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# Spectrophotometric quantitative determination of cilazapril and hydrochlorothiazide in tablets by chemometric methods

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**Abstract**

Four chemometric methods were applied to simultaneous determination of cilazapril and hydrochlorothiazide in tablets. Classical least-square (CLS), inverse least-square (ILS), principal component regression (PCR) and partial least-squares (PLS) methods do not need any priori graphical treatment of the overlapping spectra of two drugs in a mixture. For all chemometric calibrations a concentration set of the random mixture consisting of the two drugs in 0.1 M HCI and methanol (1:1) was prepared. The absorbance data in the UV–Vis spectra were measured for the 15 wavelength points (from 222 to 276 nm) in the spectral region 210–290 nm considering the intervals of $\Delta\lambda = 4$ nm. The calibration of the investigated methods involves only absorbance and concentration data matrices. The developed calibrations were tested for the synthetic mixtures consisting of two drugs and using the *Maple V* software the chemometric calculations were performed. The results of the methods were compared each other as well as with HPLC method and a good agreement was found. 

*Keywords:* Chemometric methods; Quantitative determination; Cilazapril; Hydrochlorothiazide; Tablets

## 1. Introduction

In the pharmaceutical formulations the combination of cilazapril (CA) and hydrochlorothiazide (HCT) is widely given to patients as antihypertensive and diuretic agent. On the other hand, these drugs are becoming important for the quality control in the commercial pharmaceutical tablets.

During the last decade the powerful chemometric methods classical least-square (CLS), inverse least-square (ILS), principal component regression (PCR) and partial least-squares (PLS) were used in the spectral data analysis for the mixtures containing two or more compounds with overlapping spectra [1–5]. These methods have a huge range of applications, e.g. spectrophotometric [6–9] chromatographic [9] and electrochemical [10] quantitative analysis.

During the last years the quantitative analysis of HCT in its binary mixtures with benazepril by

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chemometric methods [11], by spectrophotometry [12–15], and by HPLC [15–19], with amiloride by spectrophotometry [18–21], by chemometric method [22] and by HPLC [23,24], with captoril by spectrophotometry [25,26] and by HPLC [26–28], with enalapril maleate by spectrophotometry [29] and by HPLC [30,31], with lisinopril by spectrophotometry [32] and by HPLC [33], with spironolactone by partial least-square method [34] by flow injection analysis and spectrophotometry [35,36] and by HPLC [37], with cilazapril by spectrophotometry [36] and by HPLC [38], with ramipril by spectrophotometry [36], with fosinopril by spectrophotometry [39,40] and by HPLC [40], with losartan by HPLC [41–43], with triamterene by spectrophotometry [37] and by HPLC [44], with chlorothiazide by HPLC [45,46], with reserpine by HPLC [47,48], with propanolol by spectrophotometry [49] and by HPLC [50], with bevantolol by chromatographic and spectrophotometric methods [51], with valsartan by HPLC [52], with dihydralazine sulfate by conventional and differential pulse polarography [53] have been reported in the literature.

The chemometric techniques are based on a solid mathematical and statistical background and we believe that it is necessary to explain clearly the steps we followed in all methods. On the other hand, the fundamental advantages of our investigated methods are the simultaneously analysis of the several mixture components without any chemical pre-treatment and during a short period of time, as well as no expensive costs and complex instruments are required.

In this study four chemometric methods were applied to analyse the synthetic mixtures and tablets consisting of HCT and CA in the presence of interferences of the absorption spectra and to compare the obtained results with those given by HPLC literature method.

## 2. Experimental

### 2.1. Instruments

To record the absorption spectra and their absorbance measurements we used a Shimadzu UV-160 double beam UV–Visible spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a HP DeskJet 600 printer.

### 2.2. Pharmaceutical tablet formulations

A commercial pharmaceutical formulation (Inhibace Plus® Tablet produced by Roche Pharm. Turkey. Batch no.10 212) containing 12.5 mg HCT and 5 mg CA was analysed by the proposed chemometric methods.

