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Artigo

SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF EZETIMIBE AND SIMVASTATIN IN PHARMACEUTICAL PREPARATIONS USING CHEMOMETRIC TECHNIQUES

Ismail Murat Palabiyik* and Feyyaz Onur
Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey
Ceren Yardimci and Nuran Özaltin
Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100 Sihhiye, Ankara, Turkey

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Two spectrophotometric methods are described for the simultaneous determination of ezetimibe (**EZE**) and simvastatin (**SIM**) in pharmaceutical preparations. The obtained data was evaluated by using two different chemometric techniques, Principal Component Regression (PCR) and Partial Least-Squares (PLS-1). In these techniques, the concentration data matrix was prepared by using the mixtures containing these drugs in methanol. The absorbance data matrix corresponding to the concentration data matrix was obtained by the measurements of absorbances in the range of 240 – 300 nm in the intervals with $\Delta\lambda$ = 1 nm at 61 wavelengths in their zero order spectra, then, calibration or regression was obtained by using the absorbance data matrix and concentration data matrix for the prediction of the unknown concentrations of **EZE** and **SIM** in their mixture. The procedure did not require any separation step. The linear range was found to be 5 – 20 μg mL^{-1} for **EZE** and **SIM** in both methods. The accuracy and precision of the methods were assessed. These methods were successfully applied to a pharmaceutical preparation, tablet; and the results were compared with each other.

Keywords: ezetimibe; simvastatin; chemometric techniques.

INTRODUCTION

Ezetimibe (**EZE**) (Figure 1a), is the first in a new class of cholesterol absorption inhibitors that blocks the intestinal absorption of dietary and biliary cholesterol, without affecting the uptake of triglycerides or fat soluble vitamins. Simvastatin (**SIM**) (Figure 1b), is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the last regulated step in the synthesis of cholesterol.[1] The administration of a new agent with a novel mechanism of action, **EZE**, with a well-characterized and effective statin, **SIM**, in a single tablet now appears to provide enhanced treatment without compromising safety. **EZE/SIM** has also been associated with other beneficial effects on lipids, and it achieves greater efficacy than monotherapy with the use of lower, safer doses of the statin.[2]

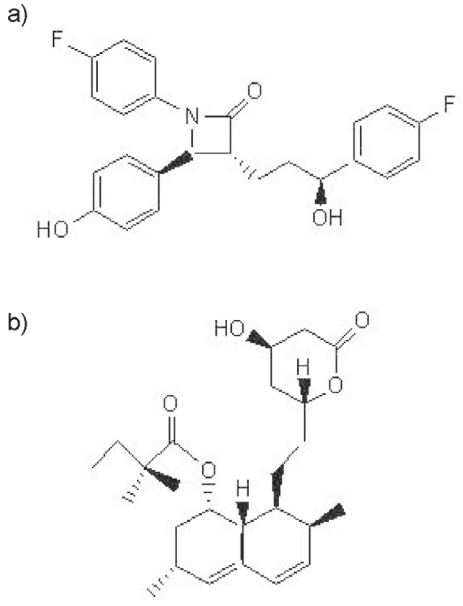

Figure 1. *The structures of (a) ezetimibe and (b) simvastatin*

Literature survey reveals that several methods for the determination of **EZE** in pharmaceutical preparations or in biological fluids including liquid chromatography (LC)[3] and liquid chromatography-tandem mass spectrometry (LC/MS/MS).[4,5] Several methods have also been described for the determination of **SIM**, such as LC,[6,7] LC/MS/MS,[8-12] micellar electrokinetic chromatography (MEKC),[13] spectrophotometry[14] and gas chromatography (GC).[15] To our knowledge, there have been no reports for the simultaneous determination of both drugs in pharmaceutical preparations.

