Short communication

Chemometric resolution of a mixture containing hydrochlorothiazide and amiloride by absorption and derivative spectrophotometry

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Abstract

Four chemometric techniques, classical least squares (CLS) and inverse least squares (ILS) and principal component regression (PCR) and partial least squares regression (PLSR) were applied to the absorption and derivative spectrophotometric determinations of amiloride and hydrochlorothiazide in a pharmaceutical preparation. Four chemometric calibrations for both zero-order and first derivative spectra were constructed by measuring the absorbance and their $dA/d\lambda$ values at 34 points in the wavelength range 205–395 nm for a training set containing 2–10 μg/ml amiloride and 4–28 μg/ml hydrochlorothiazide corresponding to 25 point mixture design. The building chemometric calibrations were confirmed by using the synthetic mixtures containing two drugs. The results obtained by the proposed techniques based on the use of the measurements at the absorption spectra and at the first derivative spectra were statistically compared with each other. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

A mixture of amiloride and hydrochlorothiazide is usually used as an antihypertensive and diuretic agent in the pharmaceutical preparations.

Quantitative determination of hydrochlorothiazide in its binary combination with benazepril by spectrophotometry [1–5] and by HPLC [5–9], with amiloride by spectrophotometry [8–12] and by HPLC [12–14], with captoril by spectrophotometry [15,16] and by HPLC [16–18], with enalapril maleate by spectrophotometry [19] and by HPLC [20,21], with lisinopril by spectrophotometry [22] and by HPLC [23], with spironolactone by partial least square method [24], by flow injection analysis and spectrophotometry [25,26] and by HPLC [27], with cilazapril by spectrophotometry [26,28], with ramipril by spectrophotometry [26], with fosinopril by spectrophotometry [29,30] and by HPLC [30], with losartan by HPLC [31–33], with triamterene by spectrophotometry [27] and by HPLC [34], with chlorothiazide by HPLC

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[35,36], with reserpine by HPLC [37,38], with propranolol by spectrophotometry [39] and by HPLC [40], with bevantolol by chromatographic and spectrophotometric methods [41], with valsartan by HPLC [42], with dihydralazine sulfate by conventional and differential pulse polarography [43] have been described in the literature.

In the last few years, the chemometric calibration techniques, such as classical least squares (CLS), inverse least squares (ILS), principal component regression (PCR) and partial least squares regression (PLSR), have widely been applied for the spectophotometric resolution of mixtures containing two or more compounds without a preliminary separation [44–49]. The chemometric regression techniques and their applications have been demonstrated in the spectrophotometric [50–54], chromatographic [54] and electrochemical [55] determinations. On the other hand the chemometric techniques 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 in the presence of the spectral interference, the errors of calibration model are minimized by measuring the absorbances at many points in the wavelength range of the zero order and derivative spectra.

Derivative spectrophotometry gives a satisfactory resolution for the binary mixture systems, eliminate the interference from sample turbidity as well as the effect of excipients placed in the commercial products removes the noise peaks coming from instrument and medium. However, this method has limits for some analytical problems, when the analysis conditions are not appropriate.

In this investigation, the validation of four chemometric calibration techniques for the absorption spectra and first derivative spectra were realized by analyzing synthetic mixtures containing hydrochlorothiazide and amiloride in different proportions. All the developed techniques were applied also to the chemometric determination of two drugs in pharmaceutical preparation marketed in Turkey.

2. Experimental

2.1. Instruments

Shimadzu UV-160 double beam UV-Visible spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software equipped with an HP OfficeJet Pro 1150C was used. The absorption spectra were recorded over the wavelength range 205–395 nm using a reagent blank (a mixture of 80% (v/v) methanol and 20% (v/v) buffer solution at 1500 nm/min and stored in the computer. The additional softwares MAPLE V and SPSS 10.0 were used for the estimation of the spectral data and the statistical analysis, respectively.

2.2. Pharmaceutical tablet formulation

A commercial pharmaceutical formulation Moduretic® tablet (produced by Merck Sharp Dohme Pharm. Ind., Turkey. Batch no. 1020483) containing 5 mg of amiloride (AM), 50 mg of hydrochlorothazide (HY) and excipients (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil and magnesium stearate)/per tablet was analyzed by the proposed chemometric techniques.

