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Simultaneous resolution of a binary mixture of captopril and hydrochlorothiazide in tablets by bivariate and multivariate spectral calibrations

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The multivariate spectral calibration methods, two-linear regression-calibration (bivariate calibration (BC)) and multi-linear regression-calibration (MLRC) are proposed for the simultaneous resolution of a binary mixture of hydrochlorothiazide (HCT) and captopril (CTP), which have closely overlapping spectra. The BC and MLRC calibration algorithms are described for the two-component system, HCT-CTP. Some alternative methods, classical least squares (CLS), inverse least squares (ILS), principal component regression (PCR) and principal least squares (PLS) methods, were also used to determine HCT and CTP in the mixture. Using a synthetic mixture of the two drugs, all the methods were validated and applied to tablets. The BC and MLRC methods which are very rapid, and easy to apply, yet not expensive, are powerful tools with very simple mathematical contents for the quantitative analysis. Data treatment was performed using MAPLE V, EXCEL and SPSS 10.0 Software.

1. Introduction

With the development of chemometric techniques such as the CLS, ILS, PCR and PLS methods, many problems of the simultaneous analysis of two-component or multi-component mixtures have been solved [1–5]. Although these methods are very easy to apply to spectrophotometric [6–10], chromatographic [10] and electrochemical [11] quantitative analysis, they require data processing with powerful software as well as the manipulation of abstract vector space and its application to regression analysis.

In spectrophotometric studies, derivative spectrophotometric methods have been used for the quantitative resolving of binary mixtures. Unfortunately, in some cases these two methods have a great disadvantage: the higher derivative process diminishes the peak amplitude and it is difficult to find zero-crossing points and the sensitivity of the method decreases. On the other hand, the ratio spectra derivative method leads in some cases to an infinite value for ratio spectra.

Other spectrophotometric methods such as dual wavelength spectrophotometry [12–14], pH-induced differential spectrophotometry [15], multicomponent analysis programs [16–17], and multi-wavelength linear regression analysis [18] have been reported in the literature for the simultaneous determination of compounds in mixtures. Recently, López-de-Alba et al. developed the bivariate calibration method for the resolution of two-component mixtures by spectrophotometry [19–22]. This method is based on the use of four linear regression calibration equations with two calibrations for each component at two selected wavelengths. The MLRC method was developed from the method of López-de-Alba et al. for the multiresolution of two-component mixtures and multi-component mixtures.

Among the various analytical techniques available for the simultaneous determination of HCT and CTP in their binary mixture are spectrophotometry [23–24], and HPLC [24–26].

The aim of the present work is the application of BC and MLRC methods to the resolution of a binary mixture containing HCT and CTP without requiring a chemical pretreatment and a graphical procedure for the overlapping spectra. CLS, ILS, PCR and PLS calibrations were studied as alternative methods for the quantitative resolution of mixtures of the drugs under consideration. All of the proposed methods were also applied to commercial tablets.

2. Investigations, results and discussion

2.1. Methods

A linear regression equation between two variables, concentration and absorbance, for the spectrophotometric determination of an analyte X at wavelength λ_i can be defined by the equation:

$$A_{Xi} = b_{Xi}C_X + a_{Xi} \quad (1)$$

Where, A_{Xi} is the absorbance of the analyte X at wavelength λ_i , C_X is the concentration of the analyte X (the concentration units are $\mu\text{g/mL}$ in this study), b_{Xi} is the slope of the linear regression equation, and a_{Xi} is the intercept of the regression model. These slope values indicate the difference between the real and modelled systems.

2.1.1. Bivariate calibration method

In this method [19–22], if the absorbance values of a mixture of two analytes (X, and Y) are measured at two wa-

wavelengths ($\lambda_i = 1$ and 2), the following equations can be written for a two-component analysis:

$$\begin{aligned} A_{\text{mix}_1} &= b_{X_1}C_X + b_{Y_1}C_Y + a_{XY_1} \\ A_{\text{mix}_2} &= b_{X_2}C_X + b_{Y_2}C_Y + a_{XY_2} \end{aligned} \quad (2)$$