Determination of the ingredients in a pharmaceutical preparation becomes more difficult as the number of components in the mixture increases. Also, dosage forms contain excipients, which might furthermore interfere with the analysis of the active ingredients. Chemometric techniques (multivariate calibration techniques), based on the computer aided instrumentation and algorithms, are employed for the analysis of multicomponent samples. A certain number of calibration methods are available as affordable commercial software is used with existing instruments. The most popular among them include **PCR** (principal component regression) and **PLS** (partial least squares). Multivariate calibration techniques for the resolution of mixtures of analytes with overlapped spectra become a useful tool for developing new analytical methods. All of the chemometric spectral analysis techniques are useful for the resolution of spectral bands overlapping in quantitative determination. Main advantage of these techniques is the simultaneous analysis of the mixture components without chemical pre-treatment or graphical procedure of spectra such as derivative and ratio spectra derivative. They also require shorter time, less

*e-mail: mpala@pharmacy.ankara.edu.tr

costs and simple instrumentation.

The aim of this study was to develop two chemometric techniques in spectrophotometry that allow the simultaneous determination of **EZE** and **SIM** in pharmaceutical preparations.

EXPERIMENTAL

Apparatus

Spectrophotometric measurements were carried out using an Agilent 8453 model UV-VIS spectrophotometer with a diode array detector (190-1100 nm). A 10 mm quartz cell was used.

In chemometric procedure, Multivariate Analysis Add-in for Excel v1.3[16] software was used.

Reagents

EZE and **SIM** reference standards were kindly supplied by Refik Saydam Hygiene Center (Ankara, Turkey) and Eczacibasi Ilaç Sanayi (Istanbul, Turkey), respectively. Pharmaceutical preparation containing **EZE** and **SIM** (Inegy ® containing 10 mg EZE and 20 mg SIM/tablet) were obtained from local pharmacies. All other chemicals were analytical reagent grade.

Standard and sample solutions

Standard solutions

Standard stock solutions (1000 μg mL^{-1}) of **EZE** and **SIM** were prepared separately in methanol. These solutions were kept at +4 °C. Various aliquots of standard solutions were taken, then diluted to 10 mL with methanol to give a final analyte concentration desired.

Sample preparation

Ten tablets were weighed and finely powdered in a mortar. A quantity of the powder equivalent to one tablet was accurately weighed and transferred to a 100 mL volumetric flask including methanol. The flask was sonicated for 15 min and diluted to the mark with methanol. Then an aliquot was centrifuged at 5000 rpm for 10 min. Appropriate amount of clear supernatant was transferred to a 10 mL flask and diluted with methanol. Then the absorbances of these solutions were measured.

Principal Component Regression (PCR)

The original data obtained in absorbances (*A*) and concentrations (*C*) of analytes were reprocessed by standardizing as A_0 and C_0, respectively. Using the ordinary linear regression with coefficients a and b:

$$C = a + b \text{ x A} \quad (1)$$

$b = P \text{ x } q$, where P is the matrix of eigenvectors and q is the C – loadings given by $q = D \text{ x } T^T \text{ x } A_0$. Here T^T is the transpose of the score matrix T. D is a diagonal matrix with the inverse of the selected eigenvalues as components. Knowing b one can easily find a by the formula $a = C_{mean} - A^T_{mean} \text{ x } b$, where A^T_{mean} represents the transpose of the matrix with the entries of the mean absorbance values and C_{mean} is the mean concentration of the calibration set.

Partial Least-Squares (PLS-1)

In the spectral work, the following steps can explain the fundamental concept of PLS-1.

In the UV-Vis spectra, the absorbance data (*A*) and concentration data (*C*) are standardized to give data matrix A_0 and vector C_0. The orthogonalized PLS algorithm has the following steps.

