Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 168 (2016) 118–122

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Development and validation of simple spectrophotometric and chemometric methods for simultaneous determination of empagliflozin and metformin: Applied to recently approved pharmaceutical formulation

Bassam M. Ayoub *

Pharmaceutical Chemistry Department, Faculty of Pharmacy, British University in Egypt (BUE), El-Sherouk City, Cairo 11837, Egypt
The Center for Drug Research and Development (CDRD), Faculty of Pharmacy, British University in Egypt (BUE), El-Sherouk City, Cairo 11837, Egypt

ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form 18 May 2016
Accepted 4 June 2016
Available online 6 June 2016

Keywords:
Empagliflozin
Metformin
Simultaneous equation
Chemometry
Partial least squares
Pharmaceutical preparation

ABSTRACT

New univariate spectrophotometric method and multivariate chemometric approach were developed and compared for simultaneous determination of empagliflozin and metformin manipulating their zero order absorption spectra with application on their pharmaceutical preparation. Sample enrichment technique was used to increase concentration of empagliflozin after extraction from tablets to allow its simultaneous determination with metformin without prior separation. Validation parameters according to ICH guidelines were satisfactory over the concentration range of 2–12 μg mL^{-1} for both drugs using simultaneous equation with LOD values equal to 0.20 μg mL^{-1} and 0.19 μg mL^{-1}, LOQ values equal to 0.59 μg mL^{-1} and 0.58 μg mL^{-1} for empagliflozin and metformin, respectively. While the optimum results for the chemometric approach using partial least squares method (PLS-2) were obtained using concentration range of 2–10 μg mL^{-1}. The optimized validated methods are suitable for quality control laboratories enable fast and economic determination of the recently approved pharmaceutical combination Synjardy® tablets.

1. Introduction

Empagliflozin (EMP), (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-[[4-[(3*S*)-oxolan-3-yl] oxyphenyl] methyl] phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol (Fig. 1-a) is a new antidiabetic drug from the gliflozin class, recently approved for the treatment of type 2 diabetes [1]. Metformin hydrochloride (MET), *N*,*N*-dimethylimidodicarbonimidic diamide (Fig. 1-b) is the drug of choice in mixed therapy for treatment of type 2 diabetes [2].

According to the literature review, only one chromatographic method was developed for simultaneous determination of EMP and MET [3]. The aim of the new methods is to present the first spectrophotometric and chemometric methods for simultaneous determination of EMP and MET in their pharmaceutical formulation. The major advantages over the reported method [3] are being fast economic methods with lower LOD and LOQ values. A well established sample enrichment technique namely spiking technique was used to enable the analysis of EMP present in low concentration.

The first developed spectrophotometric method is the simultaneous equation method, If a sample contains two drugs (X and Y) with different λ_{max}, Concentration of both drugs (C_x, C_y) can be deduced by a simultaneous equation, using absorptivities of X at λ_1 and λ_2 (ax_1, ax_2), absorptivities of Y at λ_1 and λ_2 (ay_1, ay_2) and absorbance of the sample at λ_1 and λ_2 (A_1, A_2),

$$C_x = (A_2 * ay_1) - (A_1 * ay_2) / (ax_2 * ay_1) - (ax_1 * ay_2), C_y$$
$$= (A_1 * ax_2) - (A_2 * ax_1) / (ax_2 * ay_1) - (ax_1 * ay_2)$$

The requirements are satisfied as λ_{max} of EMP and MET are relatively dissimilar and they do not interact chemically so the total absorbance is the sum of the individual absorbances.

The developed chemometric method is the (partial least squares) PLS-2 method that calculates the number of factors on all the components simultaneously, and one weighed number of factors is optimized [4]. PLS is related to PCR (principal component regression) in that the spectral decomposition is performed on the basis of the maximum variance between spectral data and information about the concentrations is not used, while PLS uses both spectral data and concentration data in modeling.

* Pharmaceutical Chemistry Department, Faculty of Pharmacy, British University in Egypt (BUE), El-Sherouk City, Cairo 11837, Egypt
E-mail addresses: bassam.ayoub@bue.edu.eg, drbassamchemistry@hotmail.com.

http://dx.doi.org/10.1016/j.saa.2016.06.010
1386-1425/

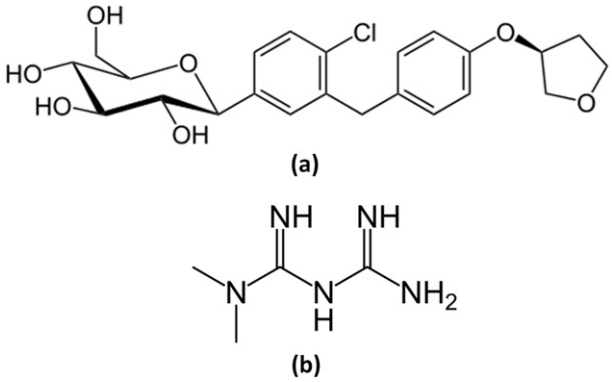

Fig. 1. Chemical structures of empagliflozin (a) and metformin (b).