Where A_{mix_1} and A_{mix_2} represent the absorbances of the mixture of analytes X and Y at the two-wavelengths, $b_{X_{1,2}}$ and $b_{Y_{1,2}}$ are the slopes of the linear regression equations of X, and Y, respectively; and a_{XY_1} and a_{XY_2} are the sums of the intercepts of linear regression equations at the two wavelengths ($a_{XY_1} = a_{X_1} + a_{Y_1}$ and $a_{XY_2} = a_{X_2} + a_{Y_2}$). Equation (2) can be formulated in matrix notation as:

$$\begin{bmatrix} A_{\text{mix}_1} \\ A_{\text{mix}_2} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} \\ b_{X_2} & b_{Y_2} \end{bmatrix} \cdot \begin{bmatrix} C_X \\ C_Y \end{bmatrix} + \begin{bmatrix} a_{XY_1} \\ a_{XY_2} \end{bmatrix} \quad (3)$$

If the absorbance matrix, A_{mix} and the intercept matrix, a_{XYZ} are matrices of the same size, then the difference $A_{\text{mix}} - a_{XY}$ is the matrix obtained by subtracting the entries of a_{XY} from the corresponding entries of A_{mix} . According to this procedure, the following equation can be written as:

$$\begin{bmatrix} A_{\text{mix}_1} - a_{XY_1} \\ A_{\text{mix}_2} - a_{XY_2} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} \\ b_{X_2} & b_{Y_2} \end{bmatrix} \cdot \begin{bmatrix} C_X \\ C_Y \end{bmatrix} \quad (4)$$

or, more simply:

$$(A_{\text{mix}_1} - a_{XY})_{2 \times 1} = K_{2 \times 2} \cdot C_{2 \times 1} \quad (4a)$$

The matrix, b , corresponding to the slope values of linear regression equations is called matrix, K :

$$K = \begin{bmatrix} b_{X_1} & b_{Y_1} \\ b_{X_2} & b_{Y_2} \end{bmatrix} \quad (5)$$

In this case, to calculate the concentrations of the analytes, X and Y, in a binary mixture, the matrix, $(A_{\text{mix}} - a_{XY})_{2 \times 1}$, is multiplied by the inverse $(K^{-1})_{2 \times 2}$ of the matrix $K_{2 \times 2}$ and it can be written as:

$$C_{2 \times 1} = (K^{-1})_{2 \times 2} \cdot (A_{\text{mix}} - a_{XY})_{2 \times 1} \quad (6)$$

This procedure is the mathematical basis of the BC method for two-component analysis.

As explained here, this calibration model can be applied easily to resolution of the two-component or binary mixtures. The choice of optimum wavelengths plays an important role in the application of this method to a binary mixture analysis. For this reason, Kaiser's method [27–28] was applied to the selection of the optimum set of wavelengths in order to provide the best sensitivity and selectivity.

The sensitivity matrices K (square matrix) (eq. 5) are formed by taking all the pairs of pre-selected wavelengths for binary mixtures.

The matrices K of the slope values obtained in the linear regression equations of the individual analytes, X and Y at two selected wavelengths (1 and 2) are considered as the sensitivity parameter [19–22]. The sensitivity parameter is used to compare different two-wavelength sets. The sensitivity of a two-component mixture analysis is defined as the absolute value of the determinant of the sensitivity matrix K . For this reason, the determinant values of the matrices K corresponding to different two-wavelength sets are calculated for the selection of the working wavelength set. The calculated maximum determinant value permits the optimum wavelength set to be selected. The method is based on using the linear regression lines for each compound at two selected wavelengths.

2.1.2. Multi-linear regression-calibration method

If the absorbance values of a mixture of two analytes (X and Y) are measured at n wavelengths ($\lambda_i = 1, 2, \dots, n$), the following set of equations can be written for a two-component analysis:

$$\begin{aligned} A_{\text{mix}_1} &= b_{X_1}C_X + b_{Y_1}C_Y + a_{XY_1} \\ A_{\text{mix}_2} &= b_{X_2}C_X + b_{Y_2}C_Y + a_{XY_2} \\ \dots &\dots \dots \dots \\ A_{\text{mix}_n} &= b_{X_n}C_X + b_{Y_n}C_Y + a_{XY_n} \end{aligned} \quad (7)$$

Where A_{mix_1} , A_{mix_2} , $\dots$, and A_{mix_n} are the absorbances of the mixture of analytes X and Y at selected wavelengths (from λ_1 to λ_n); $b_{X_{1,2,\dots,n}}$ and $b_{Y_{1,2,\dots,n}}$ are the slopes of linear regression equations of X and Y, corresponding to selected wavelengths, respectively; and a_{XY_1} , a_{XY_2} , $\dots$ and a_{XY_n} are the sums of intercepts of linear regression equations at n wavelengths ($a_{XY_1} = a_{X_1} + a_{Y_1}$, $a_{XY_2} = a_{X_2} + a_{Y_2}$, $a_{XY_n} = a_{X_n} + a_{Y_n}$).

