Iranian J. Math. Chem. 11(1) March (2020) 43 − 55

Iranian Journal of Mathematical Chemistry

Journal homepage: ijmc.kashanu.ac.ir

A Robust Spectrophotometric Method using Least Squares Support Vector Machine for Simultaneous Determination of Anti–Diabetic Drugs and Comparison with the Chromatographic Method

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ARTICLE INFO

Article History:

Received: 18 December 2019
Accepted: 9 January 2020
Published online: 30 March 2020
Academic Editor: Saeed Masoum

Keywords:

Spectrophotometric
Least squares support vector machine
Metformin
Pioglitazone
High-Performance Liquid Chromatography

ABSTRACT

In the present paper, the simultaneous spectrophotometric estimation of Metformin (MET) and Pioglitazone (PIO) in an anti-diabetic drug called Actoplus MET based on least squares support vector machine (LS-SVM) was proposed. The optimum gamma (γ) and sigma (σ) parameters were found to be 825 and 90 with the root mean square error (RMSE) of 0.1343 for MET, as well as 1000 and 350 with RMSE=0.4120 for PIO. Also, the mean recovery values of MET and PIO were 99.81% and 100.19%, respectively. Ultimately, the real sample was analyzed by High-Performance Liquid Chromatography (HPLC) reference method and the proposed procedure. Then, one-way analysis of variance (ANOVA) test at the 95% confidence level was performed on achieved results from HPLC and LS-SVM methods. The statistical data of these methods showed that there were no significant differences between them.

1. INTRODUCTION

One of the lifelong diseases is diabetes, which known with high levels of sugar in the blood. Generally, diabetes is divided into 3 important groups: type I, type II, and gestational. Among them, type II diabetes has been involved 90% of diabetic patients [1].

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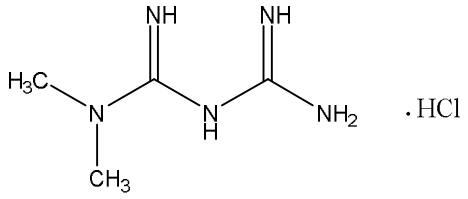
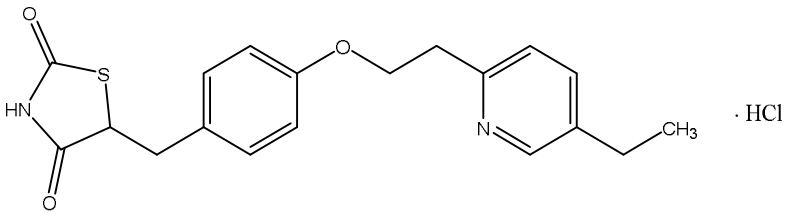
DOI: 10.22052/ijmc.2020.212363.1477

Disorder of insulin secretion and excessive glucose production by the liver lead to type II diabetes. Also, insulin-sensitive organs in the body are fatty tissue and skeletal muscles [2]. In order to treat diabetes type II, oral antidiabetic drugs are widely used such as Metformin (MET) [3]. Its duty is to suppress excessive hepatic glucose production. Decreasing fasting plasma glucose is the main effect of it [4]. MET is chemically known as N, N dimethylimidodicarbonimidic diamide hydrochloride (Figure 1a) [5]. Another drug is Pioglitazone (PIO) from the thiazolidinedione group that used for control of type II diabetes. Also, pioglitazone has useful effects in nonalcoholic steatohepatitis [6]. It is chemically 5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy]phenyl] methyl]-2,4-thiazolidinedione hydrochloride (Figure 1b) [7]. Two or more antihyperglycemic drugs can be adjusted optimal glycemic of type II diabetes patients [8]. So, the combination of MET and PIO is available commercially as a single tablet formulation in the ratio 850: 15 mg (MET: PIO).



(a)



(b)

Figure 1. Chemical structure of (a) MET and (b) PIO.

