Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(10):12-14
(http://derpharmachemica.com/archive.html)

Application of Spiking Technique Coupled with Derivative Spectrophotometry for the Analysis of a Novel Anti-diabetic Combination of Two Coformulated Drugs with Highly Different Concentrations

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ABSTRACT

A new first derivative spectrophotometric method was developed for simultaneous determination of empagliflozin and metformin with successful application on their pharmaceutical preparation. A well-established spiking technique was used to increase the concentration of empagliflozin after extraction from tablets to eliminate the deviation from Beer's law which occurs in case of low contribution. Scan of the zero order spectra of the studied drugs was carried out in the range from 200 to 300 nm using Spectra Manager II software® and then the amplitudes of the first derivative spectra were measured against methanol as blank at 223.5 nm and 233.5 nm for metformin and empagliflozin, respectively. Validation parameters were satisfactory over the concentration range of 2-12 µg/mL for both drugs using the proposed economic first derivative spectrophotometric method.

Keywords: Spiking Technique; Economic spectrophotometric method; First Derivative Spectrophotometry.

INTRODUCTION

Empagliflozin (figure 1) is a highly selective sodium glucose transporter-2 inhibitor that improves serum glucose levels by inducing glucosuria. Taken orally, it is rapidly absorbed with linear pharmacokinetics consistent in Asian and Caucasian populations. Empagliflozin treatment demonstrates consistent reductions in hemoglobin A1c, fasting plasma glucose, body weight, and blood pressure in individuals with type 2 diabetes. Improvements in glycemic control and metabolic end points are evident with empagliflozin monotherapy or as add-on to metformin (figure 2). The nonglycemic effects of empagliflozin with consistent improvements in blood pressure, body weight, and waist circumference provide additional rationale for use in patients with type 2 diabetes. Moreover, treatment with empagliflozin has recently shown significant reductions in both microvascular and macrovascular complications of diabetes [1].

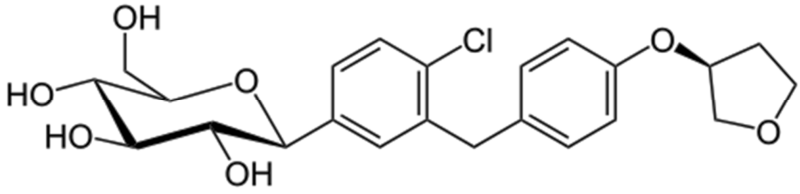

Figure 1: Chemical structure of empagliflozin

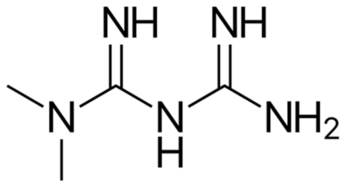

Figure 2: Chemical structure of metformin

Although many analytical procedures were reported for the analysis of the common recently approved anti-diabetic combinations [2-3], and some methods were reported for determination of empagliflozin alone [4-5] or linagliptin alone [6-13] or binary mixture of empagliflozin and linagliptin [14], or binary mixture of linagliptin and metformin [15-24], only one chromatographic method [25] was described for simultaneous determination of empagliflozin and metformin. Furthermore, simultaneous equation and PLS-2 methods manipulating zero order spectra of the drugs were used [26]. The aim of the present work is to develop a new spectrophotometric method that manipulates first derivative spectra of the drugs as an economic fast alternative. Direct UV-absorbance measurement is subject to interference from excipients and degradation products. Among the techniques used to eliminate such interference is derivative spectrophotometry without need for derivatization reaction to enhance the maximum absorption that was required with similar gliptin, saxagliptin, with low lambda max value at 205 nm [27]**.**

MATERIALS AND METHODS

Instrumentation and software

JASCO V630 Double-beam UV–Vis spectrophotometer (S/NC367961148) and Spectra Manager II software were used.

Reference samples and working solutions

Empagliflozin (99.81 %), metformin (100.65 %) and Synjardy® tablets nominally containing 12.5 mg of Empagliflozin and 500 mg of metformin per tablet were supplied from Boehringer Ingelheim pharmaceutical company (Germany). Working solutions (20 µg mL^{-1}) were prepared in methanol (analytical grade).

Extraction of the drugs from Synjardy® tablets

An amount of powdered tablets equivalent to 1.25 mg of empagliflozin and 50 mg of metformin was made up to 50 mL with methanol, sonicated for 30 minutes, filtered and then 0.5 ml of the extract was transferred to a 50 mL volumetric flask, spiked with 5 ml of empagliflozin working solution and finally completed to volume with methanol.

