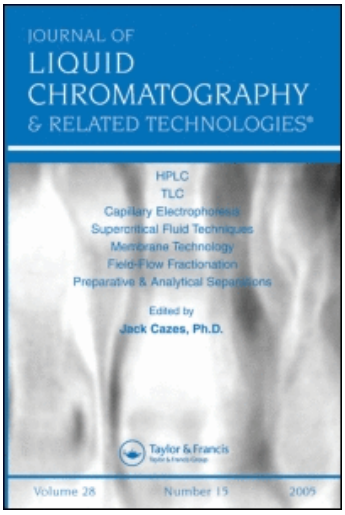

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A New Application of Chemometric Techniques to HPLC Data for the Simultaneous Analysis of a Two-Component Mixture

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A New Application of Chemometric Techniques to HPLC Data for the Simultaneous Analysis of a Two-Component Mixture

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Abstract: A new chemometric approach using high performance liquid chromatography (HPLC) with photodiode array (PDA) detection was developed and applied to the simultaneous determination of enalapril maleate (EA) and hydrochlorothiazide (HCT) in tablets. Chemometric calibration techniques, classical least squares (CLS), principle component regression (PCR), and partial least squares (PLS) were subjected to the peak area at multiwavelength PDA detector responses. The combination of HPLC and chemometric calibration techniques was called HPLC-CLS, HPLC-PCR, and HPLC-PLS. For comparison purposes, the HPLC method called classical HPLC method was used for the confirmation of the results obtained from combined HPLC-chemometric calibration techniques. A good chromatographic separation between two drugs and internal standard (IS) was achieved using a Waters Symmetry® C18 Column 5 μm 4.6 × 250 mm and a mobile phase consisting

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of 0.2 M acetate buffer and acetonitrile (v/v, 60 : 40). The multiwavelength PDA detection was done at 230 (A), 240 (B), 250 (C), 250 (D), 240 (E) nm wavelengths, and peak area was recorded for the concentration set in the mobile phase. Three HPLC-chemometric calibrations and a classical-HPLC method were tested by analyzing the synthetic mixture of EA and HCT in the presence of losartan potassium (IS). The proposed methods were applied to real samples containing the present two drugs. The obtained results were statistically compared with each other.

Keywords: HPLC-chemometric calibration, Multiwavelength HPLC data processing, Quantitative determination, Enalapril maleate, Hydrochlorothiazide, Pharmaceutical dosage form

INTRODUCTION

Enalapril maleate, a synthetic peptidic derivative, is an inhibitor of an angiotensin converting enzyme which reduces the plasmatic activity of rennin. Hydrochlorothiazide is a diuretic of the class of benzothiadiazines. The combination of these two drugs is used in the treatment of hypertension. In the literature, several simultaneous quantitative analyses of binary mixture containing EA and HCT has been carried out by spectrophotometric methods[1,2] and by HPLC methods.[2–4]

As it is known in the method development studies, many new analytical approaches, applications, and techniques have been elaborated for the resolution of analytical problems such us quantitative and qualitative analysis of analytes in complex mixtures. These new developed approaches, applications, and techniques are used for solving the basic problems of complex mixture analysis.

Generally, HPLC is the current method of choice for the analysis of multi compounds in pharmaceutical formulations, since it gives the accurate, sensitive, and reproducible quantitative analysis of compounds. The classical HPLC method uses the peak area determined at one specific wavelength to construct the linear regression functions. This method undoubtedly provides more sensitive determination than the spectrophotometric methods, but the calculations at one specific wavelength causes some errors for the construction of linear regression lines. In the case of a single wavelength detector response, classical HPLC give us some chromatographic area errors arising from injection, instrumental fluctuations, and other sources. All these mentioned reasons affect the result of an analysis. On the other hand, the simultaneous use of chromatograms obtained at multiwavelength PDA detector response will eliminate the errors of single regression functions based on single wavelength.

In recent years, chemometric calibrations such as classical least-squares (CLS), inverse least squares (ILS), principle component regression (PCR), and partial least squares (PLS) has been applied to the analysis of the analytical data obtained from many instruments.[5–10] Chemometric calibration tech-

niques have been particularly subject to the resolution of overlapping spectra for the determination of active compounds in samples containing two or more compounds.[10–14]

The main aim of our study is to apply the chemometric calibration techniques to the multivariate chromatographic data at the multiwavelength set. This increases the performance of the HPLC in the analysis of complex mixtures.