In matrix terms, the above multi-equation system (7) can be formulated as:

$$\begin{bmatrix} A_{\text{mix}_1} \\ A_{\text{mix}_2} \\ \dots \\ A_{\text{mix}_n} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} \\ b_{X_2} & b_{Y_2} \\ \dots & \dots \\ b_{X_n} & b_{Y_n} \end{bmatrix} \cdot \begin{bmatrix} C_X \\ C_Y \end{bmatrix} + \begin{bmatrix} a_{XY_1} \\ a_{XY_2} \\ \dots \\ a_{XY_n} \end{bmatrix} \quad (8)$$

which can be simplified to

$$\begin{bmatrix} A_{\text{mix}_1} - a_{XY_1} \\ A_{\text{mix}_2} - a_{XY_2} \\ \dots \\ A_{\text{mix}_n} - a_{XY_n} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} \\ b_{X_2} & b_{Y_2} \\ \dots & \dots \\ b_{X_n} & b_{Y_n} \end{bmatrix} \cdot \begin{bmatrix} C_X \\ C_Y \end{bmatrix} \quad (9)$$

or in a compact form

$$(A_{\text{mix}_1} - a_{XY})_{n \times 1} = K_{n \times 2} \cdot C_{2 \times 1} \quad (9a)$$

As explained for the calibration method above, the matrix of the slope values is called matrix K :

$$K_{n \times 2} = \begin{bmatrix} b_{X_1} & b_{Y_1} \\ b_{X_2} & b_{Y_2} \\ \dots & \dots \\ b_{X_n} & b_{Y_n} \end{bmatrix} \quad (10)$$

The matrices, $(A_{\text{mix}} - a)_{n \times 1}$ and $K_{n \times 2}$, are multiplied by the transpose $(K')_{2 \times n}$ of the matrix $K_{n \times 2}$, and it can be written as:

$$(K')_{2 \times n} (A_{\text{mix}} - a)_{n \times 1} = (K')_{2 \times n} K_{n \times 2} \cdot C_{2 \times 1} \quad (12)$$

The concentration of the drugs X and Y in a binary mixture can be calculated by using the following formula:

$$C_{2 \times 1} = [(K')_{2 \times n} K_{n \times 2}]^{-1} \cdot (K')_{2 \times n} (A_{\text{mix}} - a_{XY})_{n \times 1} \quad (13)$$

In this case, the MLRC model involves the use of linear algebra, also known as matrix mathematics. This calibration model can be applied to the quantitative resolution of two-component mixtures and multi-component mixtures.

2.2. Results and discussion

The absorption spectra of HCT, CTP and their mixture were observed in the spectral region 215–300 nm as indi-

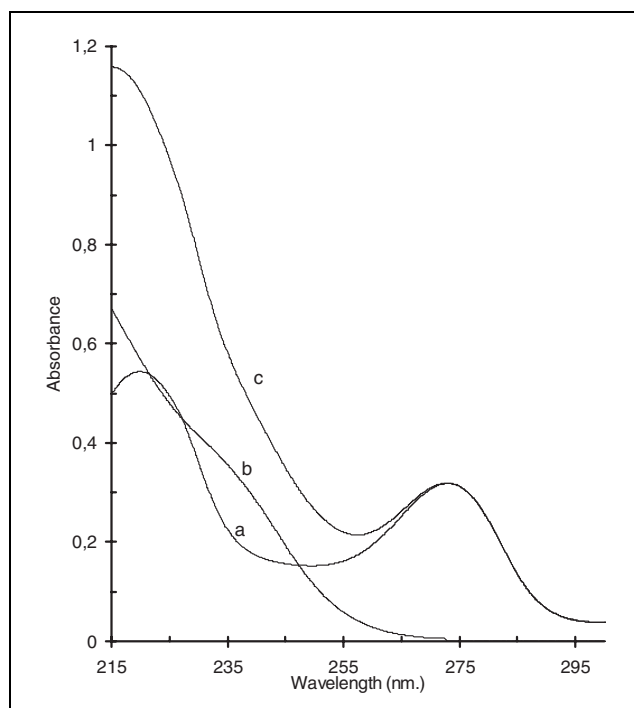


Fig. 1: Absorption spectra of a) 6 µg/mL HCT, b) 16 µg/mL CTP, and c) their mixture in 0.1 M NaOH and methanol (1 : 1).

cated in the Fig. 1. Since the spectra of two drugs overlap in the working wavelength range, it is not possible to determine HCT, and CTP simultaneously in their mixture by conventional spectrophotometric methods. In order to solve this problem, the two methods (BC and MLRC) were applied. In these methods, a standard series of solutions of HCT and CTP in 0.05 M NaOH and methanol (1 : 1) were prepared. Their absorption spectra were recorded over the wavelength range 215–300 nm against a blank (the solvent system). The alternative methods, CLS, ILS, PCR and PLS, were used. In order to validate the methods, synthetic mixture solutions of HCT and CTP were prepared according to the working range of the individual drugs.