### 2.3. Standard solutions

Stock solutions of 100 mg/100 ml HCT and CA were prepared in 0.1 M HCl and methanol (1:1). A training set consisting of 14 binary mixture solutions in the possible combinations containing 0–12 μg/ml HCT and 0–20 μg/ml CA was used for the chemometric calibrations. A validation set containing the synthetic mixtures in the range of 2–12 μg/ml for HCT and 2–20 μg/ml for CA was prepared by using the above stock solutions.

## 3. Chemometric algorithms

### 3.1. CLS and ILS

The Bouguer–Beer–Lambert law and its inverse expression of UV–Vis spectroscopy applied to multiple linear regression leads us to CLS and ILS methods, respectively. The mathematical formulations of these methods, in the matrix compact form can be written as $\mathbf{A} = K \times \mathbf{C}$ for CLS and $\mathbf{C} = P \times \mathbf{A}$ for ILS [1]. Here, the matrix $\mathbf{A}$ represents the absorbance matrix, $\mathbf{C}$ is the concentration matrix, and $K$ and $P$ are the calibration coefficients.

### 3.2. PCR

In the spectral work, the following steps can explain the fundamental concept of PCR [28]:

(a) The original data obtained in absorbances ($A$) and concentrations ($C$) of analytes were reprocessed by mean-centring as $\mathbf{A}_o$ and $\mathbf{C}_o$, respectively.

(b) The covariance dispersion matrix of the centered matrix $\mathbf{A}_o$ was computed. The normalized eigenvalues and eigenvectors were calculated starting from square covariance matrix. The number of the optimal principal components (eigenvectors) is selected by considering only the highest values of the eigenvalues. The other eigenvalues and their corresponding eigenvectors are eliminated from our study. Using the ordinary linear regression $\mathbf{C} = a + b \times \mathbf{A}$ we calculated the coefficients $a$ and $b$. To reach this objective firstly we determined the coefficient $b$ as $b = \mathbf{P} \times q$, where $\mathbf{P}$ is the matrix of eigenvectors and $q$ is the $C$-loadings given by $q = \mathbf{D} \times \mathbf{T}^T \times \mathbf{A}_o$. Here $\mathbf{T}^T$ is the transpose of the score matrix $\mathbf{T}$. $\mathbf{D}$ is a diagonal matrix having on the components the inverse of the selected eigenvalues. Knowing $b$ we can easily find $a$ by using the formula $a = C_{mean} - \mathbf{A}^T_{mean} \times b$, where $\mathbf{A}^T_{mean}$ represents the transpose of the matrix having the entries of the mean absorbance values and $C_{mean}$ is the mean concentration of the calibration set.

### 3.3. PLS

The PLS calibration technique using the orthogonalized PLS algorithm developed by Wold [20,21] and extensively discussed by Martens and Naes [12] involves simultaneously the independent and the dependent variables on the data compression and decomposition operations.

In the UV–Vis spectra, the absorbance data ($A$) and concentration data ($C$) are mean centred to give data matrix $\mathbf{A}_o$ and vector $\mathbf{C}_o$. The orthogonalized PLS algorithm has the following steps:

(a) The loading weight vector $W$ has the following expression:

$$W = \mathbf{A}'_o\mathbf{C}_o/\mathbf{C}'_o\mathbf{C}_o. \quad (1)$$

(b) The scores and loadings are given by:

$$t_1 = \mathbf{A}_o\mathbf{W}_1,$$
$$P_1 = (\mathbf{A}^T_o\, t_1)/(t^T_1\, t_1),$$
$$q_1 = (\mathbf{C}^T_o\, t_1)/(t^T_1\, t_1). \quad (2)$$

(c) The matrix and vector of the residuals in $\mathbf{A}_o$ and $\mathbf{C}_o$ are:

$$\mathbf{A}_1 = \mathbf{A}_o - t_1\, P^T_1,$$
$$\mathbf{C}_1 = \mathbf{C}_o - t_1\, q^T_1. \quad (3)$$

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

$$b = W(P^T W)^{-1} q, \quad (4)$$
$$a = C_{mean} - \mathbf{A}^T_{mean}\, b. \quad (5)$$

As in PCR method, the builded calibration equation is used for the estimation of the compounds in the samples.