In our study, CLS, PCR, and PLS calibration techniques were applied to the HPLC data set at the multiwavelengths using PDA detectors for the binary mixture analysis. These combined numerical methods with HPLC were named as HPLC-CLS, HPLC-PCR, and HPLC-PLS. The combined use of the HPLC method based on multiwavelength with chemometric calibration techniques provides the elimination or reduction of the disadvantage of single regression functions based on a single wavelength. In this study, three HPLC chemometric methods were applied to the simultaneous determination of EA and HCT in the synthetic mixtures and pharmaceutical dosage form. As an alternative method, classical HPLC was also subject to the analysis of the same samples. For a statistical comparison, t-test, F-test, and ANOVA test were applied to the obtained results. The proposed methods give us successful results.

Methodology

In this chemometric HPLC study, CLS, PCR, and PLS calibrations are applied to the ratio of the peaks area of analyzed drugs to IS at the five wavelengths using a PDA detector. The chromatograms of analyzed drugs are plotted and stored in a computer. The detector responses are measured in terms of peak area. The application procedure of the combined HPLC-chemometrics calibrations is explained in the following section for each chemometric calibration.

HPLC-CLS Method

This method contains the application of multi linear regression (MLR) to the ratio of the peak area of individual drugs. If we consider the responses as ratio values of peak area at five wavelengths (R) and 6 standard series (concentration set (C)) of analyzed drug, the following equation system can be written:

$$\begin{bmatrix} R_{11} & R_{12} & R_{13} & R_{14} & R_{15} & R_{16} \\ R_{21} & R_{22} & R_{23} & R_{24} & R_{25} & R_{26} \\ R_{31} & R_{32} & R_{33} & R_{34} & R_{35} & R_{36} \\ R_{41} & R_{42} & R_{43} & R_{44} & R_{45} & R_{46} \\ R_{51} & R_{52} & R_{53} & R_{54} & R_{55} & R_{56} \end{bmatrix} = \begin{bmatrix} K_{11} \\ K_{21} \\ K_{31} \\ K_{41} \\ K_{51} \end{bmatrix} \times [C_{11} \quad C_{12} \quad C_{13} \quad C_{14} \quad C_{15} \quad C_{16}] \tag{1}$$

Where, R_{5x6} is the matrix of the peak area responses (ratio of the peak area of analyte to the peak area of the internal standard), K_{5x1} indicates matrix of the calibration coefficients and C_{6x1} represents the concentration set of the analyzed compound.

In the compact matrix form the equation (1) can be rewritten as

$$R_{5x6} = K_{5x1}\ C_{1x6} \tag{2}$$

By using the matrix calculation we obtain immediately the values of the matrix K_{5x1} as follows:

$$K_{5x1} = R_{5x6}\ C_{6X1}^{T}\ [C_{1x6}\ C_{6X1}^{T}]^{-1} \tag{3}$$

where, C_{6x1}^{T} is the transpose of C_{1x6} and $[C_{1x6}\ C_{6X1}^{T}]^{-1}$ represents the inverse of $C_{1x6}\ C_{6X1}^{T}$.

The mathematical computation using Matlab 6.5 software is carried out by the following algorithm:

$$Ka_{1x5} = \frac{1}{[K_{1x5}^{T}\ K_{5x1}]} \times K_{1x5}^{T} \tag{4}$$

The calculated Ka_{1x5} is introduced into the following equation:

$$C_{\text{prediction}_{1xn}} = Ka_{1x5} \times R_{\text{sample}_{5xn}} \tag{5}$$

In this way the concentration of the content of analyte in the mixture is determined by multiplying Ka_{1x5} and R_{sample_5}.

HPLC-PCR Method

In the application of the HPLC-PCR, the ratio (R) of the peak area of individual drug and the drug concentration set were reprocessed by mean-centering as R_o and C_o, respectively. The covariance dispersion matrix of the centered matrix R_o was computed. The normalized eigenvalues and eigenvectors were calculated starting from square covariance matrix. The number of the optimal principal components (eigenvectors (P)) is selected by considering only the highest values of the eigenvalues. The other eigenvalues and their corresponding eigenvectors are eliminated. To reach this objective, the coefficient b defined as $b = P \times q$ is determined, where P is the matrix of eigenvectors and q is the C–loadings given by $q = D \times T^{T} \times R_o$. T^{T} is the transpose of the score matrix T, and D is a diagonal matrix having components the inverse of the selected eigenvalues. The drug content in samples was evaluated by using the $C_{\text{prediction}} = b \times R_{\text{sample}}$. PLS toolbox 3.0 in Matlab 6.5 software was used for the data treatment.