Several methods such as high performance liquid chromatography (HPLC) [9], reverse phase high performance liquid chromatography (RP-HPLC) [10-12], spectrophotometric [13,14], Thin-layer chromatography (TLC) [15], and high-performance thin-layer chromatography (HPTLC) [16] are used for simultaneous determination of MET in combination with PIO or various drugs. Among these methods, the spectrophotometric technique is accurate, simple, sensitive and rapid [17]. Due to the prior separation of binary or ternary mixtures with the spectral overlap, simultaneous spectrophotometric determination of the compounds is difficult. Spectral overlap of components in the mixture can be resolved without preliminary separation by the combination of chemometrics and

spectrophotometric techniques [18]. The aim of this study was to introduce and compare two methods, including least square support vector machine (LS-SVM) as intelligent, simple, rapid, sensitive, accurate, and low-cost method. Eventually, the HPLC method was performed on a commercial tablet and was compared with the chemometrics method by one-way analysis of variance (ANOVA) test. The results showed no significant difference between the reference method (HPLC) and proposed methods.

2. THEORETICAL BACKGROUND

LS-SVM was introduced as a new style of SVM by Suykens and Vandewalle in 1999 [19]. Indeed, SVM was improved and simplified to produce LS-SVM. An important difference exists between SVM and LS-SVM. A set of linear equations is used for training in the LS-SVM method, whereas the quadratic optimization problem is applied in SVM. It is assumed that training data in the LS-SVM model is (x_i), i=1,2,…,n, which x_i and y_i indicate the input and the output variables, respectively. LS-SVM optimization problem and the limiting conditions of it is presented as follows:

$$Min\ J\ (\omega, b, \xi) = \frac{1}{2} \parallel \omega \parallel^2 + \frac{1}{2}\gamma \sum_{i=1}^{n} \xi_i^2 \quad (1)$$

Subject to:

$$y_i\ [\ w\mathrm{T}\varphi(x_i) + b\ ] = 1 - \xi_i\ , \mathrm{i} = 1,2, \dots , n \quad (2)$$

where w belongs to H, which shows the weight vector. Higher dimensional space specifies with H. Also, γ and $\varphi(x)$ show the regularization factor and the nonlinear mapping function, respectively. Regularization factor can adjust the confidence interval of LS-SVM and the ratio of experimental risk. In addition, the deviation degree of data can measure using ξ_i, which is the slack variable for x_i. Ultimately, b demonstrates bias. Lagrange multipliers α_i, $i = 1,2, \dots , n$ proposed to solve the optimization problems and the objective function is obtained as:

$$L\ (\omega, b, \xi, \alpha) = \frac{1}{2} \parallel \omega \parallel^2 + \frac{1}{2}\gamma \sum_{i=1}^{n} \xi_i^2 - \sum_{i=1}^{n} (\alpha_i y_i [\omega^T \varphi(x_i) + b] - 1 + \xi_i). \quad (3)$$

Using the partial derivatives from Eq. 3, Karush–Kuhn–Tucker (KKT) conditions are shown as follows:

$$\frac{\partial L}{\partial \omega} = 0 \ \rightarrow \ \omega = \sum_{i=1}^{n} \alpha_i\, y_i \varphi(X_i), \quad (4)$$

$$\frac{\partial L}{\partial b} = 0 \ \rightarrow \ \sum_{i=1}^{n} \alpha_i\, y_i = 0, \quad (5)$$

$$\frac{\partial L}{\partial \xi_i} = 0 \ \rightarrow \ \alpha_i = \gamma \xi_i (6),$$

$$\frac{\partial L}{\partial \alpha_i} = 0 \ \rightarrow \ y_i [\omega^{\mathrm{T}} \varphi(x_i) + \mathrm{b}] + \xi_{\mathrm{i}} - 1 = 0. \quad (7)$$

