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Original Article

# Least-Squares Support Vector Machine and its Application in the Simultaneous Quantitative Spectrophotometric Determination of Pharmaceutical Ternary Mixture

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## Abstract

This paper proposes the least-squares support vector machine (LS-SVM) as an intelligent method applied on absorption spectra for the simultaneous determination of paracetamol (PCT), caffeine (CAF), and ibuprofen (IB) in Novafen. The signal to noise ratio (S/N) increased. Also, In the LS - SVM model, Kernel parameter ($\sigma^2$) and capacity factor (C) were optimized. Excellent prediction was shown using LS-SVM, with lower root mean square error (RMSE) and relative standard deviation (RSD). In addition, Regression coefficient ($R^2$), correlation coefficient (r), and mean recovery (%) of this method obtained for PCT, CAF, and IB. LS- SVM / spectrophotometry method is reliable for simultaneous quantitative analysis of components in commercial samples. The results obtained from analyzing the real sample by the proposed method compared to the high-performance liquid chromatography (HPLC) as a reference method. One-way analysis of variance (ANOVA) test at 95% confidence level used and results showed that there was no significant difference between suggested and reference methods.

*Key words***:** least-squares support vector machine, UV Spectroscopy, Paracetamol, Caffeine, Ibuprofen, Novafen

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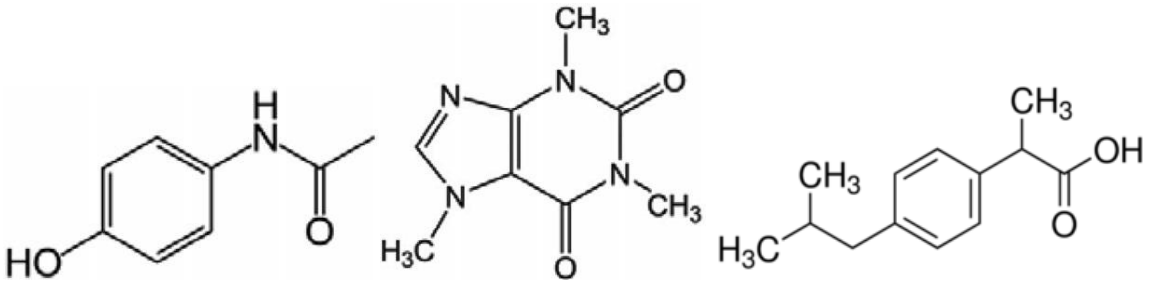
## 1. Introduction

Quantitative chemometrics methods, such as support vector machine (SVM) have been applied for the analysis of mixtures with overlapping spectra. Also, classification is a significant problem in chemometrics field, and support vector machine is a powerful tool for classification and function regression problems [1]. It is an important new methodology in the area of neural networks and nonlinear

modelling [2]. Support vector machines based on a statistical learning theory have been introduced by Vapnik [3]. SVM technique maps the input data which separate linearly into the higher dimension. The papers show that SVM were used for classification and quantification problems [4], pattern recognition problems [5], and feature selection [6]. The highly complicated quadratic programming in the original SVM is a difficult process to calculate and analyze. Therefore, Suykens et al simplified computational calculations of SVM by the performance of Least square support vector machine (LS-SVM) modified version of SVM. Actually, in LS-SVM method, a set of linear equations is applied instead of a quadratic programing problem [7]. The LS-SVM has been used in the data mining, image analysis, network security [8], and analysis of near-infrared spectra of plant samples [9]. This method has solved the over fitting problems and local minimum which can be found in artificial neural network (ANN) [10]. In the following, Novafen as a triple component drug is presented.