2. Experimental

2.1. Instrumentation

JASCO V630 Double-beam UV–Vis spectrophotometer (S/NC367961148) was used, with 1.00 cm quartz cells. Scan was carried out in the range from 200 to 300 nm at 0.1 nm intervals. Spectra Manager II software was used.

2.2. Reagents and reference samples

EMP, MET and Synjardy® tablets nominally containing 12.5 mg of EMP and 500 mg of MET per tablet were supplied from Boehringer Ingelheim pharmaceutical company (Germany). EMP and MET purity were found to be 99.81% and 100.65%, respectively according to a reported method [3]. Analytical grade methanol was purchased from S.d. Finechem Limited (Mumbai).

2.3. Preparation of stock solutions and working solutions

Stock solutions of EMP & MET (1 mg mL^{-1}) were prepared separately in methanol, sonicated for 10 min and then working solutions (20 μg mL^{-1}) were prepared by transferring 2 mL of each stock solution to a 100 mL volumetric flask and completed to volume with methanol, kept at 4 °C and discarded after two weeks.

2.4. Sample preparation and spiking sample enrichment technique

Ten tablets of Synjardy® were weighed, powdered and mixed in a mortar. An accurately weighed amount of the finely powdered tablets equivalent to 2.5 mg of EMP and 100 mg of MET was made up to 100 mL with methanol, sonicated to dissolve, filtered and then 1 mL of the extract was transferred to a 100 mL volumetric flask, spiked with 10 mL of EMP working solution and finally completed to volume with methanol. The final concentration of the diluted tablet extract was 2.25 μg mL^{-1} EMP (0.25 μg from tablet and 2 μg from spiking) and 10 μg mL^{-1} MET.

2.5. Procedure for the simultaneous equation spectrophotometric method

The zero-order absorption spectra of 5 μg mL^{-1} for each of EMP and MET were recorded in the range of 200–300 nm against methanol as a blank. EMP and MET showed the maximum absorption (λ_{max}) at 225 nm and 237 nm, respectively.

2.5.1. Linearity

Accurately measured aliquots of working solutions equivalent to 20–120 μg of EMP and MET were transferred separately into a series of 10 mL volumetric flasks, completed to volume with methanol and then the absorbance of each drug was measured at 225 nm (λ_1) and 237 nm (λ_2) using methanol as blank. Four calibration curves were obtained by plotting absorbance against concentration and the absorptivities were calculated for EMP (x) at λ_1 and λ_2 (ax_1, ax_2) and for MET (y) at λ_1 and λ_2 (ay_1, ay_2).

Table 1
Preparation of the laboratory prepared mixtures of EMP and MET.

Mixture number	(EMP:MET) ratio	EMP (μg mL^{-1})	MET (μg mL^{-1})
Mixture 1	5:1	10	2
Mixture 2	3:1	9	3
Mixture 3	1:1	5	5
Mixture 4	1:3	3	9
Mixture 5	1:5	2	10

Table 2
Calibration and validation sets for the PLS-2 method.

Mixture number	EMP	MET
1	6	6
2	6	2
3	2	2
4	2	10
5[a]	10	4
6	4	10
7	10	6
8	6	4
9[a]	4	4
10[a]	4	8
11	8	10
12[a]	10	8
13	8	6
14	6	10
15	10	10
16	10	2
17[a]	2	8
18	8	2
19	2	6
20	6	8
21[a]	8	8
22[a]	8	4
23	4	2
24[a]	2	4
25	4	6

[a] Validation set.

2.5.2. Assay of EMP and MET in lab prepared mixtures (accuracy) and Synjardy® tablets

Different ratios of the laboratory prepared mixtures were prepared as shown in (Table 1), EMP:MET (1:5, 1:3, 1:1, 3:1 and 5:1) and then the absorbances of each mixture and the diluted tablet extract prepared under Section 2.4 were measured at 225 nm (λ_1) and 237 nm (λ_2) using

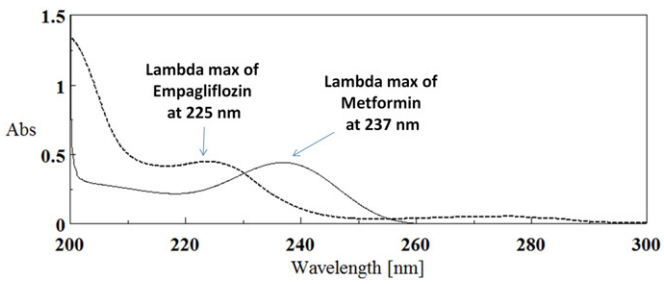

Fig. 2. Absorption spectra of EMP 5 μg mL^{-1} (- - - -) and MET 5 μg mL^{-1} (—) using methanol as blank.