Then, ω and ξ eliminate and Eq. 8 is obtained:

$$\begin{bmatrix} 0 & Y^T \\ Y & \Omega + \frac{1}{\Upsilon} I \end{bmatrix} \begin{bmatrix} b \\ a \end{bmatrix} = \begin{bmatrix} 0 \\ L_n \end{bmatrix}, \quad (8)$$

where $\Omega = ZZT = [\Omega_{ij}]_{\times n}$ indicates a symmetric matrix of size n. Based on Mercer's condition, Eq. 9 was introduced the LS-SVM model.

$$y(x) = \sum_{i=1}^{n} \alpha_i k(x, x_i) + b. \quad (9)$$

In this equation, the nonlinear kernel function is expressed by $K(x,x_i)$. The radial basis function (RBF) is one of the most effective kernel functions compared to some other kernel function. It can simplify the problem of the training proceeding for LS-SVM. So, in this study, RBF was selected as the best kernel function (Eq. 10).

$$k\ (x, x_k) = \exp(-\| x - x_i \|^2 / 2\sigma^2), \quad (10)$$

where σ shows the width parameter [20–22].

3. EXPERIMENTAL

3.1. CHEMICALS

Pure MET and PIO were obtained from Zahravi Pharmaceutical Co. Also, pharmaceutical formulation named Actoplus MET tablet was purchased from Aurobido Pharmaceuticals Co., (Culver, California) labeled to contain 850 mg MET and 15 mg PIO. Ethanol was used as solvent with 99.9% purity from Merck.

3.2. APPARATUS AND SOFTWARE

A T90 UV–V is spectrophotometer (PG Instruments) double beam with 1 cm quartz cell was used to process absorption. The HPLC system was a Varian 9001HPLC system with UV detector. LS-SVM analyses were performed by writing the program in MATLAB (R2015 a). Also, Excel 2013 was applied to carry out calculations.

3.3. PREPARATION OF STANDARD SOLUTIONS

In order to prepare stock solutions of MET and PIO, 25 mg of each component was dissolved in methanol individually and adjusted to the mark in 250 ml volumetric flask. Then, the standard solutions were prepared by dilution of the stock solution with ethanol to obtain concentrations range from 3 to 12 and 5-25 μg mL^{-1} for MET and PIO, respectively.

3.4. PREPARATION OF SYNTHETIC SOLUTIONS

In order to model and validate proposed methods, 30 synthetic mixtures containing various ratios of stock solution of both components were made into a series of 10 ml volumetric

flask. The concentration of these synthetic mixtures was selected randomly. Then, the solutions were scanned and spectra were recorded at 190-300 nm. Absorption results of mixtures along with actual concentrations of the components were entered into the MATLAB software and the LS-SVM method was performed to predict MET and PIO concentrations. The absorption spectra of 30 mixtures is shown in Figure 2.