The loading weight vector W has the following expression.

$$W = \frac{A_0^T C_0}{C_0^T C_0} \quad (2)$$

The scores and loadings are given by:

$$t_1 = A_0 W, \quad (3)$$

$$P_1 = \frac{A_0^T t_1}{t_1^T t_1}, \quad (4)$$

$$q_1 = \frac{C_0^T t_1}{t_1^T t_1}, \quad (5)$$

The matrix and vector of the residuals in A_0 and C_0 are:

$$A_1 = A_0 - t_1 p^T_1, \quad (6)$$

$$C_1 = C_0 - t_1 p^T_1 \quad (7)$$

From the general linear equation, the regression coefficients were calculated by:

$$b = W (P^T W)^{-1} q_1, \quad (8)$$

$$a = C_{mean} - A^t_{mean} b. \quad (9)$$

The builded calibration equations are used for the estimation of the compounds in the samples.[17,18]

RESULTS AND DISCUSSION

Figure 2 show the zero-order absorption spectra for **EZE** and **SIM**. In the chemometric techniques for the determination of these drugs in their binary mixture optimum conditions were investigated and absorbance data matrix were obtained by measuring of the absorbances between 240 – 300 nm in the intervals as $\Delta\lambda$ = 1 nm at 61 wavelengths in PCR (n=number of mixed standard=27) and PLS-1 (n=27) in the zero-order absorption spectra of **EZE** + **SIM** mixture in methanol. In the techniques, calibration was obtained by using the absorbance data matrix mentioned above and the concentration data matrix prepared as the concentrations in the mixtures for prediction of the unknown concentrations of **EZE** and **SIM** in their binary mixtures. We observed that good results were obtained by using standardized data in calculation procedures. Training set was designed in 27 laboratory made sample mixtures in the concentration range of 5 –20 μg mL^{-1} for **EZE** and

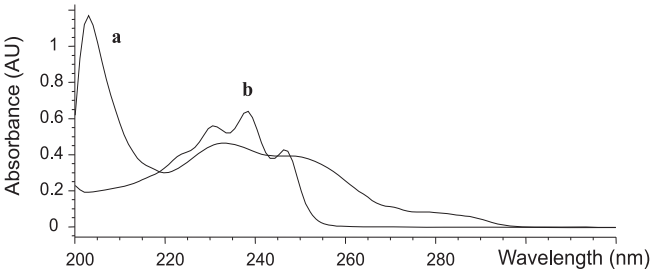

Figure 2. *Zero-order absorption spectra of a) 20 μg mL^{-1} solution of **EZE** and b) 20 μg mL^{-1} solution of **SIM** in methanol*

SIM in PCR and PLS-1 methods (Table 1).

To select the number of factors, in order to model the system without overfitting the concentration data in the PLS-1 and PCR algorithms, a cross-validation method, leaving out one sample at a time was employed using training sets. In PLS-1 technique; six factors for **EZE** and three factors for **SIM** in PCR technique; two factors for **EZE** and five factors for **SIM** in PLS-1 technique were found optimum for the determinations. RMS (root-mean-squares) errors were calculated as 0.61 for **SIM** and 0.99 for **EZE** in PCR method and 0.53 for **SIM** and 1.03 for **EZE** in PLS-1 methods with these factors. The numerical values were calculated by using software mentioned in apparatus section.

Accuracy and precision

Accuracy and precision were studied using three different solutions containing 8, 10 and 15 μg mL^{-1} of both **EZE** and **SIM** in the same solution. These solutions were analyzed in 6 independent series in the same day (intra-day) and 6 consecutive days (inter-day). Table 2 shows the results obtained for intra and inter-day accuracy and precision.

The accuracy of the proposed methods was also tested by recovery experiments. According to official validation guidelines,[19] in cases where it is impossible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product for determining recovery. For this reason, in order to know whether the excipients in the pharmaceutical preparation show any interference with the analysis, the recovery test was done by the standard addition method. The recoveries obtained after three repeated experiments were summarized in Table 3.

Analysis of pharmaceutical preparations

Developed methods were applied to the simultaneous determination of **EZE** and **SIM** in pharmaceutical preparations. Each pharmaceutical preparation was analyzed by performing 6 independent determinations. Satisfactory results were obtained for each compound and were found to be in agreement with label claims (Table 4). The developed methods were compared with each other and no significant difference was observed between them.

