectrum subtraction, empagliflozin, metformin.*

Introduction. Synjardy® is composed of empagliflozin (EG) and metformin (MT). EG is a sodium glucose co-transporter 2 inhibitor that enhances urinary glucose excretion [1]. MT is the drug of choice in mixed therapy for diabetes [1]. Only one chromatographic method [2] and one chemometric method [3] were developed for determination of EG and MT in tablets. In addition, spectrophotometric analysis of EG in binary mixture with linagliptin was reported [4]. The aim of this work is to present new methods for the analysis of EG and MT with the advantage of low cost of the developed method in comparison to the reported chromatographic method [2]. Furthermore, the proposed method in this investigation provided a lower limit of detection (LOD) and a lower limit of quantification (LOQ) than the previously reported chemometric method [3]. Also, a spiking technique was used to increase the concentration of EG after extraction from the tablets, allowing its determination despite its low contribution. This study presents two methods resolving the overlapped spectra of EG and MT by manipulating their ratio spectra, including constant multiplication (CM) coupled with spectrum subtraction (SS).

Synjardy® tablet contains EG and MT that do not interact chemically. Consequently, absorption spectra of the EG and MT mixture were divided by the spectrum of a selected divisor (EG) to get the ratio spectra after optimization of the critical factors, including the detection wavelength, scanning speed, divisor concentration, and smoothing function. The constant value was predicted from the plateau in the region where EG is extended. The previous step can be described by the following equation: (MT + EG)/EG' = MT/EG' + constant, the estimated value of the constant is equal to (EG/EG'). Consequently, constant multiplication [5] by EG' (CM method) will regenerate the original spectrum of EG followed by its subtraction (SS method) from the mixture to get the MT spectrum.

Experimental. A JASCO® double-beam UV spectrophotometer (S/N C367961148, Japan) supported with Spectra Manager® software was used. EG was certified to contain 99.70%, MT was certified to contain 99.80%, and Synjardy® tablets nominally containing 12.5 mg of EG and 500 mg of MT per tablet were supplied from Boehringer Ingelheim (Germany). Solutions of EG & MT (20 μg/mL) were prepared in methanol.

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 EG and 100 mg of MT 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 EG working solution, and finally completed to volume with methanol.

The zero-order absorption spectra of EG (8 μg/mL) and MT (8 μg/mL) were recorded separately against methanol as a blank. Overlay of both EG and MT spectra (Fig. 1) showed the maximum absorption λ_{max} at 225 and 237 nm, respectively.

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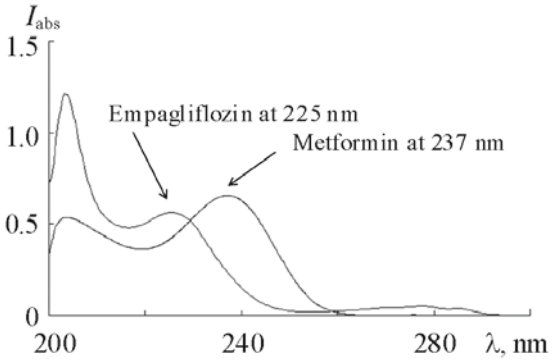

Fig. 1. Overlay of the absorption spectra of EG (8 μg/mL) and MT (8 μg/mL), using methanol as a blank.

Accurately measured aliquots of working solutions equivalent to 20–120 μg of both EG and MT were transferred separately into a series of 10 mL volumetric flasks, completed to volume with methanol. The absorbance was measured at 225 and 237 nm for EG and MT, respectively using methanol as blank. Two calibration curves were obtained by plotting absorbance against concentration for each drug.

Assay of EG and MT in 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 μg/mL and 9, 6, 3 μg/mL of EG and MT, respectively. The zero order spectra of each mixture were recorded using methanol as blank. Then the obtained spectra were divided by 12 μg/mL of EG separately in order to get the ratio spectra, which will be manipulated by CM and SS methods to calculate the corresponding concentration of each drug.

For the ratio spectra divided by EG, the constant representing the EG concentration in the mixture divided by 12 μg/mL of EG was estimated for each mixture as the straight line (plateau) that was parallel to the wavelength axis in the region where EG was extended. The constant values (plateau) obtained were multiplied by the divisor spectrum to obtain the zero-order spectrum of EG. The obtained zero-order spectrum of EG was subtracted from the mixture spectrum to obtain the zero-order spectrum of MT.

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

The absorbance spectrum of the prepared tablet extract was recorded using methanol as blank. The obtained spectrum was divided by 12 μg/mL EG in order to record the ratio spectrum, which was manipulated by CM and SS methods to calculate the corresponding concentration of each drug.

Results and Discussion. Validation parameters were investigated according to ICH guidelines [6]. The calibration curves (linearity) over the concentration range 2–12 μg/mL was validated by the obtained low values of LOD-LOQ parameters and the acceptable values of STEYX, S_b and S_a as shown in Table 1. The developed CM and SS methods were adopted successfully for determination of the investigated drugs in laboratory prepared mixtures (Fig. 2) and in Synjardy® tablets, which confirm the selectivity and specificity of the methods.