AM and HY were obtained as a donation of Merck Sharp Dohme Pharm. Ind.

2.3. Reagents

Acetic acid–sodium acetate buffer solution, 0.2 M and pH 5, was prepared by using analytical-reagent grade reagents. Stock solutions of 100 mg/ml of HY and AM were prepared in a solvent containing 80% methanol and 20% buffer solution (0.2 M, pH 5), respectively.

2.4. Standard solutions

A training set and of standard mixture solutions containing between 4–28 µg/ml HY and 2–10 µg/ml AM was made daily from stock solutions.

A validation set of ten synthetic mixtures containing various concentrations of two drugs was also prepared by using the same stock solutions.
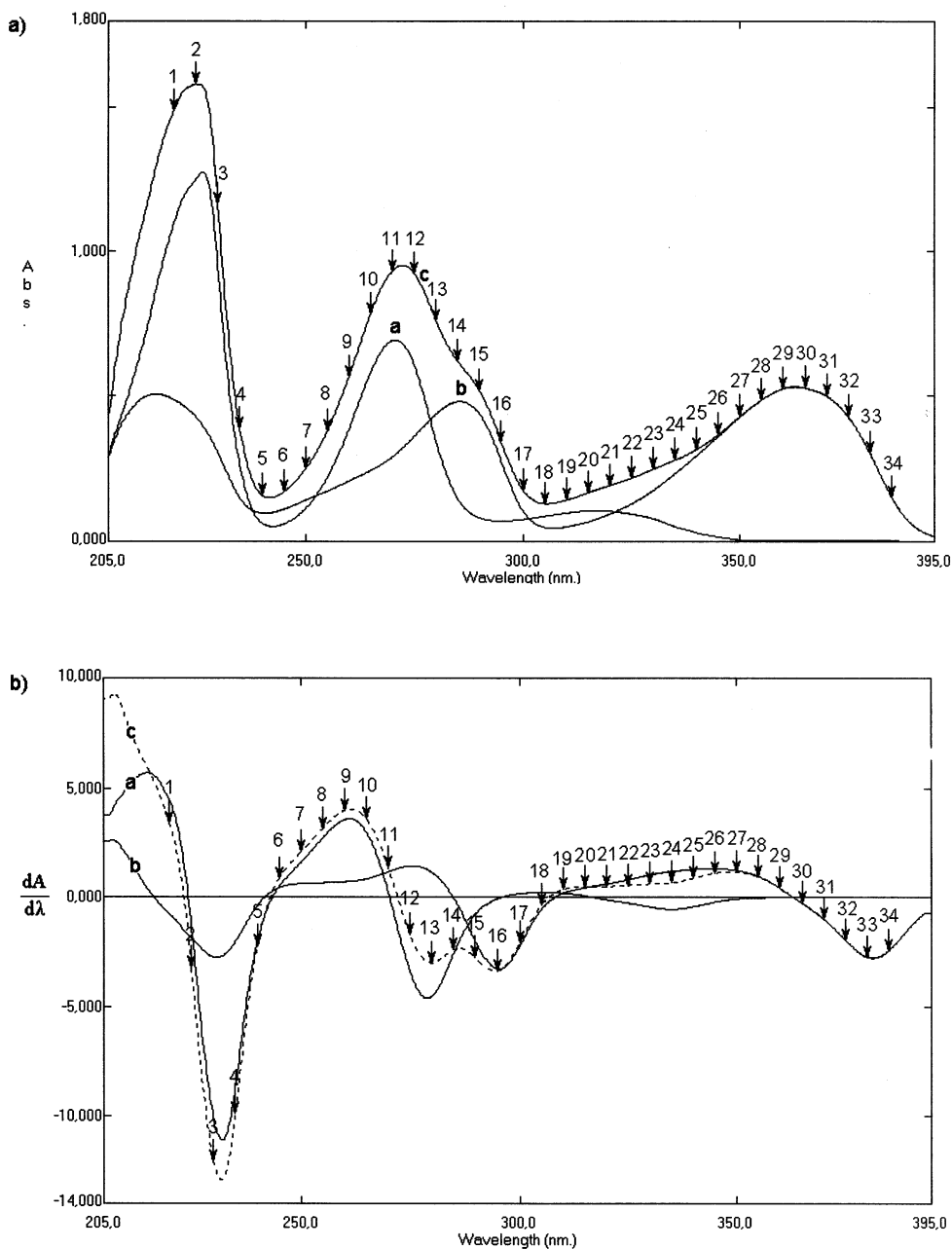
Fig. 1. Zero-order absorption spectra (a) and first derivative spectra (b) of: (a) 10 μg /ml hydrochlorothiazide; (b) 10 μg /ml amiloride; and (c) their mixture in methanol and acetate buffer solution at pH 5 (80:20). ($\downarrow$, $\downarrow$,...,$\downarrow$ corresponding to λ_1, λ_2,..., λ_{34} (from 220 to 385 nm)).