HPLC-PLS Method

The PLS calibration using the orthogonalized PLS algorithm developed by Wold[3,4] and extensively discussed by Martens and Naes[5] simultaneously involves the independent and the dependent variables on the data compression and decomposition operations.

In the HPLC data analysis, the HPLC-PLS calibration is done by decomposition of both concentration and the ratio of peak area matrix into latent variables, $R = T \times P^T + E$ and $C = U \times Q^T + F$. The linear regression, $C_{prediction} = b \times R_{sample}$, is used for the estimation of the drugs in the samples. The vector, b is given as $b = W \times (P^T \times W)^{-1} \times Q$, where W is a weight matrix.

Application of this method was performed by means of PLS toolbox 3.0 in Matlab 6.5 software.

EXPERIMENTAL

Instrumentation and Chromatography

Chromatography was performed with an Agilent 1100 series HPLC system (Agilent Technologies, Inc., California, and USA) provided with a quaternary pump, a thermostatted autosampler, a thermostatted column compartment, and a multiwavelength diode array detector (DAD). Chromatographic data were acquired and processed using HP Chem Station for LC (Rev. A0.01 [403]) software from Hawlet-Packard. The column used was a Waters Symmetry® C18 Column 5 μm 4.6 × 250 mm. The flow rate was maintained at 1.7 mL/min and the injection volume was 30 μL. The mobile phase was prepared daily and filtered through a 0.45 μm membrane filter.

Commercial Tablet Formulation

The commercial pharmaceutical formulation, Konveril® Plus tablets (produced by Nobel Pharm., Turkey, Batch no. 2D008) containing 20 mg EA and 12.5 mg HCT were analysed by using the proposed methods.

Standard Solutions

Stock solution of 12.5 mg/100 mL EA, HCT, and IS were prepared in a mixture of 0.2 M acetate buffer and acetonitrile (v/v, 60:40). A standard series (concentration set) of the solutions containing 15–40 μg/mL EA and 2.5–15 μg/mL HCT was obtained from the stock solutions. A validation set consisting of 12 synthetic mixture solutions in the working range of

15–40 μg/mL EA and 2.5–15 μg/mL HCT was prepared. For standard addition method, six solutions using the stock solutions and tablet solutions were prepared. In all the chromatographic study, 12.5 μg/mL IS as internal standard was added into each solution. All the solutions were prepared freshly and protected from light.

Tablet Analysis

Twenty tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in the mobile phase in a 100 mL calibrated flask. The solution was filtered into a 100 mL calibrated flask by a 0.45 μm membrane filter. Tablet solutions were diluted to the working concentration range of 20 μg/mL for EA and 12.5 μg/mL for HTC in a 25 mL-calibrated flask. The developed methods were applied to the final solutions of the samples.

RESULTS AND DISCUSSION

Method Development and Optimization

Introduction of multiwavelength PDA detectors to the HPLC systems make possible simultaneous chromatographic detection of samples at multiwavelengths. The obtained multiwavelength detections produce different peak area information. Simultaneous data collection at multiwavelengths provides application of multivariate calibration techniques to these HPLC data for the quantitative studies. The application of multivariate methods CLS, PCR and PLS to the obtained chromatographic data is a new idea for the simultaneous quantitative analysis of EA and HCT in samples.

The application of multivariate methods to the HPLC data requires collection of peak areas at multiwavelengths at which the sample exhibits good absorption and also requires good peak separation in the chromatograms.

The data treatments for the HPLC-chemometric calibrations need the same data process as in single wavelength HPLC calculations. The ratio of peak area was obtained by dividing each drug peak area to IS peak area. These peak area ratios as HPLC data set were used to construct the multivariate calibrations as HPLC-CLS, HPLC-PCR, and HPLC-PLS.

For a comparison of these HPLC-chemometric calibrations, the classical HPLC method based on a single wavelength detection response was also used for the analysis of the mixtures of the two drug samples. The experimental results of HPLC-chemometric calibration methods were compared with each other, as well as with those obtained by the classical-HPLC method.