Paracetamol (PCT), caffeine (CAF), and ibuprofen (IB) are active principles widely used and mostly combined in pharmaceutical preparations [11]. Paracetamol (PCT) is one of the analgesic and antipyretic agent in drug and its use for decreasing pain and fever [12]. The chemical name of it is N-(4 hydroxyphenyl)-ethanamide [13]. CAF is mainly used as the stimulus central nervous and cardiovascular systems [14]. The chemical name of CAF is 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione [15]. IBF is chemically, RS-2-(4-isobutyl-phenyl) propionic acid, a non-steroidal anti-inflammatory drug that also has good analgesic and antipyretic effects. It uses to relieve the symptoms of arthritis and dysmenorrhea [16]. The combination of PCT, CAF, and IB causes reduction pain due to headache pain, toothache, and neuro-muscular pain [17]. Figure 1 shows the chemical structures of these three drugs [18,19].

As was reported in the literature, several methods like reverse phase high-performance liquid chromatography (RP -HPLC) [20], Thin Layer Chromatography (TLC) [21], High-Performance Liquid Chromatography (HPLC) [22], and capillary electrophoresis with conductivity detection [23] were used for simultaneous determination of acetaminophen, caffeine, and ibuprofen in the tablet. These




**(a)** **(b)** **(c)**

**Figure 1**. Chemical structures of (a) Paracetamol (b) Caffeine (c) Ibuprofen.

methods need pure solvents that are harmful for the environment. Time- consuming and expensive instruments are the other disadvantage of mention methods [24,25]. On the other hand, spectrophotometry technique is a rapid, simple, and inexpensive method, but the spectral overlapping in chemical mixtures is a limiting factor for this technique. Thus, the aim of this study is to couple LS-SVM as a robust model with spectrophotometry method. So, the problem of overlapping spectral of components in ternary mixtures is solved without the need separation components of the drug. In this paper, we report the performance of LS-SVM on synthetic mixtures and the commercial capsule for determination paracetamol, caffeine, and ibuprofen simultaneously. The size of the input matrix for absorbance of 20 synthetic mixtures and concentration of components are 201×20 and 1×20, respectively. The data were divided into two part training and testing with a ratio of 70 to 30. For each dataset linear, Radial Basis Function (RBF) kernel was trained and were evaluated with a test set. Then, error model was calculated in each stage. Also, Genetic Algorithm was used to optimize width function and capacity factor, because mention parameters have great importance in LS-SVM method. Moreover, the effect of interferences was investigated in spike solutions with standard addition method. The reason for using the standard addition method is that the matrix may contain other components that interfere with the analyte signal and cause inaccuracy for determining the concentration of the analyte. On the other hand, the results obtained from analyzing the real sample by the proposed method were compared to the HPLC as a reference method by one-way analysis variance (ANOVA) test.

## 2. Materials and Methods

### *2.1. Instruments and Software*

A T90+uv/vis spectrometer (PG instruments Ltd), double beam spectrophotometer equipped with 1.0 cm quartz cells was applied. Calculations obtained in Microsoft Excel 2013 and MATLAB 2013 (version 7.11). Program of least square support vector machine was written in MATLAB environment.

### *2.2. High- Performance Liquid Chromatography (HPLC)*

A Shimadzu (Kyoto, Japan) HPLC system was used with diode array detector (DAD) and tuned at 220 nm. Chromatographic separation achieved using C18 column (250×4.6 mm, particle size 5$\mu m$). The mobile phase consisting of phosphate buffer and acetonitrile (80:20, v/v). The injection volumes of the samples and standards were 10 µl and eluted at a flow rate of 1 mL $min^{-1}$ at 40 °C [23].

### *2.3. Chemicals*

Pure paracetamol, caffeine, and ibuprofen were kindly supplied by Alhavi Pharmaceutical Company. Commercial capsule (Novafen) consists of 325 mg paracetamol, 40 mg caffeine, and 200 mg ibuprofen obtained by Alhavi Company.

Acetonitrile was purchased from Merck (Darmstadt, Germany) as a solvent.