2.2.1. Direct absorbance method

The quantitative determination of HCT in the binary mixture in the presence of CTP was carried out using direct absorbance measured at 272 nm. At this wavelength, the linear regression line for HCT was calculated as $A = 7.30 \times 10^{-3} C_{\text{HCT}} + 5.13 \times 10^{-3}$ ($r = 1.0000$) (see Table 1). The straight line obtained was tested for the synthetic mixtures containing two drugs illustrated in Table 2. Mean recovery and relative standard deviation for HCT were found to be 98.5% and 0.82% in the presence of CTP. The amount of HCT in the commercial tablets containing HCT and CTP was determined to be 25.2 mg $\pm$ 0.43 (mean $\pm$ standard deviation) for ten replicates.

2.2.2. Bivariate calibration method

As an application of this method, 15 wavelengths were considered for the binary mixture systems. The 15 linear regression equations were obtained by using the measurements of the absorbances at 15 wavelengths against the concentrations of standard solution for each compound (Table 1).

The highest values for the regression coefficients (r) were obtained for all regression equations. The detection limit (LOD) (signal to noise ratio 3 : 1) and the quantitation limit (LOQ) (signal to noise ratio 10 : 1) were computed using the data obtained from ten replicates for a standard solution of HCT (6 µg/mL) and CTP (12 µg/mL) (Table 1).

The slope values obtained from the linear regression analysis for each drug in the binary mixture of HCT and CTP were used to create the sensitivity matrices (Table 1). According to Kaiser's method [27, 28], the absolute values of the determinant of the sensitivity matrices were used to find the best sensitivity for the application of the BC model. In this procedure, it was possible to calculate 105 different pairs of the sensitivity matrices for the selection of the optimum two-wavelength set. By using the absolute values of the determinants of the sensitivity matrices, the results of applying Kaiser's method to the selection of the wavelength set for HCT-CTP mixtures are presented in Table 3. An optimum two-wavelength set, which gives the

Table 1: Linear regression analysis and its statistical results at 15 wavelengths

HCT								CTP							
λ_1 (nm)	Regression equation $A = a\ C + b$	(r)	Sr	S(b)	S(a)	LOD $\mu\text{g/mL}$	LOQ $\mu\text{g/mL}$	Regression equation	(r)	Sr	S(b)	S(a)	LOD $\mu\text{g/mL}$	LOQ $\mu\text{g/mL}$	
220	$A = 0.0873\ C + 0.0119$	0.9999	0.15	0.07	0.07	0.021	0.071	$A = 0.0375\ C - 0.0268$	0.9999	0.12	0.05	0.05	0.079	0.265	
224	$A = 0.0820\ C + 0.0153$	1.0000	0.15	0.07	0.07	0.028	0.096	$A = 0.0329\ C - 0.0253$	0.9999	0.11	0.04	0.05	0.082	0.273	
228	$A = 0.0683\ C + 0.0145$	1.0000	0.14	0.06	0.06	0.030	0.101	$A = 0.0294\ C - 0.0274$	0.9999	0.11	0.04	0.05	0.088	0.295	
232	$A = 0.0476\ C + 0.0091$	0.9999	0.12	0.05	0.05	0.015	0.050	$A = 0.0291\ C - 0.0678$	0.9959	0.11	0.04	0.05	0.281	0.937	
236	$A = 0.0331\ C + 0.0101$	0.9999	0.10	0.04	0.04	0.021	0.072	$A = 0.0235\ C - 0.0285$	0.9998	0.10	0.04	0.04	0.089	0.295	
240	$A = 0.0273\ C + 0.0079$	0.9999	0.09	0.04	0.04	0.016	0.054	$A = 0.0194\ C - 0.0245$	0.9998	0.09	0.03	0.04	0.075	0.251	
244	$A = 0.0252\ C + 0.0056$	0.9999	0.09	0.04	0.04	0.010	0.032	$A = 0.0145\ C - 0.0188$	0.9999	0.08	0.03	0.03	0.056	0.186	
248	$A = 0.0241\ C + 0.0075$	1.0000	0.08	0.04	0.04	0.015	0.051	$A = 0.0098\ C - 0.0119$	0.9999	0.06	0.02	0.03	0.035	0.116	
252	$A = 0.0243\ C + 0.0084$	0.9998	0.08	0.04	0.04	0.015	0.051	$A = 0.0059\ C - 0.0060$	0.9998	0.05	0.02	0.02	0.020	0.067	
256	$A = 0.0267\ C + 0.0022$	0.9998	0.09	0.04	0.04	0.007	0.023	$A = 0.0034\ C - 0.0020$	0.9995	0.04	0.01	0.02	0.008	0.025	
260	$A = 0.0309\ C + 0.0073$	1.0000	0.09	0.04	0.04	0.015	0.049	$A = 0.0017\ C + 0.0006$	0.9981	0.03	0.01	0.01	0.004	0.013	
264	$A = 0.0383\ C + 0.0061$	0.9999	0.10	0.05	0.05	0.011	0.036	$A = 0.0008\ C + 0.0019$	0.9950	0.02	0.01	0.01	0.006	0.020	
268	$A = 0.0461\ C + 0.0108$	1.0000	0.11	0.05	0.05	0.023	0.077	$A = 0.0004\ C + 0.0013$	0.9900	0.01	0.01	0.01	0.004	0.013	
272	$A = 0.0513\ C + 0.0070$	1.0000	0.12	0.06	0.05	0.013	0.044	–	–	–	–	–	–	–	
276	$A = 0.0496\ C + 0.0036$	0.9999	0.12	0.05	0.05	0.008	0.025	–	–	–	–	–	–	–	

