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Spectophotometric quantitative resolution of hydrochlorothiazide and spironolactone in tablets by chemometric analysis methods

Erdal Dinç *, Özgür Üstündağ

Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey

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Abstract

Spectrophotometric simultaneous determination of hydrochlorothiazide and spironolactone in tablets was performed by classical least-squares (CLS), inverse least-squares (ILS), principal component regression (PCR) and partial least-squares (PLS). The methods of the chemometric analysis do not require sample pretreatment procedure. A training set of 25 standard mixture containing both drugs was prepared in the concentration range of 2–20 μg/ml according to mixture design. The multivarate calibrations were obtained by measuring the zero-order and first-derivative absorbances at 15 points from 220 to 290 nm using the training set. The validation of the multivariate methods was realised by analysing the synthetic mixtures of hydrochlorothiazide and spironolactone. The result obtained on the synthetic mixture and tablets were statistically compared by the one-way ANOVA test. The chemometrics analysis methods were satisfactorily applied to the simultaneous determination of hydrochlorothiazide and spironolactone in the pharmaceutical tablet formulation.

Keywords: Chemometric methods; Mixture design; Hydrochlorothiazide; Spironolactone; tablet

1. Introduction

Hydrochlorothiazide (HCT) in combination with spironolactone (SP) is used as an anti-hypertensive and a diuretic agent in tablets. Simultaneous determination of HCT and SP is more often necessary in quality control.

In recent years, multivariate calibrations, such as classical least-squares (CLS), inverse least-squares (ILS), principal component regression (PCR) and partial least-squares (PLS) are started to apply to the analysis of the analytical data obtained in all the instrumentations [1–5]. The same methods and their algorithms have been apply to the simultaneous spectrophotometric determination of drugs in the pharmaceutical formulation containing two or more compounds with overlapping spectra. On the other hand the chemometric calibration methods as those enumerated above have been used extensively in quantitative spectral analysis to get selective information from unselective data. The main advantages of these techniques are the following: a higher speed of processing data concerning the values of concentrations and absorbances of compounds with strongly overlapping spectra, the errors of calibration model are minimised by measuring the absorbance values at many points in the wavelength range of the zero-order and derivative spectra.

Analytical methods using multivariate calibrations and their applications include the spectrophotometric [6–9], chromatographic [9] and electrochemical [10] for determinations of analytes in the mixtures.

Hydrochlorothiazide is found in combination with many other drugs. Quantitative analysis of the samples containing hydrochlorothiazide and benazepril is achieved by chemometry [11], spectrophotometry [12–15], HPLC [15,16], micro-bore liquid chromatography [17] and thin layer chromatography [17]. The binary mixture of hydrochlorothiazide and amiloride has been determined by spectrophotometry [18–21], chemometry [22] and HPLC [23,24]. Hydrochlorothiazide–captoril mixture is analyzed by spectrophotometry [25,26] and HPLC [26–28]. Spectrophotometry [29] and HPLC

* Corresponding author.
E-mail address: dinc@pharmacy.ankara.edu.tr (E. Dinç).

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[30,31] have been developed for determination of hydrochlorothiazide and enalapril maleate. Two different methods, spectrophotometry [32] and HPLC [33] have been applied to quantitative analysis of hydrochlorothiazide–lisinopril mixture. The determination of hydrochlorothiazide and spironolactone in samples has been carried out by partial least square method [34] and flow injection analysis and spectrophotometry [35] and HPLC [36]. Hydrochlorothiazide–cilazapril combination is determined by chemometric methods [37] and by HPLC [38]. Spectrophotometry [36] has been subjected to the determination of hydrochlorothiazide and ramipril in their mixture. The content of hydrochlorothiazide and fosinopril is determined by spectrophotometry [39,40] and HPLC [40]. Liquid chromatography [41–43] is applied to the analysis of the mixture containing hydrochlorothiazide and losartan. Quantitative determination of hydrochlorothiazide triamterene in the mixture has been reported by using spectrophotometry [37] and HPLC [44]. Hydrochlorothiazide–chlorothiazide mixture is analyzed using by HPLC [45,46], HPLC [47,48] has been used for determining the content of mixture of hydroclorothiazide and reserpine. Spectrophotometry [49] and HPLC [50] is subjected to analysis of hydrochlorothiazide and propranolol in samples. Two methods, chromatography and spectrophotometry has been used for the determination of hydrochlorothiazide and bevantolol in their mixture [51]. hydroclorothiazide and valsartan in samples is determined by using HPLC [52]. Mixtures of hydroclorothiazide and dihydralazine sulfate have been analyzed by conventional and differential pulse polarography [53].