*2.4. Standard Solutions*

Stock solutions of paracetamol, caffeine, and ibuprofen were prepared separately by dissolving 10 mg of each component in 100 mL acetonitril. Standard calibration solutions of paracetamol, caffeine, and ibuprofen in the concentration range of 10- 35, 5-19, and 10-50 $\mu$g $mL^{-1}$ were individually provided from the stock solutions. The concentration ranges follow the Beer Lambert law and amplitudes of the obtained peaks are suitable for simultaneous determination of the three components. Also, for studying the validity of the proposed method, synthetic mixtures were produced in various concentrations from stock solution.

2.5. Preparation of commercial sample

The coats of ten capsules were opened and the powder of each capsule was weighted individually. A portion of powder equivalent to the weight of one capsule was weighted and dissolved in acetonitril. This solution was filtered into a 1000 ml volumetric flask through Whattman no. 41 filter paper and then adjusted to the mark with distilled water. This solution was further diluted to get the suitable concentration for the UV measurements.

2.6. Methodology of Least-squares Support Vector Machine

Given a training data set are $S = \{(x_i,y_i) \mid i= 1, 2,3,\ldots, N\}$ where each data has d inputs ($x_i \in R^d$) and $y_i \in \{+1,-1\}$ ($x_1$, $y_1$ represent the input and output vector, respectively and R is original space) [26]. In the feature space, a linear function can be described as:

$$y(x) = \omega^T\varphi(x) + b \qquad (1)$$

$\omega$ is the normal vector of the separating hyper-plane, b is the corresponding bias term, and $\varphi(x)$ shows the high dimensional feature spaces which maps in a non-linear manner from the input space x [24,27,28].

The support vector machine method, finds the best hyperplane with maximum margin that separates the data from the two classes. Minimizing the empirical risk functional in the feature space with a squared loss causes a primal optimization problem which follows as [29]:

$$\begin{cases} Min\, J\,(\omega,\xi) = \frac{1}{2}\omega^T\omega + \frac{1}{2}\gamma\sum_{i=0}^{N}\xi_i^2 \\ \text{Subject to: } y_i\,[\,w^T\varphi\,(x_i)+b\,]=1-\xi_i\,,i= 1,2,\ldots, N \end{cases} \qquad (2)$$

$\gamma$ is a regularization parameter, $\xi_i$ is slack variable, $w^T\varphi(x_i) + b$ is a decision function of the linear classifier in a high dimensional feature space, w and b are the parameters of the decision function. To solve the optimization problem in the dual space, the Lagrangian function has been introduced:

$$L\,(\omega,b,\xi,\alpha) = J(\omega,\xi) - \sum_{i=1}^{N}\alpha_i\{\,y_i[\omega^T\varphi(x_i)+b]-1+\xi_i\} \qquad (3)$$

Where $\alpha_i$ is the langrange multiplier [30]. For optimality the Karush–Kuhn–Tucker (KKT) conditions are used:

$$\begin{cases} \dfrac{\partial L}{\partial \omega} = 0 \longrightarrow \omega = \sum_{i=1}^{N} \alpha_i y_i \varphi(x_i) \\ \dfrac{\partial L}{\partial b} = 0 \longrightarrow \sum_{i=1}^{N} \alpha_i y_i = 0 \\ \dfrac{\partial L}{\partial \xi_i} = 0 \longrightarrow \alpha_i = \gamma \xi_i \\ \dfrac{\partial L}{\partial \alpha_i} = 0 \longrightarrow \omega^T \varphi(x_i) + b = 1 - \xi_i \quad i = 1,2,3\ldots,N \end{cases} \tag{4}$$

When $\omega$ and $\xi_i$ be removed from Eq. (4) as a result, vector equation will be created and it is shown as follows:

$$\begin{pmatrix} 0 & yT \\ Y & \Omega + \gamma^{-1} I \end{pmatrix} \begin{pmatrix} b \\ \alpha \end{pmatrix} = \begin{pmatrix} 0 \\ 1 \end{pmatrix} \tag{5}$$