In our case, the main aim of the applied multivariate HPLC calibrations to the multivariate HPLC data is the elimination or reduction of the errors

occurring from sample injection and experimental environment that affect the peak area. Therefore, HPLC-chemometric calibration permits the removal or reduction of errors and residuals of calibration of the classical HPLC based on a single wavelength. Sensitivity, accuracy, and precision of the HPLC-chemometric calibrations increase in comparison with the classical-HPLC method.

In general, at least three injections should be made for good results. We also made three injections for each concentration in this study. On the other hand, we observed that single injection calculations produced satisfactory results in our multivariate calculations. This decreased the analysis time and saved some time and cost, but the single HPLC calibration method may not provide such an advantage.

During the data collection, fluctuations can occur in the instrument and produce no signal or distorted signals. If one uses single wavelength point calibration, it can lose all the information about the signal or can not produce good calibration. The HPLC multivariate models based on the use of multi-wavelength chromatograms are not affected by this situation. If this kind of situation occurs in a calibration set, the algorithms will recognize the fluctuations as noise, and will eliminate them from the calibrations.

The implementation of the multivariate calibration algorithms described in the theoretical section was explained in the following section.

Processing of HPLC Data

The concentration set consisting of the mixture solution in the concentration range of 15–40 μg/mL EA and 2.5–15 μg/mL HCT with 12.5 μg/mL IS was prepared. The peak area of the concentration set was recorded at a five-wavelength set (230, 240, 250, 260, and 270 nm) and at the retention time of 1.22 for EA, 1.90 for HCT, and 2.95 for IS. The chromatograms of the concentration set in the working range for both drugs are shown in Figure 1. The HPLC data set corresponding to the concentration set is presented in Table 1. The chemometric calibration technique, CLS, PCR, and PLS were subjected to the prepared concentration set and its measured HPLC data set. The amount of EA and HCT in samples were determined by the constructed the HPLC-chemometric calibrations.

HPLC-CLS Method

The algorithm of HPLC-CLS was briefly explained in the Experimental section. In this technique, the coefficient vector matrix (K) was calculated by using the linear equation system based on the relationship between the peak area data and concentration set, according to the HPLC data given in Table 1. This HPLC data set corresponds to the chromatograms presented