In this work, synthetic mixtures and tablets containing HCT and SP were investigated and resolved by four chemometrics methods using zero-order and first-derivative spectra. These chemometrics calibrations are based on the mathematic algorithms (see Section 3). The results obtained were compared with those of a reported method [36].

2. Experimental

2.1. Instruments

The absorption spectra and their derivative spectra were performed by using Shimadzu UV-1600 double beam UV–Vis spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software, equipped with an HP DeskJet 600. The additional Maple V and SPSS.10 softwares were used for the chemometrics and statistical treatment of data, respectively.

2.2. Pharmaceutical tablet formulation

Tablet formulation, Aldactazide® (Ali Raif Ilaç Ind., Turkey.Batch no. 1C260) containing 25 mg SP, 25 mg HCT and excipients (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil and magnesium stearate) per tablet was analysed by the proposed chemometrics methods.

2.3. Reagents

Acetic acid–sodium acetate buffer solution (0.2 M, pH 5) was prepared by using analytical-reagent chemicals. Stock solutions containing 100 mg/ml each of HCT and SP were prepared separately in methanol and buffer solution mixture (80:20).

2.4. Standard solutions

A training set of standard mixture solutions containing 2–20 μg/ml HCT and 2–20 μg/ml SP was made daily from stock solutions. A validation set of 12 synthetic mixtures containing various concentrations of two drugs was also prepared from the same stock solutions.

3. Methods

3.1. CLS and ILS methods

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 $A = K \times C$ (or $dA/d\lambda = K \times C$) for CLS and $C = P \times A$ for ILS (or $C = P \times dA/d\lambda$) [1] Here, the matrices A and $dA/d\lambda$ represent the zero-order absorbance and derivative absorbance matrices, C is the concentration matrix, and K and P are the calibration coefficients.

3.2. PCR method

In the spectral work, the following steps can explain the fundamental concept of PCR [3]:

a) The original data obtained in absorbances (A) (or derivative absorbance ($dA/d\lambda$)) and concentration (C) of analyte were reprocessed by mean-centring as A_o and C_o, respectively.
b) The covariance dispersion matrix of the centered matrix 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