C = concentration (µg/mL)

A = Absorbance values at selected wavelength for HCT and CTP

r = Regression coefficient

Sr = Standard deviation of linear regression

S(b) = Standard deviation of slope

S(a) = Standard deviation of intercept

LOD = Limit of detection

LOQ = Limit of quantification

Table 2: Recovery data obtained for the determination of HCT and CTP in different synthetic mixtures using different methods

HCT	CTP	Recoveries (%)											
		HCT						CTP					
		BC	MLRC	CLS	ILS	PCR	PLS	BC	MLRC	CLS	ILS	PCR	PLS
Added	Added												
6	4	99.6	100.3	102.0	101.3	101.3	101.5	102.5	105.00	95.5	104.0	96.5	95.3
6	8	97.3	99.5	100.3	100.8	99.5	100.0	104.3	104.9	99.6	101.9	103.4	101.1
6	12	104.4	100.0	100.0	101.0	100.0	100.0	97.0	102.7	102.3	100.7	101.8	102.4
6	16	101.0	100.3	99.5	100.7	99.8	99.7	98.7	99.3	101.3	100.4	100.2	100.8
6	20	101.7	101.2	99.5	101.0	100.8	100.2	98.6	99.5	102.9	100.4	99.0	102.0
3	12	99.5	101.0	102.7	99.7	101.0	102.3	100.9	96.4	95.3	98.8	99.1	96.3
6	12	98.4	101.8	101.8	100.8	101.3	101.8	102.7	98.6	97.8	100.6	100.8	98.0
9	12	98.6	100.3	99.9	100.9	99.7	100.0	103.0	100.1	99.8	101.3	102.3	99.3
12	12	99.5	99.9	99.3	100.4	99.5	99.5	98.0	97.8	97.3	99.9	96.4	96.3
15	12	101.1	101.1	100.2	101.0	100.3	100.5	98.0	98.9	98.8	97.7	98.3	97.1
Mean		100.1	100.5	100.5	100.8	100.3	100.5	100.4	100.3	99.1	100.6	99.8	98.9
RSD		2.01	0.70	1.19	0.45	0.73	0.98	2.61	2.91	2.66	1.69	2.35	2.65

SD = Standard deviation, RSD = Relative standard deviation

highest determinant value of the sensitivity matrices, was selected as 220 and 272 nm. With this set, the individual linear regression equations for each drug are shown in Table 1. The following set of equations was created for the BC method:

$$\begin{aligned}\lambda_1 = 220, \quad A_{\text{mix}, \lambda_1} &= 0.0873 C_{\text{HCT}} + 0.0375 C_{\text{CTP}} - 0.0148 \\ \lambda_2 = 272, \quad A_{\text{mix}, \lambda_2} &= 0.0513 C_{\text{HCT}} + 0.0000 C_{\text{CTP}} + 0.0070\end{aligned}\quad (14)$$

As described in section 2.1, the BC procedure was achieved using linear algebra, also known as matrix mathematics. The calibration constructed was applied to the analysis of synthetic mixtures and a commercial tablet formulation.