Table 1
Composition of a training set containing two drugs

Standard number	HY µg/ml	AM µg/ml
1	4.0	2.0
2	4.0	4.0
3	4.0	6.0
4	4.0	8.0
5	4.0	10.0
6	10.0	2.0
7	10.0	4.0
8	10.0	6.0
9	10.0	8.0
10	10.0	10.0
11	16.0	2.0
12	16.0	4.0
13	16.0	6.0
14	16.0	8.0
15	16.0	10.0
16	22.0	2.0
17	22.0	4.0
18	22.0	6.0
19	22.0	8.0
20	22.0	10.0
21	28.0	2.0
22	28.0	4.0
23	28.0	6.0
24	28.0	8.0
25	28.0	10.0

3. Results and discussion

Fig. 1 indicates the zero-order absorption spectra corresponding HY, AM and their mixture in methanol and acetate buffer solution at pH 5 (80:20). Under these conditions, pH 5 and methanol (20:80), the solutions of two drugs were found to be soluble and stable during 3 days at least. According to the diagram of binary matrix design [48], a training set of 25 representative mixtures at the different ratio of two drugs was prepared as in Table 1. The absorption spectra of the training set were recorded in computer and their first derivative were plotted with the interval of $\Delta\lambda = 8$ nm and scaling factor of 100 as in figure 2. The values of the absorption and first derivative absorbances were measured at 34 points with the interval of $\Delta\lambda = 5$ nm in the selected range of 220–385 nm. The obtained absorbance data were used for the proposed calibration models as explained below.

3.1. CLS and ILS for the zero-order and derivative spectra

The constant matrices K and P for both calibration models were obtained by using the linear equation system based on the use of absorption and derivative absorbance data at 34 points in the range from $\lambda_1 = 220$ nm to $\lambda_{34} = 385$ nm for the training set (calibration mixture solutions).

By inserting the values of K and P into the multi-linear regression equations, the prediction of unknown concentration of two drugs in samples was realized by measuring the zero-order and first derivative absorbance values of 34 wavelengths in the above range.

3.2. PCR and PLSR for the zero-order and the derivative spectra

According to the algorithms of PCR and PLSR, the calibrations of PCR and PLSR were built for the zero-order and derivative absorbance data at 34 points in the range of the wavelengths from 220 to 385 nm and for the training set.

By using a cross-validation, 25 calibration spectra were used for the selection of the optimum number of factors in the PCR and PLSR. For this case, the predicted concentrations of each sample in calibration step were compared with the actual concentrations. The prediction residual error sum-of-squares (PRESS) was computed by using the following formula.

$$\text{PRESS} = \sum_{i=1}^{n} (C_i^{\text{predicted}} - C_i^{\text{actual}})^2 \qquad (1)$$

In addition, two statistical criterions were considered for the calibration steps of PCR and PLSR. The first statistical parameter is the root mean square difference (RMSD). This parameter is an expression of the average error in the analysis for each component in training samples. The RMSD was obtained by the following formula [2]:

$$\text{RMSD} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (C_i^{\text{predicted}} - C_i^{\text{actual}})^2} \qquad (2)$$

Here $C_i^{\text{predicted}}$ and C_i^{actual} are the predicted and actual concentration in the calibration samples, respectively, and n is the total number of calibration samples.