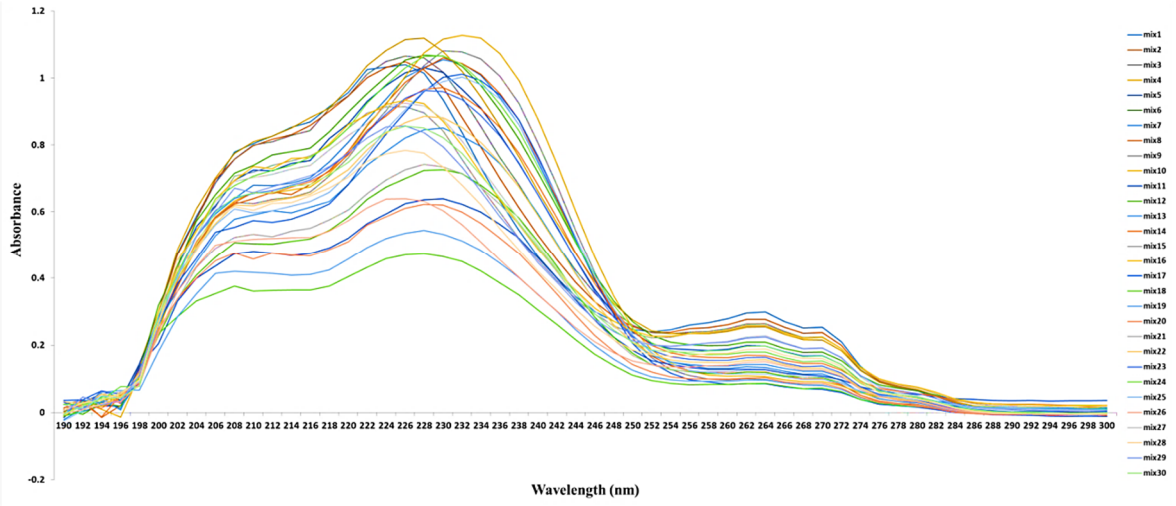


Figure 2. The absorption spectra of 30 synthetic mixtures.

3.5. Sample Preparation

Ten tablets of each Actoplus MET tablet were weighed and finely powdered. Afterward, amounts of the powder equivalent to the average weight of one tablet was dissolved in ethanol. Then, the obtained solution was filtered into 250 ml volumetric flask and adjusted to the mark. Finally, its absorption was recorded.

3.6. Chromatographic Conditions

Compounds were separated on c18 column (150×4.6 mm) with the mobile phase of acetate buffer and acetonitrile (pH 3.0) (58:42; v/v). Before the usage of the mobile phase, it was filtered and degassed. Also, the flow rate and detection were 0.8 ml $min^{-1}$and 255 nm, respectively [8].

3.7. Preparation of HPLC Standard Solutions

The stock solutions of MET and PIO were prepared individually in methanol with concentrations of 100 µg/ml^{-1}. Then, the standard solutions were provided in the range of 2-20 and 4-30 µg ml^{-1} for MET and PIO, respectively [8].

3.8. ANALYSIS OF PHARMACEUTICAL FORMULATION

Ten tablets were weighed and powdered. A quantity equivalent to one tablet was dissolved in a certain amount of methanol. Then, this mixture was shaken vigorously for 30 min. Afterward, centrifuged for 15 min. Finally, the solution was diluted with the mobile phase and injected into the HPLC system [8].

4. RESULTS AND DISCUSSION

4.1. SPECTRAL CHARACTERISTICS

The overlap spectra of both pure drugs are shown in Figure 3, which makes a problem in the analysis of MET and PIO. However, the use of LS-SVM for the MET and PIO combination facilitated their simultaneous determination.

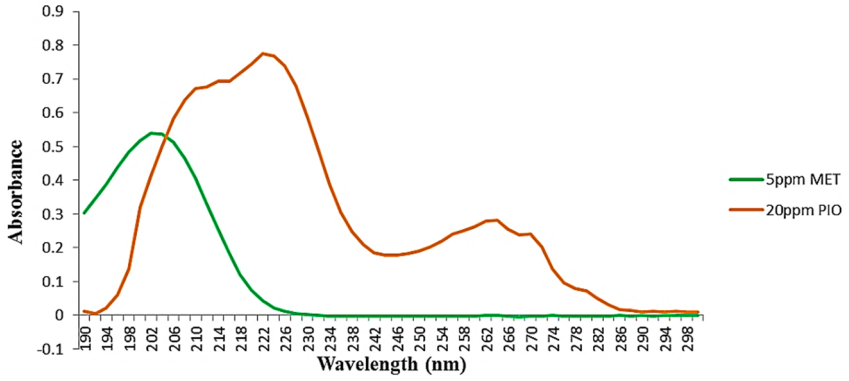


Figure 3. The absorption spectra of MET 5 μg mL^{-1} and PIO 20 μg mL^{-1}.