## 4. Results and discussion

A calibration set was randomly prepared as mixtures of HTC and CA in the possible compositions in 0.1 HCl and methanol (1:1) and it was illustrated in Table 1. The UV–Vis spectra of this calibration set were recorded in the spectrophotometer and their absorbances were measured at 15 points corresponding to the selected wavelengths from 220 to 276 nm in the region of 210–290 nm as it shown in Fig. 1. By using the correlation

Table 1
Composition of the concentration (training) set for both drugs

| Standard no: | CA (μg/ml) | HTC (μg/ml) |
|---|---|---|
| 1 | 2.0 | 10.0 |
| 2 | 4.0 | 10.0 |
| 3 | 8.0 | 10.0 |
| 4 | 12.0 | 10.0 |
| 5 | 16.0 | 10.0 |
| 6 | 20.0 | 10.0 |
| 7 | 12.0 | 0.0 |
| 8 | 4.0 | 2.0 |
| 9 | 4.0 | 4.0 |
| 10 | 4.0 | 6.0 |
| 11 | 4.0 | 8.0 |
| 12 | 4.0 | 10.0 |
| 13 | 4.0 | 12.0 |
| 14 | 0.0 | 6.0 |

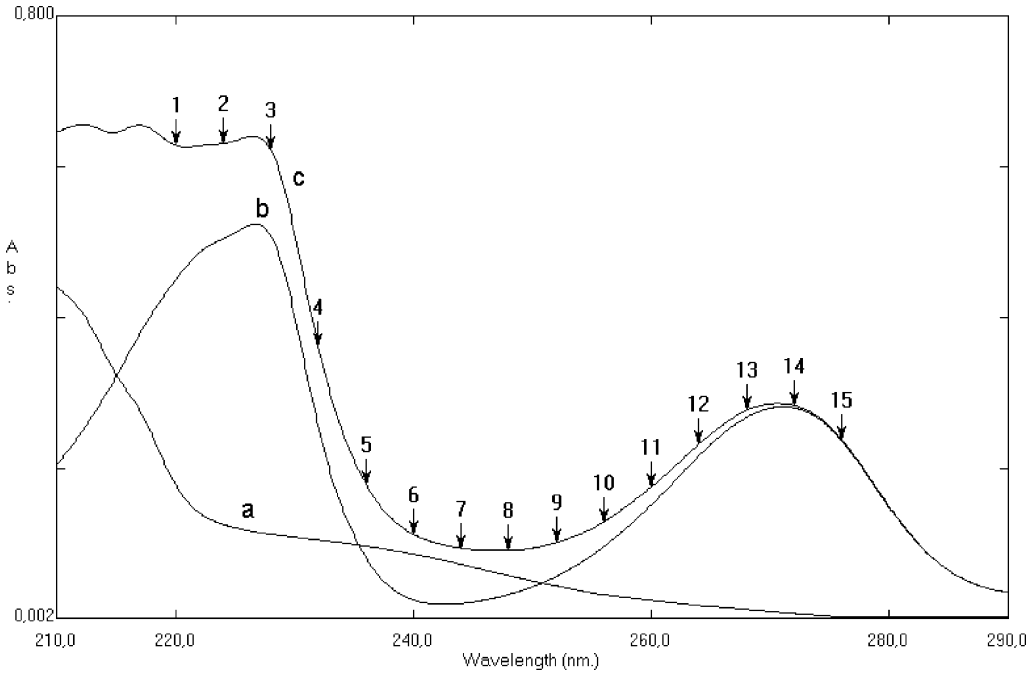


Fig. 1. Absorption spectra of (a) 12 μg/ml hidrochlorothiazide; (b) 28 μg/ml CA; and (c) their mixture in 0.1 HCl and methanol (1:1) ($\downarrow^{1}, \downarrow^{2}, \ldots, \downarrow^{15}$ corresponding to $\lambda_1$, $\lambda_2 \ldots \lambda_{15}$ (from 220.0 to 276.0 nm))