Different concentrations of EG were tried as divisors. A concentration of 12 μg/mL EG was selected as the best divisor with maximum sensitivity. The obtained absorption spectrum of the laboratory prepared mixture containing EG and MT was divided by the spectrum of the more extended EG. Then multiplication of the same divisor followed by spectrum subtraction was performed to obtain the original spectra of the drugs (Fig. 3). The absorbance values for EG and MT were measured at 225 and 237 nm, respectively. The main advantage of the CM method is the measurement of the more extended drug in one step after multiplication of the constant value by the divisor spectrum [5] followed by determination of the other drug by simple subtraction from the mixture spectrum using the SS method.

The accuracy of the results was checked by calculating the percent recovery of each drug in laboratory prepared mixtures. The mean of the recovery and standard deviations are shown in Table 1. The 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%.

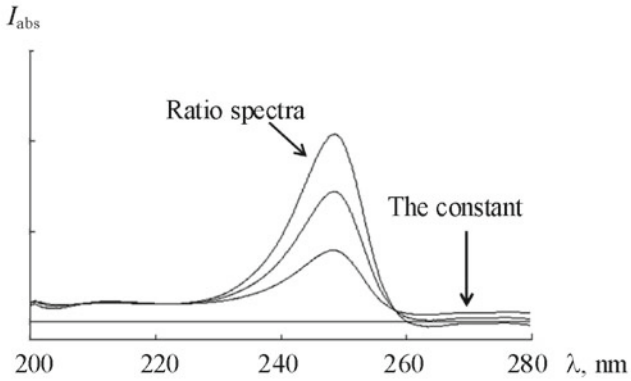

Fig. 2. The obtained ratio spectra of 2–12 μg/mL MT divided by 12 μg/mL EG in methanol.

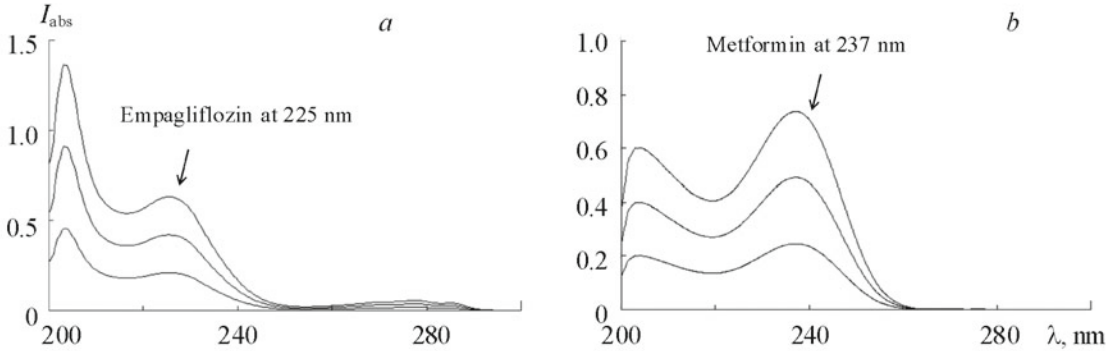

Fig. 3. The obtained EG (a) and MT (b) zero-order spectra (3, 6, and 9 μg/mL) resolved from the laboratory prepared mixtures using CM (a) and SS (b) method.

TABLE 1. Results Obtained by CM and SS Methods

Item	EG using CM	MT using SS
Wavelength of measurements, nm	225	237
Linearity range, μg/mL	2–12	2–12
Regression equation	$A_s = 0.0618C_{\mu g/ml} + 0.0031$	$A_s = 0.0814C_{\mu g/ml} + 0.0022$
Correlation coefficient r	0.9999	0.9999
Standard error of slope S_b	2.7×10^{-4}	4.20×10^{-4}
Standard error of intercept S_a	0.002	0.003
Confidence interval of the slope	$(0.0618 \pm 1.7) \times 10^{-5}$	$(0.0814 \pm 3.41) \times 10^{-5}$
Confidence interval of the intercept	$(0.0031 \pm 6.14) \times 10^{-6}$	$(0.0022 \pm 6.64) \times 10^{-6}$
Residual standard deviation of the regression line	0.0023	0.0035
LOD, μg/mL	0.12	0.14
LOQ, μg/mL	0.36	0.42
Accuracy (mean ± SD)	100.12% ± 0.33	100.23% ± 0.67
Intraday %RSD	0.10–0.13	0.09–0.23
Interday %RSD	0.14–0.27	0.11–0.26
Drug in dosage form (mean ± SD)	96.42% ± 0.57	100.28% ± 0.22

Note. A_s is the absorbance value, C is the concentration of the drug, SD is the standard deviation, and %RSD is the percent relative standard deviation.

Conclusions. The proposed methods proved to be accurate for determination of EG and MT as an economic assay. The methods were applied successfully on the pharmaceutical dosage form with acceptable validation results. The spiking technique was crucial in the developed method, which allowed the direct measurement of the minor component EG in the mixture. The developed methods could be used by QC laboratories for the analysis of recently approved Synjardy® tablets.

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