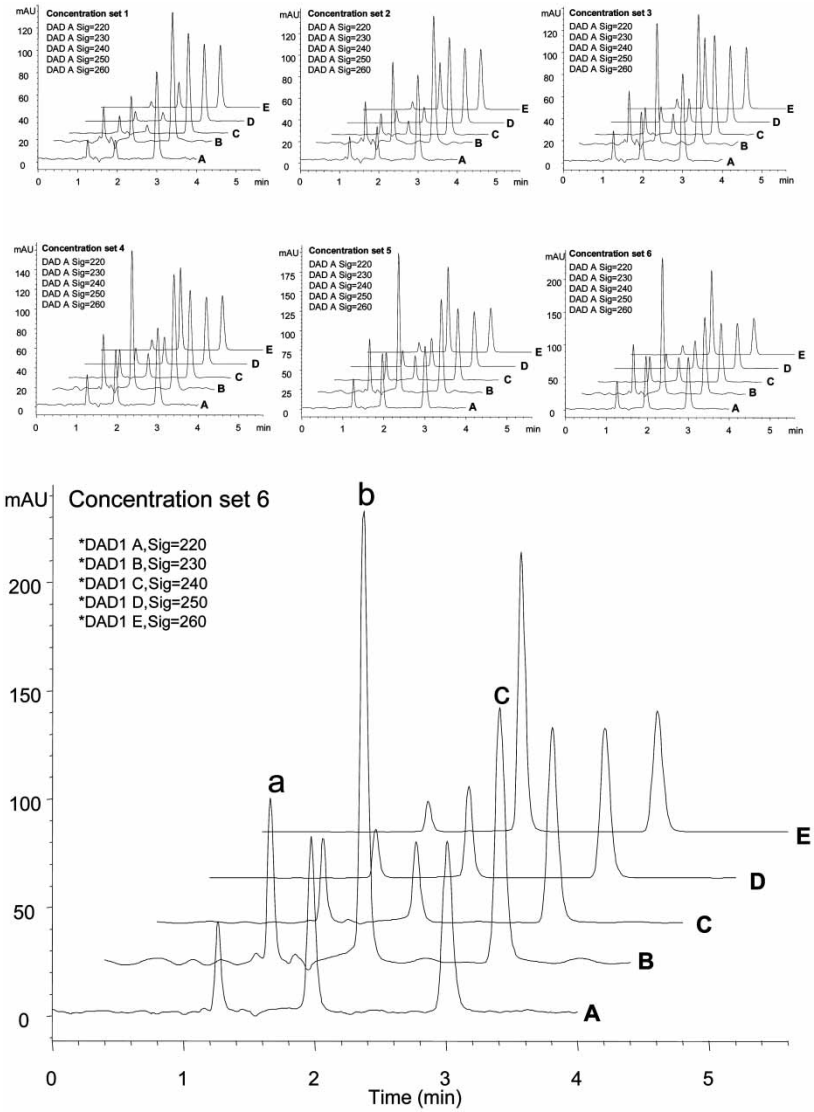

Figure 1. HPLC 3D chromatograms of concentration set 6 containing 40 μg/mL EA (a) and 15 μg/mL HCT (b) and 12.5 μg/mL IS (c), at five different wavelengths. The small chromatograms represent concentrations set 1–6.

in Figure 1. Replacing the coefficient matrix (K) into the linear equation system, the calibration of HPLC-CLS was obtained. The prediction of unknown concentration of EA and HCT in samples was carried out by the HPLC-CLS calibration. The calibration and data treatment were computed by CLS algorithm written in Matlab 6.5 software.

Table 1. The HPLC data set corresponding to the concentration set

	Concentration set			The ratio of peak areas (EA/IS)					The ratio of peak areas (HCT/IS)				
No.	EA	HCT	IS	220	230	240	250	260	220	230	240	250	260
1	15	2.5	12.5	0.1897	0.2429	0.1628	0.0819	0.0628	0.2201	0.5281	0.0791	0.0829	0.2963
2	20	5.0	12.5	0.2211	0.285	0.1709	0.1042	0.0798	0.3389	0.6519	0.1928	0.1495	0.5732
3	25	7.5	12.5	0.2419	0.2982	0.1919	0.1293	0.0999	0.4574	0.7285	0.1609	0.2279	0.8534
4	30	10.0	12.5	0.2837	0.3424	0.2235	0.1522	0.1140	0.6093	1.0375	0.2421	0.3069	1.1124
5	35	12.5	12.5	0.3212	0.3572	0.2704	0.1794	0.1358	0.7231	1.2103	0.3277	0.3817	1.4039
6	40	15.0	12.5	0.3493	0.4136	0.2820	0.2059	0.1585	0.8101	1.4885	0.4003	0.4600	1.6975

HPLC-PCR Method

The HPLC-PCR calibration was constructed by using the PCR algorithm as it was explained above. In this model, the square matrix of peak area data was obtained by decomposition of peak area values. Linear correlation between concentration set and decomposed peak area values was used to obtain the HPLC-PCR calibration. This procedure was separately repeated for EA and HCT. The obtained HPLC-PCR calibration was subjected to the determination of the above drugs in the synthetic mixtures and tablets. The data given in Table 1, corresponding to the Figure 1, was used for HPLC-PCR calibration. The PLS toolbox 3.0 in Matlab 6.5 was used for the calculation of calibration and data treatment.

HPLC-PLS Method

PLS calibration algorithm briefly described in Experimental section was applied to HPLC data summarized in Table 1, which corresponds to Figure 1. In this calibration model, both peak area data and concentration set were decomposed. HPLC-PLS calibration was obtained by using the relationship between the decomposed peak area data and concentration set. The quantitative determination of the subjected two drugs in samples was performed by the obtained HPLC-PLS calibration. The mathematical treatments have been done by means of the PLS toolbox 3.0 in Matlab 6.5.

Classical HPLC

The chromatograms corresponding to the concentration range of 15–40 μg/mL EA and 2.5–15 μg/mL HCT with 12.5 μg/mL IS were plotted by using a diode array detector at the five-wavelength set as shown in Figure 1. The detector responses were measured in terms of peak area. Separation was carried out at the ambient temperature on Waters Symmetry® C18 Column 5 μm 4.6 × 250 mm, and mobile phase consisted of 0.2 M acetate buffer and acetonitrile (v/v, 60:40). The flow rate was set at 1.7 mL/min with 30 μL as injection volume. The used IS is suitable for our case as seen in Figure 1. In fact, several mobile phases and other chromatographic conditions were tested, but the above chromatographic conditions were found to be suitable for the separation and determination of EA and HCT in their mixtures. The same conditions were used for the HPLC-chemometric calibrations. At a flow rate of 1.7 mL/min, retention times were obtained as 1.22 for EA, 1.90 for HCT, and 2.95 for IS (Figure 1).