between the calibration concentration and its absorbance data the chemometric calibrations were computed within the CLS, ILS, PCR and PLS algorithms. Below, the contents of HCT and CA in the mixtures and tablets were calculated by the chemometric calibrations. The obtained results were compared by each other and with those given by the HPLC method [38]. We observe that our recovery and tablet results are better than those provided by HPLC method. We conclude that the concentration range of our methods is suitable for determination of small quantities of subject matter drugs

$$\begin{bmatrix} \mathbf{A}_1 \\ \mathbf{A}_2 \\ \mathbf{A}_3 \\ \mathbf{A}_4 \\ \mathbf{A}_5 \\ \mathbf{A}_6 \\ \mathbf{A}_7 \\ \mathbf{A}_8 \\ \mathbf{A}_9 \\ \mathbf{A}_{10} \\ \mathbf{A}_{11} \\ \mathbf{A}_{12} \\ \mathbf{A}_{13} \\ \mathbf{A}_{14} \\ \mathbf{A}_{15} \end{bmatrix} = \begin{bmatrix} 11.86\times10^{-3} & 10.75\times10^{-2} \\ 8.89\times10^{-3} & 11.96\times10^{-2} \\ 8.03\times10^{-3} & 11.99\times10^{-2} \\ 7.19\times10^{-3} & 6.00\times10^{-2} \\ 6.29\times10^{-3} & 1.95\times10^{-2} \\ 5.57\times10^{-3} & 6.29\times10^{-3} \\ 4.47\times10^{-3} & 5.14\times10^{-3} \\ 3.81\times10^{-3} & 7.98\times10^{-3} \\ 3.00\times10^{-3} & 1.37\times10^{-2} \\ 2.23\times10^{-3} & 2.31\times10^{-2} \\ 1.73\times10^{-3} & 3.61\times10^{-2} \\ 1.42\times10^{-3} & 5.11\times10^{-2} \\ 1.07\times10^{-3} & 6.34\times10^{-2} \\ 7.19\times10^{-4} & 6.62\times10^{-2} \\ 5.33\times10^{-4} & 5.56\times10^{-2} \end{bmatrix} \begin{bmatrix} C_{\mathrm{CA}} \\ C_{\mathrm{HTC}} \end{bmatrix}$$

### 4.1. CLS method

In this technique, the coefficient matrix (**K**) was calculated by using the linear equation system between the absorbance data and training set. Replacing the coefficient matrix (**K**) into the linear equation system, the calibration of CLS can be written as:

where, $C_{\mathrm{CA}}$ and $C_{\mathrm{HTC}}$ are the concentration of HCT and CA, respectively. The absorbance values at the 15 wavelengths with the interval of $\Delta\lambda = 4$ nm in the range 210–290 nm for the samples were replaced in the above CLS calibration and the content of two drugs in synthetic mixtures and tablet was calculated.

### 4.2. ILS method

In this method, the coefficient matrix (**P**) was obtained from the linear equation system using the absorbance data and the training set. Introducing (**P**) into the linear equation system we obtain the calibration for ILS as:

$$\begin{bmatrix} C_{CA} \\ C_{HTC} \end{bmatrix} = \begin{bmatrix} 26.76 & 2.25 & -3.41 & 18.62 & 31.35 & 32.76 & 27.95 & 20.74 & 12.92 & 3.76 & -5.40 & -14.30 & -22.12 & -25.63 & -21.98 \\ -0.13 & 1.81 & 2.22 & -0.34 & -1.92 & -2.23 & -1.91 & -1.35 & -0.69 & 0.11 & 0.98 & 1.86 & 2.63 & 2.92 & 2.49 \end{bmatrix} \begin{bmatrix} \mathbf{A}_1 \\ \mathbf{A}_2 \\ \mathbf{A}_3 \\ \mathbf{A}_4 \\ \mathbf{A}_5 \\ \mathbf{A}_6 \\ \mathbf{A}_7 \\ \mathbf{A}_8 \\ \mathbf{A}_9 \\ \mathbf{A}_{10} \\ \mathbf{A}_{11} \\ \mathbf{A}_{12} \\ \mathbf{A}_{13} \\ \mathbf{A}_{14} \\ \mathbf{A}_{15} \end{bmatrix}.$$