The square of the correlation coefficient (r^2), which is, indicated the quality of fit of all the data to a straight line is calculated for the checking of each calibration. The relative error of the prediction (REP) as a second statistical parameter was also computed to control the predictive ability of the estimated calibration model for training set, as following:

$$REP = \frac{100}{\bar{C}} \sqrt{\frac{1}{n} \sum_{i=1}^{n} (C_i^{predicted} - C_i^{actual})^2} \qquad (3)$$

where $\bar{C}$ represents the mean of the predicted concentration in the calibration step for all samples.

Many number of factors for the calibrations were tested and the first five factors for PCR and five factors for PLSR were considered as suitable for the corresponding calibrations in this paper. The statistical results (PRESS, RMSD, REP, r^2, intercept and slope for the proposed calibrations) are summarized in Table 2. The statistical parameters in calibration step for the measurements zero order and first derivative spectra were compared and found to be acceptable for all the proposed calibration models.

3.3. Validation of chemometric calibrations for synthetic binary mixtures

In order to test the proposed calibrations, an independent set of the validation samples containing HY and AM in the different compositions given in Table 3, was prepared and analyzed. The means, standard deviations (S.D.) and relative standard deviations (R.S.D.) are indicated in Table 3.

The following statistical parameters were used for the validation of all the calibration techniques. The predictive applicability of a regression model is described in various ways. The most general expression is the standard error of prediction (SEP) and standard error of calibration denoted by SEC which is given in the following formula:

$$SEP (SEC) = \sqrt{\frac{\sum_{i=1}^{n} (C_i^{predicted} - C_i^{actual})^2}{n-1}} \qquad (4)$$

Here, C_i^{actual} is the actual concentration of analyte, $C_i^{predicted}$ is the predicted concentration of analyte and n is the total number of synthetic mixtures and calibration set.

In our paper, the values of SEC and SEP for CLS, ILS, PCR and PLSR were calculated by using the above formula in the calibration and the prediction steps, respectively. The SEP and SEC results and other statistical evaluations, square of correlation coefficient (r^2), intercept and slope obtained by applying CLS, ILS, PCR and PLSR to the above mentioned validation set of the synthetic mixtures were presented in Table 4. In addition, to check the precision of four calibration models, the limit of detection (LOD) and limit of quantification (LOQ) were computed by the obtained from ten replicate measurements or standard solutions of HY (25 μg/ml) and AM (2.5 μg/ml) individually as indicated in Table 4.

Table 2
Statistical results for the optimized chemometric techniques in the calibration step by using the cross-validation method

Parameters	Zero order spectra				First derivative spectra			
	PCR		PLSR		PCR		PLSR	
	HY	AM	HY	AM	HY	AM	HY	AM
PRESS	0.8101	0.2485	0.4278	0.4092	0.9888	0.5642	0.4307	0.5190
RMSD	0.1800	0.1000	0.1308	0.1279	0.1988	0.1502	0.1312	0.1440
REP	1.2080	0.5909	1.9382	0.7559	1.2452	2.5033	0.8200	2.4000
r^2	0.9990	0.9989	0.9990	0.9992	0.9998	0.9991	0.9989	0.9988
Intercept	0.0191	0.0228	0.0112	0.0541	−0.0159	−0.0243	−0.0321	0.0429
Slope	1.0121	0.9885	0.9991	1.0020	1.0008	1.0040	1.0240	0.9990

Table 3
Recovery results obtained for the determination of two drugs in synthetic mixtures by the chemometric techniques