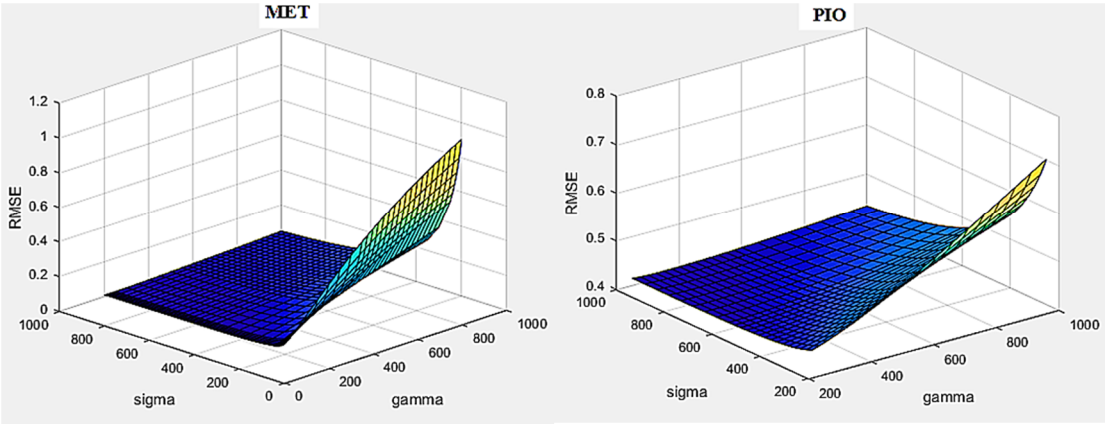
4.2. LS-SVM METHOD

Radial basis function was selected among a various kernel functions. In the LS-SVM method, the data were divided into two groups of training and test sets. In order to perform this method, two major factors should be optimized. These parameters include width parameter (σ) and the regularization parameter (γ) of the RBF kernel function. σ reflects the value of function regression error and the sensitivity of LS-SVM. The role of γ and σ for making a good LS-SVM model with high prediction accuracy and stability is crucial [23].The mentioned parameters were optimized by leave-one-out (LOO) cross-validation method based on the lowest RMSE value. Gamma parameter was optimized for MET and PIO in the range of 50-850 and 250-1000, respectively and the appropriate amount was selected. The optimum sigma parameter was also selected in the same range. As shown in

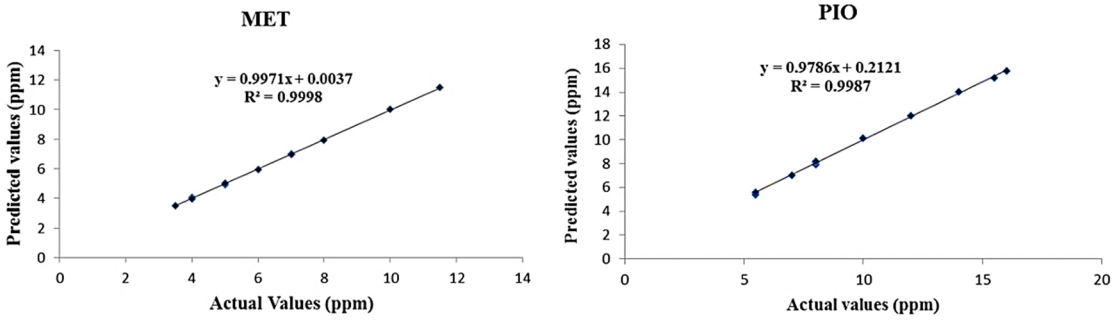
Figure 4a, for the MET component, the optimal parameters of γ and σ with RMSE equal to 0.1343 are 825 and 90, respectively. On the other hand, these optimal parameters for PIO are 1000 and 350, respectively with RMSE= 0.4120. In the optimization of the mentioned parameters, the root mean square error can be calculated by the Eq. 11.

$$RMSE = \left[\sum_{i=1}^{n} \frac{\left(y_{pred} - y_{obs} \right)^2}{n} \right]^{1/2}, \tag{11}$$

where y_{pred} and y_{obs} show the estimated value in the sample and the actual value of the sample, respectively. Also, n is the number of samples in the validation set [24].