In this calibration, $C_{CA}$ and $C_{HTC}$ are the concentration of CA and HCT, respectively. The absorbance values of the samples, at the 15 wavelengths in the spectral region from 210 to 290 nm, were replaced in the above equation and the amounts of CA and HCT in the synthetic mixtures and tablets were found.

### 4.3. PCR method

The PCR calibration was constructed by using the PCR algorithm as it was explained above. For our drugs we obtain the following:

$$C_{CA} = 0.42 + 17.09\mathbf{A}_1 + 7.58\mathbf{A}_2 + 2.78\mathbf{A}_3 + 20.67\mathbf{A}_4 + 33.89\mathbf{A}_5 + 32.88\mathbf{A}_6 + 28.28\mathbf{A}_7 + 19.32\mathbf{A}_8 + 11.03\mathbf{A}_9 + 1.95\mathbf{A}_{10} - 7.91\mathbf{A}_{11} - 15.51\mathbf{A}_{12} - 25.81\mathbf{A}_{13} - 28.81\mathbf{A}_{14} - 19\,989\mathbf{A}_{15},$$

and

$$C_{HCT} = -0.09 - 23.07\mathbf{A}_1 + 10.89\mathbf{A}_2 + 0.59\mathbf{A}_3 - 9.22\mathbf{A}_4 - 11.25\mathbf{A}_5 - 11.34\mathbf{A}_6 - 1151\mathbf{A}_7 - 11.33\mathbf{A}_8 - 8.08\mathbf{A}_9 - 6.85\mathbf{A}_{10} + 0.98\mathbf{A}_{11} - 15.64\mathbf{A}_{12} + 1.04\mathbf{A}_{13} - 4.87\mathbf{A}_{14} - 4.60\mathbf{A}_{15}.$$

Here, $C_{CA}$ and $C_{HTC}$ are the concentration of HCT and CA, respectively. The absorbance values, measured at 15 points in the range of 210–290 nm, were introduced in the above equations and the quantity of each drug in mixtures and tablets was determined.

Table 2
Statistical results of chemometric methods in the calibration step

| Component | CLS | ILS | PCR | | PLS | |
|---|---|---|---|---|---|---|
| | SEC | SEC | SEC | PRESS | SEC | PRESS |
| HCT | 0.11 | 0.11 | 0.08 | 0.08 | 0.11 | 0.16 |
| CA | 0.15 | 0.15 | 0.09 | 0.10 | 0.12 | 0.17 |

Table 3
Statistical parameters of synthetic mixtures

| Component | CLS | | | | ILS | | | | PCR | | | | PLS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SEP | *a* | *b* | *r* | SEP | *a* | *b* | *r* | SEP | *a* | *b* | *r* | SEP | *a* | *b* | *r* |
| HCT | 0.10 | 0.053 | 0.993 | 1.000 | 0.10 | 0.008 | 0.999 | 1.000 | 0.07 | −0.052 | 1.007 | 1.000 | 0.10 | 0.009 | 1.001 | 0.999 |
| CA | 0.12 | 0.081 | 0.991 | 1.000 | 0.11 | 0.000 | 1.000 | 1.000 | 0.09 | −0.066 | 1.000 | 1.000 | 0.35 | 0.051 | 1.002 | 1.000 |

*a*, intercept; *b*, slope, *r*, correlation coefficient.