In equations set (5), $\Omega_{ij}=y_i y_j \varphi(x_i)^T \varphi(x_j)$ and $\varphi(x_i)^T \varphi(x_j)=k(x_i,x_j)$, where $k(\cdot,\cdot)$ is the selected kernel function. Hence, $\Omega$ is an n×n matrix. $1= [1, 1, \cdots, 1]^T$ is a n-dimensional column vector, and I is a n×n identity matrix [31,32]. Eventually, the decision function of LS-SVM is represented as follows [29,33]:

$$f(x) = \sum_{i=1}^{N} \alpha_i k(x_i, x) + b \tag{6}$$

According to Mercer's theorem, the RBF kernel function is given by:

$$K(x_i,x_j) = \exp\left[-\|x_i - x_j\|^2 / 2\sigma^2\right] \tag{7}$$

$\sigma^2$ is a tuning parameter which is related to the radial basis function [34,35].

## 3. Results and Discussion

### *3.1. UV Spectra and Proposed Method*

Absorption spectra of standard solutions and synthetic mixtures of drugs recorded in the range of 200 – 400 nm. Figure 2 demonstrates a strong overlapping in the UV spectra of

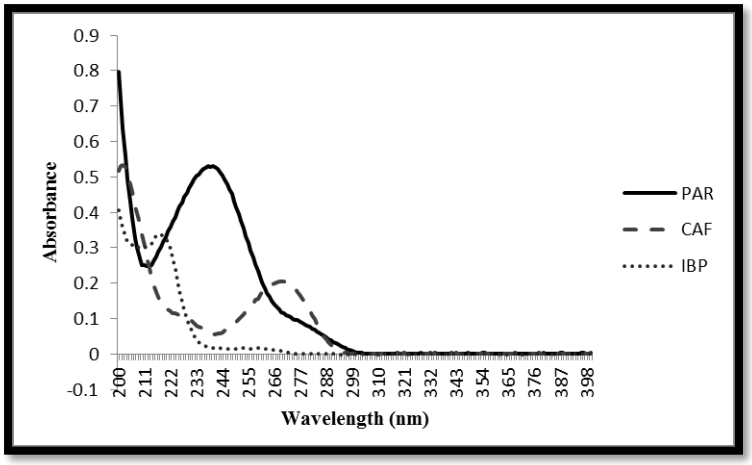



**Figure 2.** The absorption spectra of PCT 20 µg $mL^{-1}$, CAF 10 µg $mL^{-1}$and IB 20 $\mu$g $mL^{-1}$.

paracetamol, caffeine, and ibuprofen. Also, the absorption spectra of commercial capsule and 20 synthetic mixtures are shown in Figure 3 and 4 respectively. Hence, simultaneous determinations of these components by the classical spectrophotometric method were not possible to perform. Least square support vector machine was used for the spectral resolution of ternary mixtures.