For the calibration, the ratio of peak area of analyte to IS was plotted against the concentration of EA and HCT. Table 1 indicates the data of the ratio peak area obtained at the five wavelength set 220 (A), 230 (B),

Table 2. Linear regression analysis and their statistical parameters

Drug	λ	Regression equation	r	SE (m)	SE (n)	SE (r)
EA	220	$A = 0.0065C_{EA} + 0.0887$	0.9965	0.0003	0.0078	0.0057
	230	$A = 0.0064C_{EA} + 0.1481$	0.9865	0.0005	0.0152	0.0111
	240	$A = 0.0053C_{EA} + 0.0713$	0.9563	0.0053	0.0163	0.0118
	250	$A = 0.0050C_{EA} + 0.0056$	0.9994	0.0001	0.0024	0.0017
	260	$A = 0.0038C_{EA} + 0.0046$	0.9978	0.0001	0.0036	0.0026
HCT	220	$A = 0.0486C_{HCT} + 0.1010$	0.9977	0.0017	0.0162	0.0173
	230	$A = 0.0776C_{HCT} + 0.2622$	0.9830	0.0072	0.0705	0.0757
	240	$A = 0.0239C_{HCT} + 0.0246$	0.9621	0.0034	0.0330	0.0355
	250	$A = 0.0304C_{HCT} + 0.0020$	0.9997	0.0003	0.0034	0.0036
	260	$A = 0.1115C_{HCT} + 0.0137$	0.9998	0.0010	0.0097	0.0104

SE(m): Standard error of slope; SE(n): Standard error of intercept; SE(r): Standard error of regression constant.

C: Concentration (μg/mL); A: Peak area; r: Regression coefficient.

240 (C), 250 (D), and 260 (E). In the above wavelength points, five straight lines for each drug were obtained from the HPLC data given in Table1. Two equations having the highest regression coefficients at 250 nm among the calculated calibration equations were chosen for the analysis procedure of EA and HCT.

The calculated straight lines and their statistical parameters were presented in Table 2. The correlation coefficients of regression equations were found to be higher than 0.99. At the subjected wavelength point, the calibration equation achieves good linearity and successful results for EA and HCT.

The validity of the HPLC method was assessed by applying the standard addition technique for five replicates. The results are presented in Table 3. In this table, the results indicate that there is no interference from the excipients used in the formulation of the tablets.

Table 3. Statistical results for standard addition technique

	HPLC method		PCR		PLS		CLS	
	EA	HCT	EA	HTC	EA	HTC	EA	HTC
Mean recovery	105.4	100.5	103.6	100.8	103.2	101.1	104.7	99.2
SD	1.88	3.34	2.51	1.12	1.97	1.46	0.85	1.72
RSD	1.78	3.33	2.43	1.11	1.91	1.44	0.81	1.73

SD: Standard deviation; RSD: Relative standard error.

Statistical Analysis

In the HPLC-chemometrics calibrations, the predictive ability of a regression model can be defined by several ways. The most general expression is the standard error of calibrations (SEC). In our case, six chromatograms corresponding to the concentration set with IS were used in calibration steps for both drugs. The SEC values of EA and HCT were calculated by the data obtained from difference between added and predicted concentrations in the calibration steps of both drugs. The linear regression analysis and its other statistical results based on the relationship between added and predicted concentrations were obtained. Their statistical results with SEC values are given in Table 4. According to the cross validation procedure, the first three factors for HPLC-PCR and HPLC-PLS were found reliable for the prediction of both drugs. The above SEC values and other statistical values, correlation coefficient (r), slope (m), and intercept (n) were computed by the HPLC-PCR and HPLC-PLS calibrations using the first three factors.

In the chemometric calibration study, another important parameter is the standard error of prediction (SEP). The SEP values and their statistical values were calculated according to the difference between added and predicted concentrations in the synthetic mixtures. The obtained results, SEP, correlation coefficient (r), slope (m), and intercept (n) are presented in Table 4.

All the statistical data indicate that the minimum values of SEC and SEP give us acceptable results, under optimized conditions, in the calibration and prediction steps.