Table 4
Recoveries obtained for the determination of CA and HCT in different synthetic mixtures by using the proposed chemometric techniques

| Mixture | | CLS | | ILS | | PCR | | PLS | | Error % | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Added (μg) | | Recovery (%) | | Recovery (%) | | Recovery (%) | | Recovery (%) | | CLS | | ILS | | PCR | | PLS | |
| CA | HCT | CA | HCT | CA | HCT | CA | HCT | CA | HCT | CA | HCT | CA | HCT | CA | HCT | CA | HCT |
| 2 | 10 | 103.0 | 100.3 | 102.5 | 100.2 | 104.5 | 100.4 | 98.5 | 100.2 | 3.0 | 0.3 | 2.5 | 0.2 | 4.5 | 0.4 | −1.5 | 0.2 |
| 4 | 10 | 99.0 | 99.4 | 98.8 | 99.5 | 98.8 | 98.8 | 97.5 | 99.3 | −1.0 | −0.6 | −1.2 | −0.5 | −1.2 | −1.2 | −2.5 | −0.7 |
| 8 | 10 | 98.5 | 99.3 | 98.6 | 99.4 | 98.3 | 98.9 | 96.6 | 99.3 | −1.5 | −0.7 | −1.3 | −0.6 | −1.7 | −1.1 | −3.4 | −0.7 |
| 12 | 10 | 102.5 | 102.7 | 102.4 | 102.7 | 100.8 | 100.9 | 100.0 | 102.8 | 2.5 | 2.7 | 2.5 | 2.7 | 0.8 | 0.9 | 0.0 | 2.8 |
| 16 | 10 | 100.1 | 99.4 | 100.0 | 99.4 | 99.3 | 99.5 | 99.4 | 99.6 | 0.1 | −0.6 | 0.0 | −0.6 | −0.7 | −0.5 | −0.6 | −0.4 |
| 20 | 10 | 100.1 | 99.9 | 100.2 | 99.9 | 100.3 | 100.9 | 99.75 | 100.0 | 0.1 | −0.1 | 0.2 | −0.1 | 0.3 | 0.9 | −0.2 | 0.0 |
| 4 | 2 | 97.3 | 100.5 | 97.5 | 100.0 | 103.0 | 104.5 | 101.0 | 103.0 | −2.7 | 0.5 | −2.5 | 0.0 | 3.0 | 4.5 | 1.0 | 3.0 |
| 4 | 4 | 97.3 | 99.5 | 97.0 | 99.5 | 100.5 | 99.0 | 98.3 | 98.5 | −2.7 | −0.5 | −3.0 | −0.5 | 0.5 | −1.0 | −1.7 | −1.5 |
| 4 | 6 | 97.3 | 99.5 | 97.5 | 99.5 | 99.0 | 99.0 | 97.5 | 99.0 | −2.7 | −0.5 | −2.5 | −0.5 | −1.0 | −1.0 | −2.5 | −1.0 |
| 4 | 8 | 99.8 | 99.6 | 100.0 | 99.8 | 101.0 | 100.0 | 99.0 | 99.5 | −0.2 | −0.4 | 0.0 | −0.2 | 1.0 | 0.0 | −1.0 | −0.5 |
| 4 | 10 | 101.3 | 98.6 | 101.0 | 98.6 | 102.0 | 99.4 | 101.8 | 98.5 | 1.3 | −1.4 | 1.0 | −1.4 | 2.0 | −0.6 | 1.7 | −1.5 |
| 4 | 12 | 98.3 | 99.3 | 98.5 | 99.3 | 97.5 | 99.0 | 97.5 | 99.5 | −1.7 | −0.7 | −1.5 | −0.7 | −2.5 | −1.0 | −2.5 | −0.5 |
| Mean: | | 99.5 | 99.8 | 99.5 | 99.8 | 100.4 | 100.0 | 98.9 | 99.9 | −0.45 | −0.17 | 0.48 | −0.18 | 0.42 | 0.03 | −1.10 | −0.07 |
| RSD: | | 1.98 | 1.02 | 1.86 | 0.99 | 2.01 | 1.60 | 1.55 | 1.47 | | | | | | | | |

RSD, Relative standard deviation.