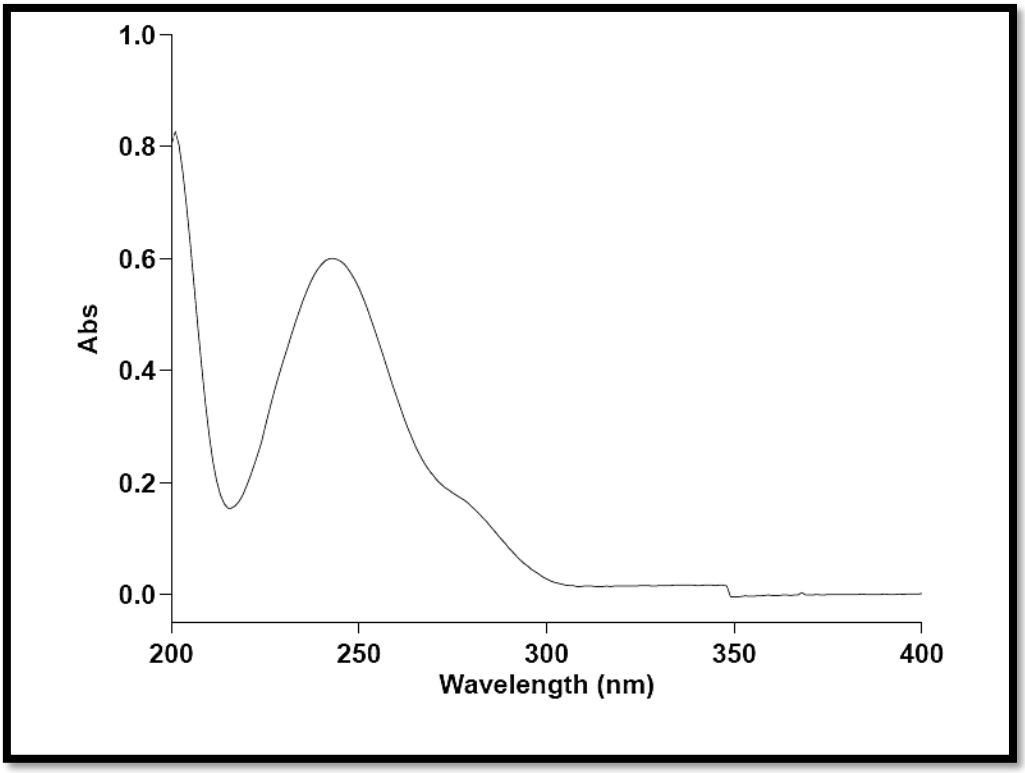



**Figure 3.** The absorption spectra of commercial Novafen as real sample.

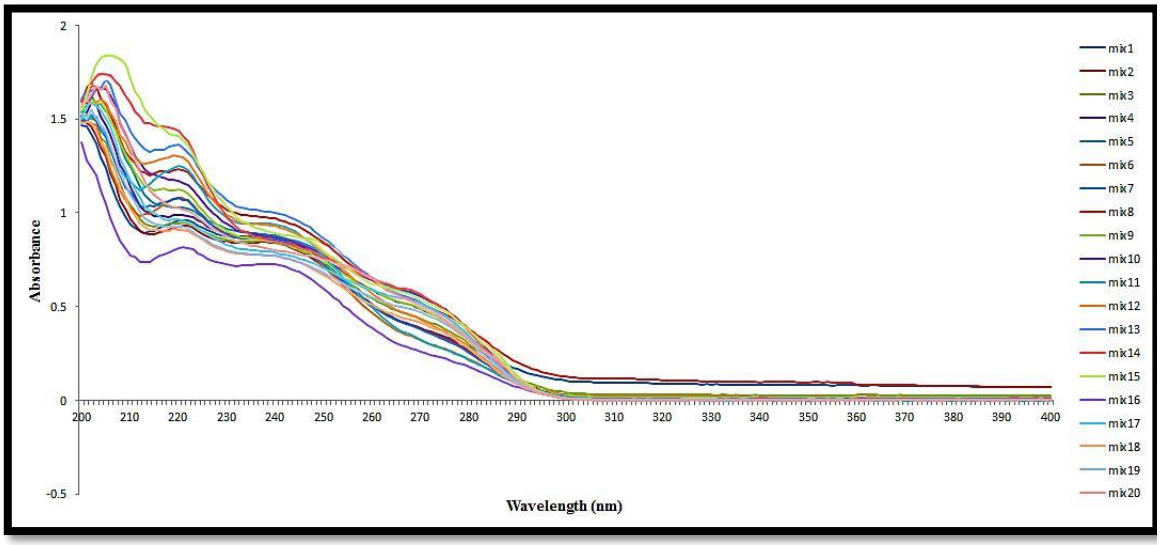



**Figure 4.** The absorption spectra of synthetic mixtures.

*3.2. Least Square Support Vector Machine Method*

In the first one, synthetic mixtures were prepared from Novafen components. Then, the LS-SVM method was used after the spectral measurement. The input of LS-SVM involves the absorption region of synthetic mixtures. In the second one, the radial basis function was selected from among a variety of kernel functions. In the last one, capacity parameter (C) and kernel parameter ($\sigma^2$) must be optimized. High values of capacity parameter cause overfitting and low values of it, creates scattering in prediction of results. To determine the optimal parameters, algorithm genetic was used and the results have been shown in table 1.