Zero order spectra

Added mixture (µg/ml)		CLS		ILS		PCR		PLSR	
HY	AM	HY	AM	HY	AM	HY	AM	HY	AM
4	2.5	99.8	102.0	99.5	101.6	100.0	98.0	97.5	97.6
10	2.5	102.9	102.0	103.0	101.6	101.0	98.0	99.7	97.8
16	2.5	103.1	101.2	103.2	102.4	101.0	97.6	102.5	98.0
22	2.5	101.5	102.8	101.5	102.4	102.3	101.6	103.8	102.8
28	2.5	99.7	100.0	100.3	100.4	102.1	100.8	100.1	101.2
25	2.0	101.7	100.5	101.6	100.0	100.8	102.5	101.2	103.5
25	4.0	101.6	101.3	98.3	101.5	102.0	100.8	101.6	101.3
25	6.0	102.0	101.8	101.9	102.0	100.2	101.7	99.0	102.0
25	8.0	97.4	100.8	98.8	98.8	100.6	101.3	98.2	101.8
25	10.0	98.8	101.2	100.6	101.2	100.2	102.1	97.8	103.1
Mean		100.8	101.4	100.6	101.2	101.0	100.4	100.1	100.9
R.S.D.[a]		1.84	0.81	1.95	1.13	0.83	1.84	2.10	2.25

First derivative spectra

Added mixture (µg/ml)		CLS		ILS		PCR		PLSR	
HY	AM	HY	AM	HY	AM	HY	AM	HY	AM
4	2.5	102.0	98.4	102.0	98.8	99.0	100.2	102.0	100.4
10	2.5	102.0	103.2	102.2	102.0	101.2	99.2	101.0	101.6
16	2.5	103.1	102.0	103.0	102.4	100.3	99.6	101.8	102.4
22	2.5	102.7	102.4	102.6	103.6	103.6	100.4	100.5	100.8
28	2.5	98.1	100.0	98.0	100.4	99.6	98.4	100.0	99.6
25	2.0	99.9	99.0	100.2	101.0	100.0	101.0	101.2	101.5
25	4.0	100.3	98.2	100.4	99.5	99.0	98.3	101.6	101.3
25	6.0	98.1	98.3	98.0	98.3	99.6	98.2	100.4	100.7
25	8.0	98.0	100.0	98.2	98.9	100.0	98.5	99.9	100.4
25	10.0	100.4	100.2	100.1	99.6	100.2	100.1	100.9	101.5
Mean		100.4	100.2	100.5	100.4	100.2	99.4	100.9	101.0
R.S.D.[a]		1.95	1.81	1.93	1.76	1.34	1.02	0.73	0.78

[a] R.S.D., Relative standard deviation.

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

Zero order spectra

Parameter	CLS		ILS		PCR		PLSR	
	HY	AM	HY	AM	HY	AM	HY	AM
SEP	0.393	0.209	0.387	0.071	0.311	0.088	0.412	0.125
SEC	0.469	0.249	0.463	0.085	0.371	0.106	0.493	0.149
R^2	0.9977	0.9999	0.9978	0.9999	0.9994	0.9997	0.9970	0.9996
Intercept	0.240	0.011	0.242	0.009	−0.052	−0.070	0.088	−0.088
Slope	0.996	1.010	0.996	1.012	1.014	1.025	0.998	1.035
LOD	0.622	0.317	0.592	0.245	0.222	0.556	0.522	0.587
LOQ	2.073	1.058	1.975	0.998	1.052	1.854	1.740	1.959

First derivative spectra

Parameter	CLS		ILS		PCR		PLSR	
	HY	AM	HY	AM	HY	AM	HY	AM
SEP	0.376	0.069	0.373	0.059	0.272	0.058	0.210	0.059
SEC	0.449	0.082	0.445	0.071	0.325	0.070	0.251	0.069
R^2	0.9979	0.9995	0.9979	0.9997	0.9987	0.9997	0.9997	0.9999
Intercept	0.460	0.044	0.461	0.061	0.118	−0.002	0.120	−0.003
Slope	0.977	0.986	0.977	0.984	0.996	0.993	1.002	1.010
LOD	0.334	0.560	0.352	0.241	0.131	0.324	0.201	0.492
LOQ	1.114	1.772	1.175	0.897	1.119	1.080	1.412	1.121

Table 5
Determination results of two drugs by using the developed techniques (mg/tablet)