### 4.4. PLS method

The corresponding calibration was obtained by using PLS algorithm and it is as follows:

$$C_{CA} = 0.43 + 26.39\mathbf{A}_1 + 2.32\mathbf{A}_2 - 3.52\mathbf{A}_3 + 18.05\mathbf{A}_4 + 31.17\mathbf{A}_5 + 32.61\mathbf{A}_6 + 27.86\mathbf{A}_7 + 20.68\mathbf{A}_8 + 12.87\mathbf{A}_9 + 3.82\mathbf{A}_{10} - 5.42\mathbf{A}_{11} - 14.22\mathbf{A}_{12} - 22.01\mathbf{A}_{13} - 25.22\mathbf{A}_{14} - 21.19\mathbf{A}_{15}$$

and

$$C_{HCT} = -0.06 - 0.08\mathbf{A}_1 + 1.826\mathbf{A}_2 + 2.24\mathbf{A}_3 - 0.29\mathbf{A}_4 - 1.88\mathbf{A}_5 - 2.20\mathbf{A}_6 - 1.88\mathbf{A}_7 - 1.33\mathbf{A}_8 - 0.68\mathbf{A}_9 + 0.11\mathbf{A}_{10} + 0.98\mathbf{A}_{11} + 1.85\mathbf{A}_{12} - 2.61\mathbf{A}_{13} + 2.87\mathbf{A}_{14} + 2.46\mathbf{A}_{15}$$

In the above system of equations, $C_{CA}$ and $C_{HTC}$ are the concentration of HCT and CA and the absorbance values where measured in the same range and the same samples as in PCR method.

### 4.5. Statistical analysis

We can define the ability of a calibration in several ways. In this subsection we calculated the estimations of the standard variation of the chemometric calibrations in the case of the investigated mixtures.

The standard error of calibration (SEC) and prediction (SEP) are given by the following expression:

$$SEC(SEP) = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{Added} - C_i^{Found})^2}{n-1}}. \quad (6)$$

Here, $C_i^{\text{Added}}$ represents the added concentration, $C_i^{\text{Found}}$ denotes the determined concentration and $n$ is the total number of samples. The numerical values of SEC were indicated in Table 2. By inspection we conclude that SEC is minimise for PCR method for both drugs. The SEP of the same mixtures are displayed in Table 3 and the similar behaviour of the values was observed as for SEC.

Table 5
Results obtained in the pharmaceutical samples (mg/tablet) by using four chemometric techniques

| Chemometric methods | CA (Label value = 5 mg per tablet) Mean[a] ± SD[b] | HCT (Label value = 12.50 mg per tablet) Mean[a] ± SD[b] |
|---|---|---|
| CLS | 4.97 ± 0.11 | 12.51 ± 0.12 |
| ILS | 5.01 ± 0.09 | 12.45 ± 0.20 |
| PCR | 5.11 ± 0.15 | 12.51 ± 0.12 |
| PLS | 4.98 ± 0.10 | 12.53 ± 0.17 |

[a] Results obtained are average of ten experiments for each technique.
[b] Standard deviation.

For PCR and PLS methods, a number of 14 calibration spectra were used for the selection of the optimum number of factors by using the cross-validation technique.

The prediction residual error sum-of-squares (PRESS) of the calibration step was calculated as:

$$\text{PRESS} = \sum_{i=1}^{n}(C_i^{\text{Added}} - C_i^{\text{Found}})^2. \quad (7)$$

The values of (PRESS) were indicated in Table 2. By using the cross validation-procedure we found that its numerical values were minimised in the case of first four factors for PCR and one factor for PLS, respectively.

In order to test our calibration methods, the validation set consisting of two drug mixtures in the various compositions was analysed and the results were given in Table 4. The maximum values of the mean percent errors corresponding to CLS, ILS, PCR and PLS for the same mixtures were completely acceptable because of their smallest values (see Table 4). The means recoveries and the relative standard deviations of our proposed methods were computed and indicated in the same table. Their numerical values were found satisfactory for the validity of all calibration methods.