2.2.3. Multi-linear regression-calibration method

This approach is analogous to the BC method, but the MLRC method involves an n-wavelength procedure instead of two-wavelengths. For this reason, the 15-wavelength set (Table 1) at the critical points, which correspond to the maximum, shoulder and minimum in the spectral range 215–300 nm were selected for the construction of the individual linear regressions for HCT and CTP in the binary mixture. As indicated in Table 1, the 15 linear regression equations of HCT and CTP, for each drug,

were obtained by measuring the zero-order absorbance values at the wavelengths set. A set of 15 equations was obtained:

$$\begin{aligned}\lambda_1 = 220, \quad A_{\text{mix}_1} &= 0.0873 C_{\text{HCT}} + 0.0375 C_{\text{CTP}} - 0.0148 \\ \lambda_2 = 224, \quad A_{\text{mix}_2} &= 0.0820 C_{\text{HCT}} + 0.0329 C_{\text{CTP}} - 0.0100 \\ \lambda_3 = 228, \quad A_{\text{mix}_3} &= 0.0683 C_{\text{HCT}} + 0.0294 C_{\text{CTP}} - 0.0129 \\ \lambda_4 = 232, \quad A_{\text{mix}_4} &= 0.0476 C_{\text{HCT}} + 0.0291 C_{\text{CTP}} - 0.0588 \\ \lambda_5 = 236, \quad A_{\text{mix}_5} &= 0.0331 C_{\text{HCT}} + 0.0235 C_{\text{CTP}} - 0.0184 \\ \lambda_6 = 240, \quad A_{\text{mix}_6} &= 0.0273 C_{\text{HCT}} + 0.0194 C_{\text{CTP}} - 0.0165 \\ \lambda_7 = 244, \quad A_{\text{mix}_7} &= 0.0252 C_{\text{HCT}} + 0.0145 C_{\text{CTP}} - 0.0132 \\ \lambda_8 = 248, \quad A_{\text{mix}_8} &= 0.0241 C_{\text{HCT}} + 0.0098 C_{\text{CTP}} - 0.0044 \\ \lambda_9 = 252, \quad A_{\text{mix}_9} &= 0.0243 C_{\text{HCT}} + 0.0059 C_{\text{CTP}} - 0.0024 \\ \lambda_{10} = 256, \quad A_{\text{mix}_{10}} &= 0.0267 C_{\text{HCT}} + 0.0034 C_{\text{CTP}} - 0.0002 \\ \lambda_{11} = 260, \quad A_{\text{mix}_{11}} &= 0.0309 C_{\text{HCT}} + 0.0017 C_{\text{CTP}} - 0.0078 \\ \lambda_{12} = 264, \quad A_{\text{mix}_{12}} &= 0.0383 C_{\text{HCT}} + 0.0008 C_{\text{CTP}} - 0.0080 \\ \lambda_{13} = 268, \quad A_{\text{mix}_{13}} &= 0.0461 C_{\text{HCT}} + 0.0004 C_{\text{CTP}} - 0.0120 \\ \lambda_{14} = 272, \quad A_{\text{mix}_{14}} &= 0.0513 C_{\text{HCT}} + 0.0000 C_{\text{CTP}} - 0.0070 \\ \lambda_{15} = 276, \quad A_{\text{mix}_{15}} &= 0.0496 C_{\text{HCT}} + 0.0000 C_{\text{CTP}} - 0.0036\end{aligned}\quad (15)$$

Table 3: Application of Kaiser's method to the selection of the wavelength set for HCT-CTP mixtures by using the absolute values of determinants sensitivity matrices ($K \times 10^{-3}$)