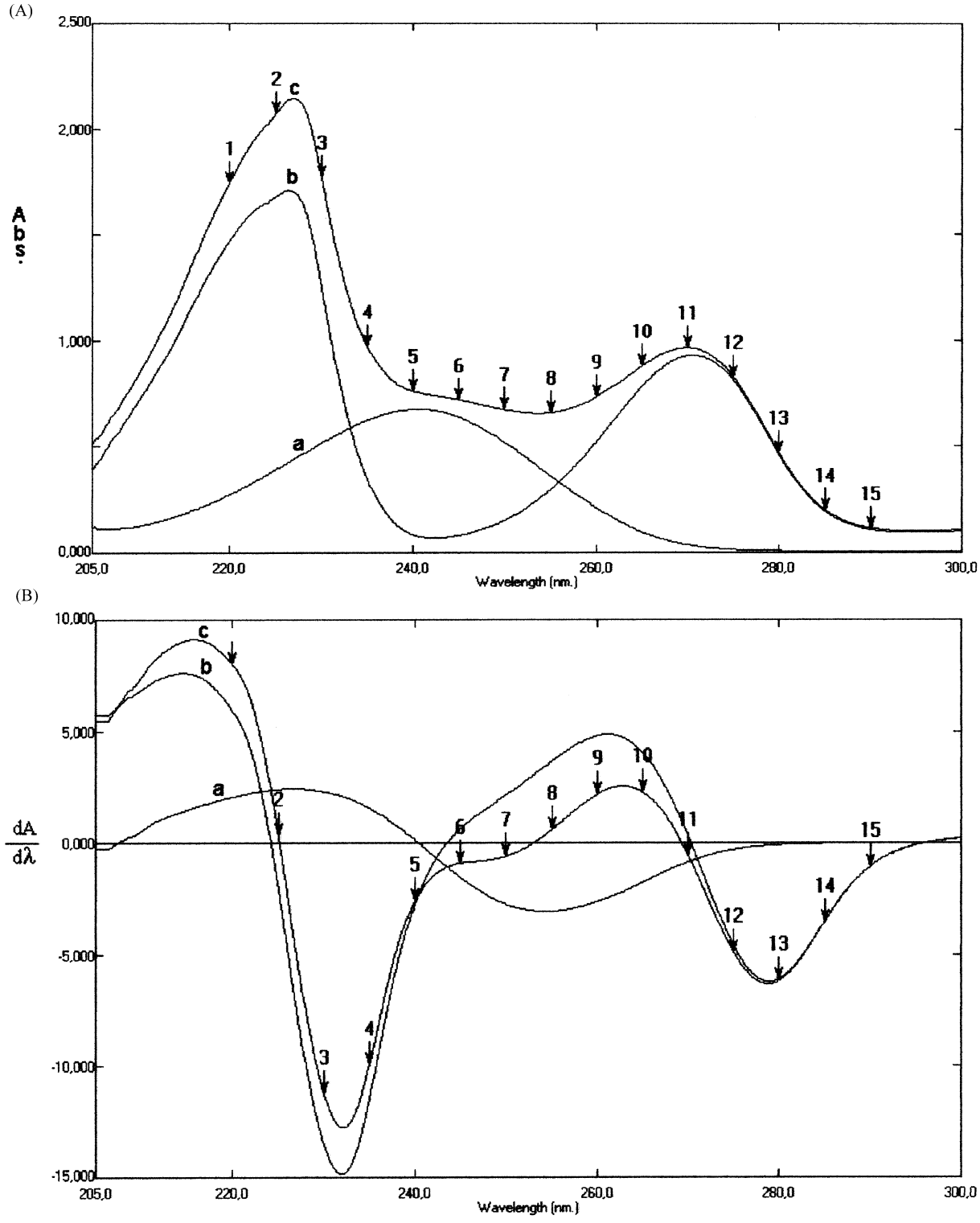

Fig. 1. Absorption spectra (A) and derivative spectra (B) of (a) SP, (b) HCT and (c) their mixture (1↓, 2↓,..., 15↓ corresponding to λ_1, $\lambda_2 \ldots \lambda_{15}$ (from 220.0 to 290.0 nm)).

the eigenvalues. The other eigenvalues and their corresponding eigenvectors are eliminated from our study. Using the ordinary linear regression $C = a + b \times A$ we calculated the coefficients, a and b. To reach this objective firstly, we determined the coefficient b as $b = P \times q$, where P is the matrix

Table 1
Concentration matrix of the mixtures containing two drugs

Standard no.	HCT (μg/ml)	SP (μg/ml)
1	2.0	2.0
2	2.0	5.0
3	2.0	10.0
4	2.0	15.0
5	2.0	20.0
6	5.0	2.0
7	5.0	5.0
8	5.0	10.0
9	5.0	15.0
10	5.0	20.0
11	10.0	2.0
12	10.0	5.0
13	10.0	10.0
14	10.0	15.0
15	10.0	20.0
16	15.0	2.0
17	15.0	5.0
18	15.0	10.0
19	15.0	15.0
20	15.0	20.0
21	20.0	2.0
22	20.0	5.0
23	20.0	10.0
24	20.0	15.0
25	20.0	20.0

of eigenvectors and q is the C-loadings given by $q = D \times T^{T} \times A_{o}$. Here T^{T} is the transpose of the score matrix T. 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 $a = C_{mean} - A^{T}_{mean} \times b$, where A^{T}_{mean} represents the transpose of the matrix having the entries the mean absorbaces values and C_{mean} is the mean concentration of the calibration set.