### 4.6. Recovery of tablet formulation

To check the validity of the chemometric methods using standard addition method, the standard of two pure drugs as equal to content of the tablet

formulation were added to the tablets. The results and their standard deviations corresponding to CLS, ILS, PCR and PLS calibrations were found to be $4.98 \pm 0.12$, $5.0 \pm 0.11$, $4.90 \pm 0.10$ and $5.04 \pm 0.15$ mg for CA per tablet, and $12.41 \pm 0.11$, $12.44 \pm 0.06$, $12.47 \pm 0.16$ and $12.54 \pm 0.12$ mg for HCT per tablet, respectively. The recovery results were obtained in the average of five replicate for each drug.

### 4.7. Tablet analysis

Ten tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in 0.1 M HCl and methanol (1:1) in a 100 ml calibrated flask by sonication. The solution was filtered into a 100 ml calibrated flask through Whatman No. 42 filter paper and diluted to an appropriate volume with the same solvent. The proposed techniques were applied to the analysis of tablets. The experimental results of tablet formulation were presented in Table 5.

The results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. The HPLC method was previously applied to same tablet formulation and its results were given as $98.5\% \pm 0.38$ for CA and $99.1\% \pm 1.15$ for HTC [38]. It was observed that our tablet results indicated the harmony with those given by HPLC method.

### 4.8. ANOVA test

In this study, to compare the differences among methods, an ANOVA test was applied to four sets of 10 sub-samples for each drug in tablet formulation. For this reason, Snedecor's $F$-values were computed and compared with the standard tabulated value using a significance level of $P = 0.05$. The same computation process was repeated for both drugs. From standard table, for $n_1 = 3$ and $n_2 = 28$ ($P = 0.05$), the value of $F$ is given as 2.95. The experimental (calculated) $F$-values did not exceed the tabulated value of $F$ in the analysis of variance, indicating that there was no significant difference among the methods.

ANOVA's results were illustrated in Table 6. The numerical values of all statistic parameters indicated that the investigated techniques are suitable for the determination of both drugs in the tablet formulation.

## 5. Conclusions

Four chemometric methods were applied to UV–Vis spectra of two drugs overlap in the spectral region of 210–290 nm (Fig. 1). The corresponding calibrations indicated good results both for the mixtures and for the tablets. We observe that the detection limit of our proposed methods is 2 μg/ml for CA and HTC as well as HPLC method developed in [38] indicates 15 μg/ml for CA and 10 μg/ml for HTC. From this comparison we conclude that our investigated methods are better than HPLC method in this case. On the other hand, the recoveries and the tablet results presented in this study are comparable with those delivered by HPLC method.

The proposed chemometric methods can be applied for the routine analysis of two drugs in the tablet formulation without any a priori chemical separation and without time consuming.

These simple and confident chemometric techniques are suitable for the quality control of both drugs in samples.

Table 6
ANOVA test for the results of CA and HCT obtained in the synthetic mixtures by using three chemometric techniques

| Source of variation | Sum of squares | | Degrees of freedom | | Mean squares | | $F$-test | |
|---|---|---|---|---|---|---|---|---|
| | CA | HCT | CA | HCT | CA | HCT | CA | HCT |
| Between groups | $6.9 \times 10^{-2}$ | $5.0 \times 10^{-2}$ | 3 | 3 | $2.3 \times 10^{-2}$ | $1.7 \times 10^{-2}$ | 1.61 ($F_{\text{theor}} = 2.95$) | 1.13 ($F_{\text{theor.}} = 2.95$) |
| Within groups | 0.402 | 0.417 | 28 | 28 | $1.4 \times 10^{-2}$ | $1.5 \times 10^{-2}$ | ($P = 0.05$) | ($P = 0.05$) |
| Total | 0.472 | 0.468 | 31 | 31 | | | | |

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