$\lambda_1 \lambda_2$	220	224	228	232	236	240	244	248	252	256	260	264	268	272	276
220	0.0	2.0	0.1	7.6	8.1	6.7	3.2	0.5	4.0	7.0	10.1	13.7	16.9	19.2	18.6
224		0.0	1.6	8.2	8.4	6.9	3.6	0.1	3.2	6.0	8.8	11.9	14.8	16.9	16.3
228			0.0	5.9	6.3	5.2	2.5	0.4	3.1	5.5	7.9	10.7	13.3	15.1	14.6
232				0.0	1.6	1.3	0.4	2.3	4.3	6.2	8.2	10.8	13.2	14.9	14.4
236					0.0	0.0	1.1	2.4	3.8	5.1	6.7	8.7	10.7	12.1	11.7
240						0.0	0.9	2.0	3.1	4.3	5.5	7.2	8.8	10.0	9.6
244							0.0	1.0	2.0	3.0	4.1	5.4	6.6	7.4	7.2
248								0.0	1.0	1.8	2.6	3.6	4.4	5.0	4.9
252									0.0	0.7	1.4	2.1	2.6	3.0	2.9
256										0.0	0.6	1.1	1.5	1.7	1.7
260											0.0	0.4	0.7	0.9	0.8
264												0.0	0.2	0.4	0.4
268													0.0	0.2	0.2
272														0.0	0.0
276															0.0

Table 4: Composition of training set for the chemometric methods

Mixture no.	HCT	CTP
1	3	0
2	15	0
3	0	4
4	0	20
5	6	4
6	6	8
7	6	12
8	6	16
9	6	20
10	0	8
11	3	12
12	6	12
13	9	12
14	12	12
15	15	12
16	9	0

This MLRC approach was applied to the resolution of binary mixtures and tablets containing HCT and CTP.

2.2.4. CLS, ILS PCR and PLS methods

The CLS, ILS, PCR and PLS methods, also known as chemometric methods, were applied [1–10] described. A training set containing HCT and CTP was prepared with various random concentration ratios (Table 4). Multivariate calibrations were obtained by measuring the zero-order absorbances at 13 points from 220 nm to 280 in the 215–300 nm wavelength range using the training set. Quantitative determinations of HCT and CTP in the mixtures were successfully carried out by these methods.

We can define the capability of a calibration in several ways. In this paragraph we calculate the estimations of the standard variation of the chemometric calibrations in the case of the mixtures investigated.

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

$$\text{SEC (SEP)} = \sqrt{\frac{\sum_{i=1}^N (C_i^{\text{Added}} - C_i^{\text{Found}})^2}{n - 1}} \quad (16)$$

Here, C_i^{Added} represents the added concentration, C_i^{Found} denotes the determined concentration and n is the total number of samples. The numerical values of SEC are indicated in Table 5. By inspection we conclude that SEC is minimised for the methods for both drugs. The standard errors of prediction (SEP) of the same mixtures are shown in Table 6 and similar behaviour of the values is observed as for SEC.

For the PCR and PLS methods, 16 calibration spectra were used to select 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:

$$\text{PRESS} = \sum_{i=1}^n (C_i^{\text{Added}} - C_i^{\text{Found}})^2 \quad (17)$$

The values of (PRESS) were indicated in Table 5. By using the cross validation-procedure we found that its numerical values were minimised in the case of first three factors for PCR and one factor for PLS respectively.

2.2.5. Validation

The methods was validated by analysing the synthetic mixtures of HCT and CTP. To check the validity of the calibration models, simultaneous resolution of the synthetic mixtures containing various concentrations of HCT and CTP was carried out by BC, MLRC and other methods. Results are summarized in Table 2. The mean recoveries and the relative standard deviations were calculated. The results obtained can be considered satisfactory in the case of spectral overlapping between HCT and CTP. The numerical values were found satisfactory for the validity of all the calibration methods. In addition, the accuracy and precision of the BC, MLRC and chemometric methods were confirmed by applying the standard addition technique. Recoveries were within 99.6%, with standard deviations ranging from 0.7–1.8%. The results for each drug were obtained with as average of five replicates for five levels, repeated three times. It was observed that the excipients in the tablets do not interfere with the analysis of the drugs.

The experimental results for commercial tablets are given in Table 7. The results of all the methods were very close

Table 5: Statistical calculations obtained in the calibration step

Parameters	HCT				CTP			
	CLS	ILS	PCR	PLS	CLS	ILS	PCR	PLS
R	0.9999	1.0000	0.9999	0.9999	0.9987	0.9999	0.9992	0.9980
Intercept	−0.0374	0.0169	−0.0209	−0.0398	0.1788	0.0650	0.0028	0.0935
Slope	1.0041	1.0037	1.0015	1.0047	0.9849	0.9986	1.0038	0.9965
PRESS	0.0686	0.0441	0.1130	0.1189	1.8004	0.1401	0.2859	0.6220
SEC =	0.0676	0.0542	0.0868	0.0890	0.3464	0.1129	0.1612	0.2378

r = correlation coefficient

Table 6: Statistical calculations obtained in the prediction step

Parameters	HCT				CTP			
	CLS	ILS	PCR	PLS	CLS	ILS	PCR	PLS
R	0.9999	1.0000	0.9999	0.9999	0.9981	0.9994	0.9985	0.9980
Intercept	−0.0723	0.0063	−0.0367	−0.0484	0.5955	−0.0952	−0.0518	0.4830
Slope	1.0068	0.9911	1.0028	1.0028	0.9542	1.0052	1.0060	0.9666
SEP	0.0687	0.0758	0.0519	0.0616	0.3338	0.1465	0.2357	0.3144