The root mean square error (RMSE) of the PCT, CAF, and IB were 0.232, 0.126, and 0.237 respectively.

The root mean square was error used and calculated by the following equation:

$$RMSE = \left[ \sum_{i=1}^{n} \frac{(y_{pred} - y_{obs})^2}{n} \right]^{1/2} \quad (8)$$

Where $y_{pred}$ is estimated value in the sample, $y_{obs}$ is the actual value of the sample and n is the number of samples [36].

Also, regression coefficient ($R^2$), correlation coefficient (r) between actual and predicted values, recovery (%) and relative standard deviation (R.S.D.) were calculated and the results were summarized in table 2 and 3. The

**Table 1**. Optimal values for each parameter using genetic algorithm.

| Parameters | **PCT** | **CAF** | **IB** |
|---|---|---|---|
| $\sigma^2$ | 25.16 | 0.0072 | 0.0022 |
| C | 40.928 | 3.507 | 25.139 |

**Table 2**. The statistical results of calibration standard solutions graphs obtained by the LS-SVM method .

| | **LS-SVM** | | |
|---|---|---|---|
| | **PCT** | **CAF** | **IB** |
| Linear range ($\mu$g mL$^{-1}$) | 10-35 | 5-19 | 10-50 |
| Slope (a) | 0.9944 | 0.9909 | 0.9901 |
| Intercept (b) | 0.1627 | 0.0912 | 0.2439 |
| Regression coefficient ($R^2$) | 0.9994 | 0.9972 | 0.9992 |
| Correlation coefficient (r) | 0.9997 | 0.9981 | 0.9996 |
| LOD ($\mu$gmL$^{-1}$) | 1.00 | 0.67 | 0.86 |
| LOQ ($\mu$gmL$^{-1}$) | 3.01 | 2.03 | 2.31 |

**Table 3.** Recovery data obtained by application of the LS-SVM method to the some synthetic mixtures.

| Actual values ($\mu$g/ml) | | | Predicted values ($\mu$g/ml) | | | Recovery (%) | | |
|---|---|---|---|---|---|---|---|---|
| PCT | CAF | IB | PCT | CAF | IB | PCT | CAF | IB |
| 10 | 10 | 10 | 9.781 | 10.201 | 10.203 | 97.81 | 102.01 | 102.03 |
| 12 | 12 | 5 | 12.234 | 12.011 | 5.111 | 101.95 | 100.09 | 102.23 |
| 35 | 5 | 20 | 34.813 | 4.969 | 19.969 | 99.46 | 99.39 | 99.84 |
| 25 | 10 | 17 | 25.050 | 9.859 | 17.359 | 100.20 | 98.59 | 102.70 |
| 20 | 5 | 19 | 20.360 | 5.134 | 18.874 | 101.80 | 102.68 | 99.33 |
| Mean recovery (%) | | | | | 100.24 | 100.55 | 101.22 | |
| R.S.D | | | | | 1.71 | 1.72 | 1.50 | |

**Table 4.** Results obtained by the LS-SVM and reference methods to the commercial capsule.

| Method | **LS-SVM** | | | **HPLC** | | |
|---|---|---|---|---|---|---|
| | PCT | CAF | IB | PCT | CAF | IB |
| Label claim(mg) | 325 | 40 | 200 | 325 | 40 | 200 |
| Amount found (mg)[a] | 324.47 | 39.57 | 199.31 | 324.58 | 39.6 | 199.29 |
| Recovery (%) | 99.83 | 98.92 | 99.65 | 99.87 | 99.00 | 99.64 |
| SD | 0.026 | 0.047 | 0.040 | 0.056 | 0.02 | 0.050 |
| RSD (%) | 0.008 | 0.118 | 0.020 | 0.017 | 0.050 | 0.025 |