3.3. PLS method

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

In the spectrophotometry, the PLS calibration is done by decomposition of both concentration and absorbance matrix into latent variables, $A = TP^{T} + E$ and $C = UQ^{T} + F$. The vector, b is given as $b = W(P^{T}W)^{-1}Q$, where W is a weight matrix. By using the linear regression $C = a + b \times A$, the constant, a is calculated by $a = C_{mean} - A^{T}_{mean} \times b$. As in PCR method, the PLS calibration is used for the estimation of the compounds in the samples.

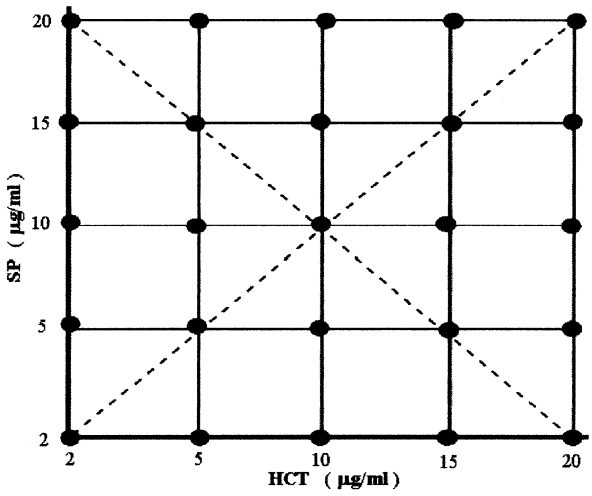

Fig. 2. Training set diagram consisting of the standard mixtures of HCT and SP in the calibration step.

4. Results and discussion

Fig. 1(a,b) shows the zero-order and first derivative spectra for HCT and SP as well as their corresponding binary mixture in methanol and acetate buffer solution of pH 5 (80:20). As shown, HCT exhibits two absorption maxima at 226.4 and 270.6 nm while SP gives an absorption maximum at 240.4 nm. The spectra of HCT and SP are overlapped in the absorption maxima as explained above. For this reason, four chemometrics calibrations using the zero-order and first derivative spectra were separately applied to the simultaneous determination of drugs in mixtures. Here, the chemometrics calibrations based on the first-derivative measurements were used to compare with those of zero-order measurements. The absorbance matrix was obtained by measuring the zero-order and first-derivative absorbances at the 15 wavelength from 220 to 290 nm as shown the same figure.

4.1. Training set design

A training set design of the concentration data corresponding to the HCT-SP mixtures was organized statistically to maximize the information content in the spectra and to minimize the error of the multivariate calibrations [5] as shown in Table 1. The training set of 25 standard mixture solutions which contains the concentrations with different ratio of HCT and SP was symmetrically prepared in the 2–20 μg/ml range of two drugs and it was illustrated in the diagram (see Fig. 2).

4.2. CLS method

In this method, the coefficient matrix (K) was calculated by using the linear equation system between training set and the absorbance data, and first-derivative

Table 2
Statistical results for PCR and PLS in the calibration step

	Zero-order spectra				First-derivative spectra			
	PCR		PLS		PCR		PLS	
Parameters	HCT	SP	HCT	SP	HCT	SP	HCT	SP
PRESS	0.681	0.101	1.331	0.174	0.745	0.407	0.421	0.322
SEC	0.172	0.094	0.245	0.129	0.177	0.130	0.133	0.114
R^2	0.9991	0.9999	0.9997	0.9993	0.9994	0.9998	0.9997	0.9997
Intercept	−0.0080	−0.0441	0.1907	0.1877	0.0198	−0.0301	−0.0192	0.0440
Slope	1.0030	1.0031	0.9895	0.9871	1.0045	1.0037	0.9961	0.9954

Table 3
Statistical results for CLS and ILS in the calibration step

	Zero-order spectra				First-derivative spectra			
	CLS		ILS		CLS		ILS	
Parameters	HCT	SP	HCT	SP	HCT	SP	HCT	SP
SEC	0.324	0.151	0.322	0.154	0.283	0.231	0.280	0.238
R^2	0.9985	0.9999	0.9985	0.9999	0.9987	0.9993	0.9987	0.9993
Intercept	−0.1880	−0.0804	−0.1880	−0.0804	−0.1795	−0.1873	−0.1795	−0.1873
Slope	1.0124	1.0055	1.0124	1.0055	1.0065	1.0128	1.0065	1.0128

absorbances. Replacing the coefficient matrix (K) into the linear equation systems, the calibrations of CLS for the zero-order spectra and first-derivative spectra were obtained. The zero-order absorbance and first-absorbance values at the 15 wavelengths from 220 to 290 nm in the 205–300 nm range for the samples were replaced in the CLS calibrations and the amounts of both drugs in synthetic mixtures and tablets were computed.