Drugs	Zero order spectra				First derivative spectra				HPLC[c] [12]
	CLS	ILS	PCR	PLSR	CLS	ILS	PCR	PLSR	
HY Mean[a] ± S.D.[b]	51.0 ± 0.80	51.1 ± 0.89	50.9 ± 0.61	50.8 ± 0.91	49.8 ± 0.55	49.7 ± 0.60	50.6 ± 0.35	50.4 ± 0.43	49.6 ± 0.95
AM Mean ± S.D.	5.1 ± 0.10	5.1 ± 0.09	4.9 ± 0.09	4.9 ± 0.09	5.0 ± 0.09	5.0 ± 0.08	5.0 ± 0.07	5.0 ± 0.06	4.98 ± 0.15

[a] Mean is average of eight experiments for each technique.
[b] S.D., Standard deviation.
[c] Literature method.

As can be seen, all the statistic parameters indicate that all techniques are applicable for the determination of two drugs in synthetic mixtures.

3.4. Tablet analysis

Twenty tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet (ten time) was transferred to 100 ml calibrated flask and mechanically dissolved in 80% (v/v) methanol and 20% (v/v) buffer solution (acetic acid–sodium acetate (0.2 M, pH 5). The content of flask was filtrated into a 100 ml calibrated flask through a Whatman® no. 42 filter paper. The residue was washed three times with 5 ml and the volume was completed to 100 ml with the same solvent. A portion of 5 ml of this solution was transferred to 100 ml-calibrated flask and made up to volume with solvent. All the techniques were applied to the prepared solutions.

In the next step, recovery studies of standard additions to commercial tablet preparation were realized. After dilution, the recovery results for the validation of CLS, ILS, PCR and PLSR were found to be 50.9 ± 0.5, 51.0 ± 0.8, 51.07 ± 0.06 and 50.0 ± 0.6 for HY, and 5.06 ± 0.04, 5.05 ± 0.03, 5.1 ± 0.48 and 4.9 ± 0.17 for AM, respectively. These recovery results were obtained in the average of five replicate for each drug added in commercial tablet formulation.

The results of the chemometric techniques and HPLC [12] for the same tablet formulation were shown in Table 5, and we conclude that they are very close to each other.

In order to compare the results obtained by applying the proposed techniques to the above mentioned pharmaceutical preparation, Snedecor's F values for the proposed techniques-zero order spectra and the proposed techniques-first derivative spectra were separately computed by using one-way ANOVA of four set of ten sub-sample for each drug, respectively. Although the F-values for the zero-order spectra and first derivative spectra were found as 0.676 and 0.862 for HY; 1.358 and 0.601 for AM, respectively, the value of F from standard table, for $n_1 = 3$ and $n_2 = 36$, and with a 5% level significance is given as 2.80. The experimental or calculated F-values did not exceed the tabulated values of F in the ANOVA, indicating that there was no significant difference between the techniques.

4. Conclusions

Although the absorption spectra of both drugs were superposed in the working wavelengths, chemometric calibration techniques using the absorption and derivative spectrophotometry were successfully applied to the simultaneous determination of HY and AM in synthetic mixtures and tablets.

PCR and PLSR depends on the number of factors to be retained in the process of chemomet-

ric calibration techniques and their results are superior with respect to those given by CLS and ILS. Despite of the fact that PCR and PLSR are considered much more complex than CLS and ILS we found out that our obtained results are very close to each other.

For the determination of amiloride and hydrochlorothiazide in their mixture by HPLC [12] given in Section 1, the proposed calibration models can be used as alternative determination techniques for two drugs in the same commercial pharmaceutical preparation.

When the chemometric calibrations suggested by us for the simultaneous determination of HY and AM compared with the HPLC [12] given in Section 1 (see the results indicated in Table 5), our calibration techniques could be given as alternative determination techniques for two drugs in the same pharmaceutical preparation. We observed that the chemometric analysis techniques do not require any separation procedure and do not influence any excipient for the quantitative determination of both drugs.

Consequently, we strongly believe that the developed techniques in this paper can be used for the quality control of HY and AM in the mixtures and pharmaceutical preparation.

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