[a] Mean value of the three determinations

regression coefficient was 0.9994, 0.9972, and 0.9992 for PCT, CAF, and IB respectively. Also, the figures of merit consist of limit of detection (LOD) and Limit of quantitation (LOQ) for these components calculated as shown in Table 2. Values of LOD and LOQ showed this method is satisfactory. In addition, relative standard deviation is less than 2 for these components. Eventually, the high values of the regression coefficient, excellent recoveries, low RSD, and RMSE demonstrated that LS-SVM had a powerful ability for predicting the Novafen contents in a drug by increasing the dimension space of the data as mentioned in the methodology section.

### *3.3. Reference Method*

The HPLC was used in this work as the reference method. The retention times of PCT, CAF, and IB were at 6.9, 8.5, and 13.7 min, respectively.

### *3.4. Analysis of Commercial Formulation*

Commercial novafen capsule was produced by Alhavi company and analyzed by using LS-SVM, and HPLC methods. The results are

shown in table 4. In the LS - SVM method, recovery was excellent for PCT, CAF, and IB. Also, the standard deviation (SD) value of novafen contents was less than 0.05. Moreover, this technique has low RSD.

*3.5. Analysis of Variance*

Obtained results of the proposed model and HPLC as a reference method were compared with each other by one-way ANOVA test. Since the calculated F-values (P = 0.05) were less than the critical F-values. No significant differences were observed. The results of this comparison are summarized in table 5.

*3.6. Inferences Effect*

To investigate the effect of excipients on the quantitative analysis, the standard addition

**Table 5.** The ANOVA test results by applying two methods to the real samples.

| Source of variation | SS | df * | MS | F Calculated | F Critical |
|---|---|---|---|---|---|
| **Between groups** | | | | | |
| PCT | 0.002756 | 2 | 0.001378 | 0.154613 | 5.143253 |
| CAF | 0.000289 | 2 | 0.000144 | 0.022298 | 5.143253 |
| IB | 0.002422 | 2 | 0.001211 | 0.389286 | 5.143253 |
| **Within groups** | | | | | |
| PCT | 0.053567 | 6 | 0.008911 | | |
| CAF | 0.038867 | 6 | 0.006478 | | |
| IB | 0.018667 | 6 | 0.003111 | | |
| **Total** | | | | | |
| PCT | 0.056222 | 8 | | | |
| CAF | 0.039156 | 8 | | | |
| IB | 0.021089 | 8 | | | |

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.

**Table 6.** Recovery results obtained from the standard addition technique by application of the proposed method.

| | PCT | CAF | IB |
|---|---|---|---|
| Mean recovery (%) | 100.55 | 98.73 | 99.91 |
| RSD | 0.254 | 1.57 | 0.430 |

method was applied. Pure components of the drug were mixed up with the commercial form, then the prepared solutions were analyzed. Mean recoveries and relative standard deviations of this technique are reported in table 6. The results show that the proposed method has no interference or systematic error.

## 4. Conclusion

This paper proposes the application of LS-SVM as a powerful and rapid method, coupled with spectrophotometry technique to determine the Novafen contents in synthetic mixtures and commercial capsule simultaneously. Therefore, the signal to noise ratio (S/N) increased. The data were divided into two groups: the train and test. The parameters of the model were optimized with training and were evaluated with the test set. The validation results of LS-SVM model with lowest RMSE, RSD, and high correlation coefficient show that the mention technique with good prediction ability is suitable for the simultaneous determination of the PCT, CAF, and IB in the commercial samples. The obtained results from the proposed method were compared with the high performance liquid chromatography (HPLC) as a reference method with one way analysis of variance (ANOVA) at the 95 % confidence level and it was no significant difference between them. Finally, LS-SVM / spectrophotometry method is promising technique to be used for the simultaneous determination of PCT, CAF, and IB for the quality control and routine analysis of the mixtures and commercial products, because they are simple, robust, fast and also they use an inexpensive instrument.

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