4.3. ILS method

In the calibration equation, the coefficient matrix (P) was obtained from the linear equation system using the absorbance and first-derivative absorbance corresponding to the training set. Introducing (P) into the equation system gives the ILS calibrations. The absorbance values of the samples, at the 15 wavelengths in the spectral region from 210 to 290 nm, were introduced into the obtained caibrations and the quantity of SP and HCT in the synthetic mixtures and tablets were predicted.

4.4. PCR method

The PCR calibration was constructed by using the PCR algorithm as explained in Section 3.2. The zero-order and first derivative absorbances measured at 15 points in the range of 210–290 nm, were introduced in the PCR calibtations and the quantity of each drug in mixtures and tablets was determined.

4.5. PLS method

The corresponding calibration was obtained by using PLS algorithms as explained in Section 3.3. The zero-order and first-derivative absorbances were measured in the same range and on the same sample as in PCR method.

4.6. 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:

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

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 were indicated in Tables 2 and 3. By inspection we conclude that SEC is minimum for PCR method for both drugs. The standard errors of prediction (SEP) of the same mixtures are displayed in Table 4 and the similar behaviour of the values was observed as for SEC.

Table 4
Statistical parameters obtained by applying the chemometric calibrations to the synthetic mixtures

Parameter	Zero-order spectra								First-derivative spectra							
	CLS		ILS		PCR		PLSR		CLS		ILS		PCR		PLSR	
	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP
SEP	0.101	0.182	0.100	0.195	0.164	0.21	0.622	0.176	0.261	0.340	0.262	0.342	0.131	0.218	0.201	0.244
R^2	0.9996	0.9990	0.9996	0.9988	0.9984	0.9990	0.9986	0.9990	0.9990	0.9988	0.9990	0.9988	0.9988	0.9990	0.9994	0.9986
(a)	0.0224	−0.0102	0.0224	−0.0399	−0.0253	0.0215	−0.0201	−0.1454	0.3150	0.2669	0.3150	0.2669	−0.0844	−0.2233	0.1340	0.1186
(b)	1.0029	1.0082	1.0029	1.0102	1.0133	1.0196	1.0077	1.0170	0.9897	0.9939	0.9897	0.9939	0.9898	1.0145	1.0020	1.0032

a = intercept, b = slope, r = correlation coefficient.

For PCR and PLS methods, a total 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:

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

The values of (PRESS) are 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.

Validation of the calibration methods was carried out by resolution of ten synthetic mixtures in various compositions within the working concentration range for both drugs. The results obtained are given in Tables 5 and 6. The maximum values of the mean percent errors corresponding to CLS, ILS, PCR and PLS for the same mixtures are completely acceptable because of their smallest values (see Tables 5 and 6). The mean recoveries and the relative standard deviations of the proposed methods were computed and were indicated in Tables 5 and 6. Their numerical values were found to be satisfactory for the validity of all calibration methods. The same tablet formulation was simultaneously determined by the reported HPLC method and its recovery and relative standard results were found as 100.5±0.5% with the lamp on and 100.6±0.4% with the lamp off for HCT and 100.2±0.6% for SP [36]. It was observed that our results on tablet formulation indicated good agreement with those given by HPLC method [36].

4.7. Standard addition method for validation

The interference of excipients and tablet additives for two drugs was tested for the application of the proposed methods to a commercial tablet formulation and no interference was observed according to the experimental results. For this reason, the standard solutions of pure drugs corresponding to tablet content were added to the tablet solutions and analysed by the CLS, ILS, PCR and PLS methods. In this case, the mean and relative standard deviation of CLS, ILS, PCR and PLS calibrations was found as 98.2±1.55, 99.0±1.85, 102.3±2.10 and 96.8±1.08 for SP and 103.1±2.41, 102.4±1.89, 101.8±2.11 and 104.2±2.64 for HCT, respectively. The recovery results are an average of 5 replicates for each drug.