Table 7: Results obtained for the pharmaceutical samples (mg/tablet) by the proposed methods

	HCT (mean $\pm$ SD)							CTP (mean $\pm$ SD)					
	BC	MLRC	CLS	ILS	PCR	PLS	DAM	BC	MLRC	CLS	ILS	PCR	PLS
Mean	25.4	25.2	25.5	25.3	25.7	25.2	25.2	51.1	50.7	49.9	50.1	50.4	25.2
SD	0.72	0.61	0.82	0.25	0.61	0.63	0.43	1.47	1.56	1.02	1.24	1.81	0.64
RSD	2.83	2.42	3.22	0.99	2.37	2.50	1.71	2.88	3.08	2.04	2.48	3.59	2.54
SE	0.42	0.35	0.47	0.14	0.35	0.36	0.24	0.85	0.90	0.59	0.72	1.05	0.37
CL ($p = 0.05$)	0.84	0.71	0.95	0.29	0.71	0.73	0.56	1.71	1.81	1.19	1.44	2.11	0.75

SD = Standard deviation, RSD = Relative standard deviation, SE = Standard error, CL = Confidential limit ($P = 0.05$), DMA = Direct absorbance method

to each other as well as to the labelled values for the commercial tablet. The statistical parameters indicated that the methods used are suitable for the determination of two drugs in the tablet formulation.

To compare the differences between the six methods, a one-way ANOVA test was carried out using the parallel results obtained by applying the methods to 10 samples for each drug in a tablet formulation. For this purpose, Snedecor's F-values were computed and compared with the standard tabulated value ($p = 0.05$). The same computation process was repeated for three compounds. In the standard table, for $n_1 = 5$ and $n_2 = 54$ ($P = 0.05$), the F-value is 2.37. ANOVA test results were found to be 0.74 for HCT and 1.51 for CTP. The experimental (calculated) F-values did not exceed the tabulated F-value in the variance analysis. It was observed that there was no significant difference between the methods.

3. Experimental

3.1. Instruments

A Shimadzu UV-160 double beam UV-VIS spectrophotometer with a fixed slit width (2 nm) connected to a computer running Shimadzu UVPC software and a HP DeskJet 600 printer were used to record the absorption spectra. The application of Kaiser's method, and the regression and statistical analyses were achieved by using MAPLE V, EXCEL and SPSS 10.0 software, respectively.

3.2. Commercial tablet formulation

A commercial tablet formulation (Captiohexal[®] comp 50/25 tablets produced by HEXAL AG Pharm. Ind., Holzkirchen, Germany, Batch no. 04UV32) consisting of 50 mg of CTP and 25 mg HCT per tablet was studied. The active compounds were kindly donated by Turkish pharmaceutical industry firms.

3.3. Standard solutions

Stock solutions containing 100 mg/100 mL HCT and CTP were prepared in 0.05 M NaOH and methanol (1:1). In the BC and MLRC method, the standard series of the solutions containing 3–15 $\mu\text{g/mL}$ HCT and 4–20 $\mu\text{g/mL}$ CTP was obtained from the stock solutions. In the chemometric methods, a training set was randomly prepared in the concentration range of 0–15 $\mu\text{g/mL}$ HCT and of 0–20 $\mu\text{g/mL}$ CTP in various combinations. A validation set consisting of 10 synthetic mixture solutions in the working range of 3–15 $\mu\text{g/mL}$ HCT and 4–20 $\mu\text{g/mL}$ CTP was prepared using the same stock solutions. All the solutions were prepared freshly and protected from light.

3.4. Procedure

20 tablets (Captiohexalcomp[®] tablets) were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in

0.05 M NaOH and methanol (1:1) in a 100 ml calibrated flask with the aid of mechanical shaking for 20 min. This solution was filtered into a 100 ml calibrated flask through Whatman No. 42 filter paper. The residue was washed three times with the solvent system used. The resulting solution was diluted to an appropriate volume with the same solvent. The analysis of the sample solutions was carried out by using the BC, MLRC and other chemometric methods.

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