Procedure

Accurately measured aliquots equivalent to 20-120 µg of empagliflozin and metformin were transferred separately into a series of 10 mL volumetric flasks, completed to volume with methanol and then the zero order absorption spectra were recorded against methanol as blank in the wavelength range 200 - 300 nm and then the amplitudes of the first derivative spectra were measured at 223.5 nm and 233.5 nm for metformin and empagliflozin, respectively (figure 3). The amplitudes were plotted against the corresponding concentrations to construct the calibration curves.

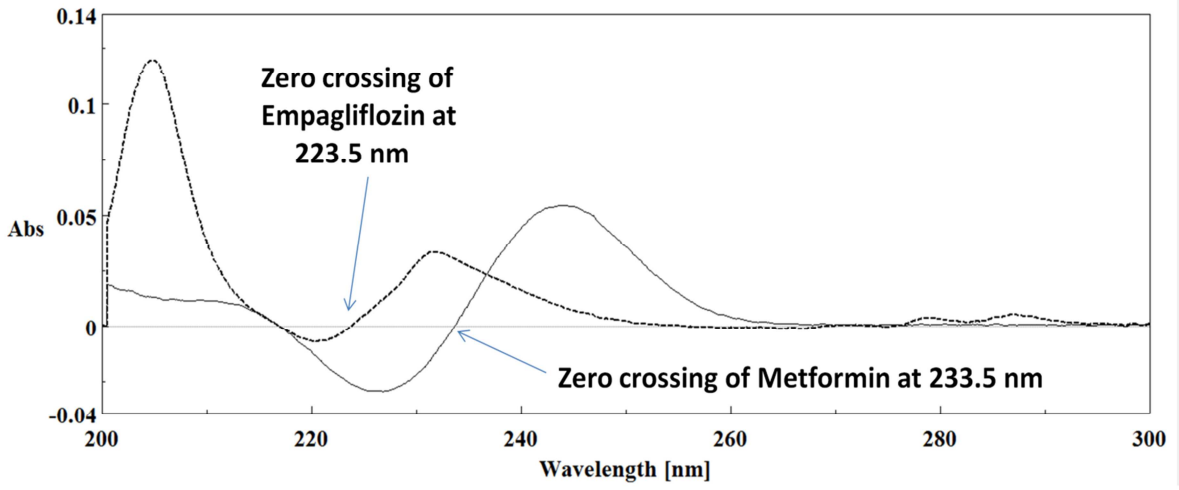

Figure (3): First derivative spectra of empagliflozin 5 µg/mL (- - - -) and metformin 5 µg/mL (⟶) using methanol as blank

Method Validation

Validation parameters were satisfactory over the concentration range of 2-12 µg/mL for both drugs using the proposed economic first derivative spectrophotometric method. A linear correlation was obtained between the amplitude values and its corresponding concentrations and the regression equation was then computed. LOD and LOQ values were found to be (0.51 µg/mL – 1.52 µg/mL) and (0.31 µg/mL – 0.94 µg/mL) for empagliflozin and metformin, respectively. Accuracy was confirmed by calculating the percent recovery of each drug in three laboratory prepared mixtures containing (2.5, 5, 10 µg/mL), and (10, 5, 2.5 µg/mL) of empagliflozin and metformin, respectively in order to obtain ratios of (1:4, 1:1 and 4:1) for the investigated binary mixture. The mean of the percent recovery was between 98.22 and 102.33 % and standard deviation was below 2 %. Precision was confirmed by applying intraday and interday criteria. The percent relative standard deviation (% RSD) of recoveries was found to be less than 2% in the three investigated laboratory prepared mixtures. Statistical comparison with the reported methods [25-26] using *t*-test, *F*-test and ANOVA analysis confirmed that there is no significant difference between the methods making the proposed experiment a fast economic alternative.

Assay of Synjardy® tablets

The diluted tablet extract prepared under (experimental section) was recorded against methanol as blank in the wavelength range 200 - 300 nm and then the amplitudes of the first derivative spectra were measured at 223.5 nm and 233.5 nm for metformin and empagliflozin, respectively. The concentrations of drugs were calculated using the corresponding regression equations. The mean of the percent recovery and standard deviation were 94.47 % ± 0.85 and 101.33 % ± 0.46 for empagliflozin and metformin, respectively.

CONCLUSION

Derivative spectrophotometry is a well-established technique for assaying the drugs in mixtures and in pharmaceutical formulations; enhancing the resolution of overlapping bands. It can be applied for determination of a drug in the presence of another by selecting a wavelength; where contribution of one compound is almost zero while the compound to be determined has a reasonable value. First derivative technique showed that; metformin could be determined by measuring the amplitude at 223.5 nm while empagliflozin could be determined by measuring the amplitude at 233.5 nm with the advantage of narrowing the spectral band and increased sensitivity.

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