(a)



(b)

Figure 4. (a) Parameter optimization response surface for MET and PIO (b) Predicted values against actual values.

Based on the obtained optimal factors, the simultaneous measurement of both drugs was performed in synthetic mixtures and the results of prediction of several mixtures are presented in Table 1. Very low errors and the ideal percentage of the recovery for MET and PIO represent the effective performance of this method. Also, the predicted concentration chart based on actual concentration for analytes is demonstrated in Figure 4b, which indicates the high ability of this method for predicting the concentration of the components.

Table 1. Obtained recovery and RMSE by application of the LSSVM in synthetic mixtures.

Mixtures (μg/ml)		Predicted (μg/ml)		Recovery (%)	
MET	**PIO**	**MET**	**PIO**	**MET**	**PIO**
8	12	7.946	12.046	99.32	100.38
10	8	10.002	7.942	100.02	99.27
11.5	5.5	11.491	5.391	99.92	98.01
5	5.5	4.945	5.589	98.90	101.62
7	8	6.998	8.137	99.97	101.71
3.5	16	3.520	15.802	100.59	98.76
4	15.5	4.051	15.174	101.28	97.89
5	7	5.014	7.013	100.28	100.19
6	8	5.938	8.212	98.98	102.65
7	10	6.947	10.117	99.25	101.17
4	14	3.977	14.064	99.43	100.46
Mean recovery				99.81	100.19
RMSE				0.038	0.151

4.3. METHOD VALIDATION

The statistical values of LS-SVM and HPLC methods are reported in Table 2. The Limits of detection (LOD) and limits of quantitation (LOQ) of the proposed and reference methods were calculated and are represented in this table. LOD is the analyte concentration producing a signal equal to the blank signal, y_B, plus three standard deviations of the blank signal, s_B:

$$\mathrm{LOD} = y_B + 3s_B \tag{12}$$

Similarly, LOQ is the analyte concentration giving a signal equal to the blank signal, y_B, plus ten standard deviations of the blank signal, s_B [25]:

$$\mathrm{LOQ} = y_B + 10s_B \tag{13}$$

Also, the correlation coefficients were calculated, which indicates good linearity. The obtained results confirm that the suggested method is suitable for the simultaneous determination of MET and PIO.

Table 2. The statistical results of calibration standard solutions graphs obtained by the proposed and reference methods.

Parameter	LS-SVM		HPLC	
	MET	PIO	MET	PIO
Linear range(μgml^{-1})	3-12	5-25	2-20	4-30
Slope (a)	0.0773	0.0355	1×10^{-5}	3×10^{-5}
Intercept (b)	0.0416	0.0836	1.2185	0.4272
Regression coefficient (R^2)	0.997	0.9912	0.9989	0.999
Correlation coefficient (r)	0.9984	0.9956	0.9994	0.9995
LOD (µgml^{-1})	0.092	0.173	1.66	0.877
LOQ (µgml^{-1})	0.211	0.383	2.62	1.92

4.4. Results of Commercial Tablet

The Actoplus MET tablet was analyzed by HPLC as a reference method and its chromatogram is shown in Figure 5. The retention time for metformin and pioglitazone was 1.8 and 4.846 min, respectively. Then, the LS-SVM method was used to determine these drugs in pharmaceutical formulation, as well as investigation of the applicability of it. The results of three replicate measurements are listed in Table 3. RSD values of MET and PIO were less than 1 in the LS-SVM method. Also, in order to evaluate the presence or absence of a significant difference between the LS-SVM and HPLC methods, one-way ANOVA test was accomplished. As shown in Table 4, there are no significant errors in the determination of MET and PIO at 95% confidence level by these two methods because the critical F-values (9.552094) was found greater than the calculated F-values (0.185226 for MET and 0.776810 for PIO). It can be concluded that the proposed methods are sufficiently accurate for the simultaneous determination of both drugs.