Method Validation

The validation of HPLC-CLS, HPLC-PCR, HPLC-PLS, and classical-HPLC was carried out by their performance for obtaining reliable results of analysis. For this purpose, twelve different synthetic mixtures in the concen-

Table 4. Statistical calculations for the calibration techniques in the calibration and prediction steps

	Classical HPLC		HPLC-PCR		HPLC-PLS		HPLC-CLS	
Parameter	EA	HTC	EA	HTC	EA	HTC	EA	HTC
SEP	0.5223	0.3575	0.3916	0.2739	0.3633	0.2693	0.4635	0.275
SEC	0.56393	0.40405	0.3957	0.08202	0.29957	0.0629	0.53341	0.3595
r	0.9957	0.9830	0.9996	0.9978	0.9991	0.9979	0.9987	0.9972
n	0.0038	0.0776	1.0258	0.9833	1.0183	0.9803	1.0394	0.9798
m	0.0046	0.2622	−0.8872	0.0466	−0.5865	0.0771	−0.7454	0.1562

SEP: Standard error of prediction; SEC: Standard error of calibration.
n: Intercept; m: Slope.

tration range of 15–40 μg/mL EA and 2.5–15 μg/mL HCT with 12.5 μg/mL IS were analyzed by the proposed calibration method. The mean recoveries and the relative standard deviations of our proposed methods were computed and presented in Table 5. Their numerical values were found satisfactory for the validity of HPLC-CLS, HPLC-PCR, HPLC-PLS, and classical-HPLC. Reliable accuracy and higher precision in the application of these methods were observed for the analysis of both drugs. During the analysis procedure, interference and systematical errors were not observed.

Another parameter for validity of the developed methods is the standard addition technique. The standard of two pure drugs equal to the content of the tablet formulation was added to the tablet solutions in the working concentration range. The results and their standard deviations were calculated and presented in Table 3. Recovery results were obtained on the average of five replicates for each drug. Good agreement was observed for the standard addition assay results by application of these methods.

Tablet Analysis

HPLC-CLS, HPLC-PCR, HPLC-PLS, and classical-HPLC techniques were applied to the quantitative analysis of EA and HCT in tablets. The experimental results of the pharmaceutical dosage forms are presented in Table 6. The results of all methods were very close to each other, as well as to the label value of the commercial pharmaceutical dosage forms. Good agreement was observed for all the applied methods.

Results Evaluation by Statistical Tests

Three statistical tests, t-test, F-test, and ANOVA tests were applied to tablet assay results for the significance of differences between the methods. First, in the t-test and F-test HPLC-CLS, HPLC-PCR, and HPLC-PLS were compared with classical-HPLC. Their theoretical and calculated results are presented in Table 6. Second, a one way ANOVA test was subjected to the assay results obtained from all the methods. The theoretical and calculated F-, t-, and ANOVA tests are illustrated in Table 6. The calculated statistical test values did not exceed the theoretical statistical values, indicating that there was no significant difference among the methods. The numerical values of all statistical tests indicated that the investigated techniques are suitable for the determination of both drugs in the pharmaceutical dosage form.

CONCLUSIONS

In analytical studies, the HPLC method is a comparison method for the analysis of samples. In this presented study, the HPLC method was denoted as the classical HPLC method. For good separation and determination, it is

Table 5. Recovery data obtained from the synthetic mixtures by the proposed methods