4.8. Tablet analysis

Ten tablets were weighed accurately 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

Table 5
Multivariate spectral analysis of SP and HCT in the synthetic binary mixtures by zero-order spectra

Mixture		CLS		ILS		PCR		PLS		Error (%)							
Added (μg)		Recovery (%)		Recovery (%)		Recovery (%)		Recovery (%)		CLS		ILS		PCR		PLS	
SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT
10	2	99.9	95.0	99.9	95.0	100.9	101.0	98.6	95.0	0.1	5.0	0.1	5.0	−0.9	−1.0	1.4	5.0
10	6	101.0	103.0	101.0	102.7	102.5	104.0	100.3	100.5	−1.1	−2.7	−1.0	−2.7	−2.5	−4.0	−0.3	−0.5
10	10	101.0	101.1	101.2	101.1	102.8	100.6	100.8	100.4	−1.2	−1.1	−1.2	−1.1	−2.8	−0.6	−0.8	−0.4
10	14	99.0	100.1	98.4	100.0	100.3	98.9	98.3	99.9	0.2	0.0	1.6	0.0	−0.3	1.1	1.7	0.1
10	18	99.0	101.0	98.8	100.2	101.1	100.7	99.2	100.4	1.2	−0.2	1.2	−0.2	−1.1	−0.7	0.8	−0.4
10	20	99.0	101.4	98.3	100.4	100.8	102.6	98.9	100.7	1.7	−0.4	1.7	−0.4	−0.8	−2.6	1.1	−0.7
2	10	102.0	102.1	103.0	102.1	103.0	101.4	97.5	100.2	−3.0	−2.1	−3.0	−2.1	−3.0	−1.4	2.5	−0.2
6	10	100.9	101.3	102.3	100.4	104.5	100.0	101.0	99.0	−2.3	−0.4	−2.3	−0.4	−4.5	0.0	−1.0	1.0
10	10	100.3	99.9	101.3	99.9	102.9	99.9	100.8	99.2	−1.3	0.1	−1.3	0.1	−2.9	0.1	−0.8	0.8
14	10	102.1	101.0	103.4	100.8	104.6	105.0	103.4	105.9	−3.4	−0.8	−3.4	−0.8	−4.6	−5.0	−3.4	−5.9
18	10	100.8	100.0	101.1	99.7	102.1	100.1	101.0	100.1	−0.8	0.3	−1.1	0.3	−2.1	−0.1	−1.0	−0.1
20	10	100.4	101.0	100.4	100.7	101.6	99.6	100.6	101.5	−0.4	−0.7	−0.4	−0.7	−1.6	0.4	−0.6	−1.5
Mean		100.5	100.6	100.8	100.2	102.3	101.2	100.0	100.2	−0.86	−0.25	−0.76	−0.25	−2.26	−1.15	−0.03	−0.23
RSD		1.07	1.95	1.70	1.87	1.40	1.83	1.59	2.42								

RSD, relative standard deviation.

Table 6
Multivariate spectral analysis of SP and HCT in the synthetic binary mixtures by first-derivative spectra