Table 3. Results of analyzing commercial tablet by proposed methods Claim label (mg): 850 mg MET, 15 mg PIO in each tablet.

	MET		PIO	
Method	LSSVM	HPLC	LSSVM	HPLC
Found (mg)[a]	849.29	844.70	14.96	14.50
Recovery (%)	99.91	99.37	99.75	96.68
RSD (%)	0.630	1.75	0.064	0.503

[a]Mean value of the three measurements

Table 4. One-way ANOVA results by applying the proposed methods to the real sample.

Source of variation	SS	df *	MS	F Calculated	F Critical
Between groups					
MET	4.238152	2	2.119076	0.185226	9.552094
PIO	0.257778	2	0.128889	0.776810	9.552094
Within groups					
MET	34.32141	3	11.44047		
PIO	0.497763	3	0.165921		
Total					
MET	38.55956	5			
PIO	0.7555416	5			

SS, sum of squares; df, degree of freedom; MS, mean squares.
* Degree of freedom for between groups: *h*-1; Within Groups: *h* (*n*-1); Total: hn-1; h, number of methods; n, number of samples of each method.

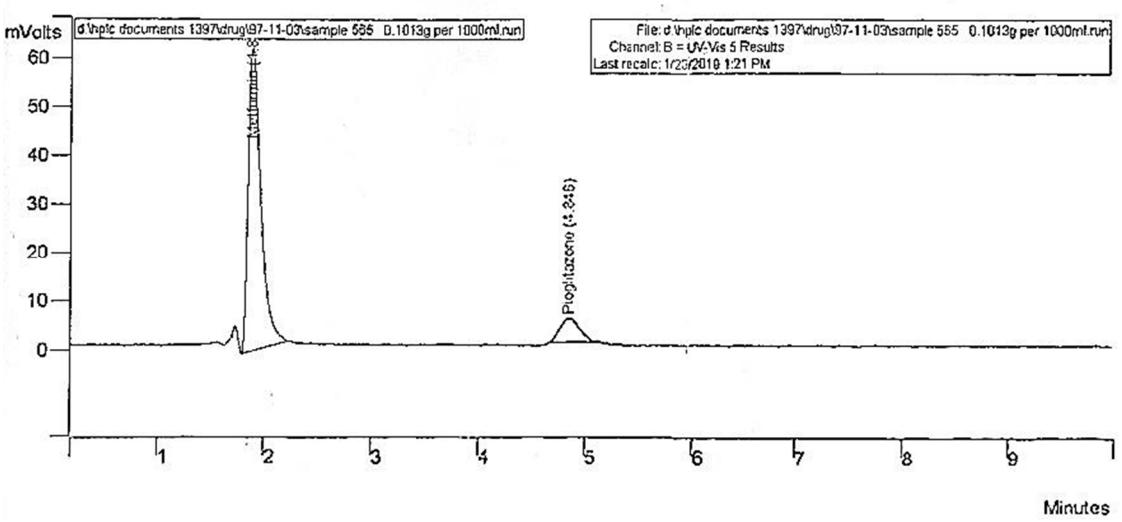


Figure 5. Chromatogram obtained from the commercial tablet containing: 850 mg of MET and 15 mg of PIO.

5. CONCLUSION

In this study, the absorption spectra of MET and PIO has a strong overlap with each other in the binary mixture. Hence, a UV-Vis spectrophotometric method was usedby LS-SVM as an intelligent method for determination of MET and PIO in Actoplus MET tablet. Due to the spectral resolution of this method, the simultaneous quantitative determination of both components is possible. Comparing the obtained results of the LS-SVM method and the HPLC as a reference method, indicate no significant differences between them. Thus, it can be concluded that the developed method is accurate and precise. Moreover, the proposed procedure is simple, fast, economic, and does not require initial pretreatment steps for

quality control and routine analysis of binary and ternary mixtures in pharmaceutical formulations.

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