	Added (mg/mL)		Found (mg/mL)								Recovery (%)							
			HPLC method		PCR		PLS		CLS		HPLC method		PCR		PLS		CLS	
No.	EA	HTC	EA	HTC	EA	HTC	EA	HTC	EA	HTC	EA	HTC	EA	HTC	EA	HTC	EA	HTC
1	15.0	12.5	15.56	11.98	14.38	12.03	15.20	12.05	15.12	12.37	103.74	104.36	95.9	96.2	101.3	96.4	100.8	99.0
2	20.0	12.5	19.84	12.60	19.71	12.34	19.69	12.30	20.71	12.26	99.20	99.22	98.6	98.7	99.9	99.7	105.2	99.7
3	25.0	12.5	24.36	12.23	24.91	12.33	24.75	12.30	25.22	12.13	97.43	102.23	99.6	98.6	99.4	99.7	101.9	98.6
4	30.0	12.5	29.68	13.01	29.96	12.18	29.76	12.19	30.53	12.66	98.94	96.04	99.9	97.5	99.3	100.1	102.6	103.9
5	35.0	12.5	34.31	12.00	34.70	12.79	34.96	12.79	35.50	12.93	98.03	104.15	99.1	102.3	100.7	100.0	101.6	101.1
6	40.0	12.5	40.46	12.44	40.28	12.60	40.49	12.59	40.96	12.38	101.16	100.46	100.7	100.8	100.5	99.9	101.2	98.3
7	20.0	2.5	19.62	2.38	19.87	2.22	20.16	2.26	19.57	2.71	98.11	104.98	99.4	88.8	101.5	101.6	97.0	120.0
8	20.0	5.0	20.23	5.23	19.55	5.29	20.17	5.28	20.21	5.09	101.16	95.60	97.8	105.8	103.2	99.9	100.2	96.3
9	20.0	7.5	19.78	7.83	19.52	7.54	19.37	7.54	20.34	7.20	98.91	95.76	97.6	100.6	99.2	100.0	105.0	95.5
10	20.0	10.0	19.02	9.65	19.62	9.72	19.50	9.73	20.12	9.75	95.12	103.60	98.1	97.2	99.4	100.1	103.2	100.3
11	20.0	12.5	19.56	11.97	19.97	12.18	19.68	12.22	19.13	12.76	97.79	104.39	99.9	97.5	98.5	100.3	97.2	104.4
12	20.0	15.0	19.42	14.70	19.22	14.71	19.48	14.68	19.88	14.56	97.10	102.01	96.1	98.1	101.3	99.8	102.1	99.2
										Mean	98.9	101.1	98.5	98.5	100.4	99.8	101.5	101.4
										RSD	2.28	3.56	1.53	4.11	1.31	1.19	2.51	6.33

Table 6. The experimental results obtained in tablets by proposed methods

	Tablet							
	Classical HPLC		PCR		PLS		CLS	
	EA	HTC	EA	HTC	EA	HTC	EA	HTC
Mean	21.31	11.56	20.99	12.33	20.86	12.38	20.54	12.45
SD	0.42	0.66	0.39	0.28	0.42	0.25	0.35	0.27
RSD	1.98	5.67	1.84	2.24	2.01	2.05	1.68	2.19
SE	0.15	0.23	0.14	0.10	0.15	0.09	0.12	0.10
CL (P = 0.05)	0.29	0.45	0.27	0.19	0.29	0.18	0.24	0.19
ANOVA test	2.6819	1.5671	2.6819	1.5671	2.6819	1.5671	2.6819	1.5671
$F_{theoretical}$	2.8165	2.8165	2.8165	2.8165	2.8165	2.8165	2.8165	2.8165
$F_{calculated}$	—	—	2.2389	1.4965	2.0379	1.8600	1.1436	1.4976
$F_{theoretical}$			2.8179	2.8179	2.8179	2.8179	2.8179	2.8179
$t_{calculated}$	—	—	0.0985	0.2574	0.1266	0.0172	0.4139	0.2570
$t_{theoretical}$			2.8179	2.8179	2.8179	2.8179	2.8179	2.8179

Label claim: 12.5 mg/tab HCT and 20 mg/tab.

not possible to find the chromatographic condition and optimization in every case. For this reason, the HPLC-chemometric calibration technique has an important role for the evaluation of chromatograms at the multiwavelength points in the presence of PDA responses. As an alternative, the combined calibration technique, HPLC-CLS, HPLC-PCR, and HPLC-PLS calibration models were proposed for simultaneous prediction of drug amounts in samples. In addition, multivariate HPLC calibration using special mathematical algorithms enables reduction of the errors and residuals, compared with calibration of classical HPLC based on a single wavelength.

The selection of a single wavelength point sometimes causes time consumption because of multiple injections to find the best wavelength point. The use of multivariate HPLC models for the calibrations does not require searching for the best wavelength point, because all the scanned wavelength points are included in the calibration. This is a superiority of the chemometric HPLC method over classical HLPC based on single wavelength detector response.

This study indicates the evaluation of HPLC data for chemometric calibrations. In our case, a good chromatographic separation and higher peak area was obtained by satisfactory optimization and conditions. Good agreement was observed in the results of the HPLC-chemometric approaches. This new application of a chemometric calibration technique to the HPLC data set is an alternative model for the minimization of experimental errors in chromatographic analysis.

The HPLC-chemometric calibration techniques can be successfully applied to the routine and quality control analysis of the subjected drugs and other drugs in samples.

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