Mixture		CLS		ILS		PCR		PLS		Error (%)							
Added (μg)		Recovery (%)		Recovery (%)		Recovery (%)		Recovery (%)		CLS		ILS		PCR		PLS	
SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT	SP	HCT
10	2	104.0	100.5	103.7	100.0	97.2	97.5	103.2	96.0	−3.7	0.0	−3.7	0.0	2.8	2.5	−3.2	4.0
10	6	103.0	105.0	103.1	104.7	99.1	98.5	103.5	103.3	−3.1	−4.7	−3.1	−4.7	0.9	1.5	−3.5	−3.3
10	10	105.0	104.0	103.0	103.5	99.8	97.7	103.4	102.8	−3.0	−3.5	−3.0	−3.5	0.2	2.3	−3.4	−2.8
10	14	104.0	100.0	103.8	101.4	97.5	97.1	103.3	101.3	−3.8	−1.4	−3.8	−1.4	2.5	2.9	−3.3	−1.3
10	18	102.5	101.1	102.5	100.9	97.9	99.3	102.4	101.2	−2.5	−0.9	−2.5	−0.9	2.1	0.7	−2.4	−1.2
10	20	98.5	99.0	98.5	99.2	98.5	98.7	97.3	99.9	1.5	0.8	1.5	0.8	1.5	1.3	2.7	0.1
2	10	100.0	104.0	102.5	104.3	99.0	97.9	102.5	102.2	−2.5	−4.3	−2.5	−4.3	1.0	2.1	−2.5	−2.2
6	10	100.5	103.0	103.8	102.6	98.0	96.1	100.5	101.2	−3.8	−2.6	−3.8	−2.6	2.0	3.9	−0.5	−1.2
10	10	104.0	102.0	103.6	102.4	99.0	96.8	100.1	101.7	−3.6	−2.4	−3.6	−2.4	1.0	3.2	−0.1	−1.7
14	10	101.4	103.0	101.6	103.3	102.6	101.8	101.6	100.5	−1.6	−3.3	−1.6	−3.3	−2.6	−1.8	−1.6	−0.5
18	10	100.6	103.0	100.2	102.5	99.9	96.8	100.4	103.3	−0.2	−2.5	−0.2	−2.5	0.1	3.2	−0.4	−3.3
20	10	100.5	101.0	100.4	100.9	100.5	98.9	100.7	102.0	−0.4	−0.9	−0.4	−0.9	−0.5	1.1	−0.7	−2.0
Mean		102.0	102.1	102.2	102.1	99.1	98.1	101.6	101.3	−2.23	−2.14	−2.23	−2.14	0.92	1.91	−1.58	−1.28
RSD		1.99	1.79	1.72	1.69	1.49	1.51	1.86	1.97								

RSD, relative standard deviation.

Table 7
Results obtained in the pharmaceutical samples by using four chemometric calibrations (label claim: 25 mg for each drug per tablet)

Drug	Zero-order spectra				First-derivative spectra			
	CLS	ILS	PCR	PLS	CLS	ILS	PCR	PLS
HCT mean [a] ± SD [b]	25.5 ± 0.1	25.0 ± 0.1	25.2 ± 0.3	25.0 ± 0.3	25.1 ± 0.2	25.0 ± 0.1	25.1 ± 0.2	25.0 ± 0.3
SP mean [a] ± SD [b]	24.8 ± 0.2	24.8 ± 0.1	24.7 ± 0.1	24.8 ± 0.2	24.8 ± 0.4	24.8 ± 0.3	24.7 ± 0.5	24.8 ± 0.2

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

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 the tablet formulation, and the results are presented in Table 7.

The results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. In addition, we observed that our results were in agreement with those of literature methods [35,36].

4.9. ANOVA test

In order to compare the differences among methods, the one-way ANOVA test was applied to four sets (ten replicate) obtained from the four calibrations for each drug in tablets. In this procedure, Snedecor's F-values were computed and compared with the tabulated F-value ($p = 0.05$). The same computation process was repeated for both drugs. In standard table, for $n_1 = 3$ and $n_2 = 28$ ($P = 0.05$), the F-value is given as 2.95. The results of ANOVA test were found to be 1.61 for SP and 1.13 for HCT. The experimental (calculated) F-values did not exceed the tabulated F-value in the variance analysis. It was concluded that there is no significant difference among the methods.

The numerical values of all statistic parameters indicated that our methods are suitable for the simultaneous determination of both drugs in tablets.

5. Conclusions

Four chemometric methods were applied successfully to simultaneous determination of HCT and SP in mixtures and tablets. It was observed that the results obtained were comparable with those given by HPLC method [36]. On the other hand, the fundamental advantages of our investigated methods are the simultaneous analysis of the mixture of the subject matter drugs, without chemical pretreatment, speed of analysis and cost effectiveness. Results also showed that the developed methods are more reliable than other spectrophotometric method [35].

Finally, all the developed methods can be strongly applied to a routine analysis, quality control of mixtures and commercial preparation containing two subject matter drugs.

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