Table 1. Training set using in PCR and PLS-1 method for **EZE** and **SIM**

Mixture No	EZE	SIM
1	10	10
2	10	20
3	5	5
4	5	20
5	20	8
6	8	20
7	20	10
8	10	8
9	5	5
10	5	15
11	15	20
12	20	15
13	15	10
14	10	20
15	20	20
16	20	5
17	5	15
18	15	5
19	5	10
20	10	15
21	15	15
22	15	8
23	8	5
24	5	8
25	8	10
26	10	0
27	0	10

Table 2. Accuracy and precision data for **EZE** and **SIM**

	Intra-Day			Inter-Day		
Method			PCR			
Added (μg mL^{-1})	Found[a] (μg mL^{-1})	Precision[b] (R.S.D. (%))	Accuracy[c] (bias %)	Found (μg mL^{-1})	Precision (R.S.D. (%))	Accuracy (bias %)
EZE						
8	8.11 ± 0.06	1.39	1.46	8.18 ± 0.00	0.10	2.26
10	9.98 ± 0.00	0.04	-0.17	10.23 ± 0.01	0.23	2.30
15	14.51 ± 0.05	0.56	-3.25	14.99 ± 0.03	0.36	-0.07
SIM						
8	7.82 ± 0.00	0.03	-2.19	7.62 ± 0.00	0.02	-4.73
10	9.92 ± 0.00	0.13	-0.85	9.64 ± 0.01	0.12	-3.51
15	14.95 ± 0.00	0.02	-0.34	14.65 ± 0.00	0.05	-2.35
Method			PLS			
EZE						
8	8.10 ± 0.02	0.49	1.25	8.18 ± 0.00	0.10	2.25
10	9.94 ± 0.01	0.22	-0.58	10.23 ± 0.01	0.22	2.30
15	14.84 ± 0.01	0.11	-1.03	14.85 ± 0.02	0.28	-1.03
SIM						
8	8.13 ± 0.11	2.43	1.57	7.62 ± 0.00	0.02	-4.73
10	10.12 ± 0.12	2.01	1.15	9.65 ± 0.01	0.12	-3.51
15	15.09 ± 0.06	0.69	0.62	14.65 ± 0.00	0.05	-2.35

[a] Mean ± Standard Error . [b] RSD % : Relative Standard Deviation. [c] Bias % : [(Found – Added) / Added] x 100

Table 3. Recovery results for **EZE** and **SIM** (n=3)

	EZE		**SIM**	
	PCR	PLS-1	PCR	PLS-1
Mean	102.50	103.10	96.90	98.90
± SE [a]	± 0.55	± 0.14	± 0.04	± 0.17
RSD [b]	0.94	0.23	0.08	0.30

[a]Mean ± Standard Error, [b] RSD %: Relative Standard Deviation

Table 4. Assay results of commercial preparations ((Inegy ® containing 10 mg **EZE**/20 mg **SIM**)

	EZE		**SIM**	
Methods	**PCR**	**PLS-1**	**PCR**	**PLS-1**
Mean (mg)	9.95	10.00	19.81	19.96
± SE	± 0.02	± 0.06	± 0.07	± 0.05
RSD	0.48	1.30	0.83	0.56
Bias	0.42	-0.03	-0.94	0.22
t test	0.80		1.64	

RSD=Relative Standard Deviation, Bias % : [(Found – Added) / Added] x 100. Theoretical value for *t* at P : 0.05 level = 2.26

CONCLUSION

The proposed chemometric techniques (PCR and PLS-1) used in spectrophotometric analysis could be applied with great success for the simultaneous determination of **EZE** and **SIM** in their binary mixtures and in a pharmaceutical formulation selected, tablet. The procedures do not require any separation step. Satisfactory results were obtained by these methods but, they need softwares for the mathematical calculations. Using only zero-order spectra in the procedures and not need any other graphical mode, such as ratio mode in the instruments is the advantages for the chemometric methods when compared with ratio spectra derivative spectrophotometric methods. By not needing any time consuming sample preparation procedures and using methanol as solvent, spectrophotometric methods developed are easier and cheaper when compared with the LC methods. These two new spectrophotometric methods were found suitable for simple and precise routine analysis of the pharmaceutical preparations.

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