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Two new spectrophotometric approaches to the multicomponent analysis of the acetaminophen and caffeine in tablets by classical least-squares and principal component regression techniques

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Abstract

Classical least-squares (CLS) and principal component regression (PCR) techniques were proposed for the simultaneous analysis of tablets containing acetaminophen and caffeine without using a chemical separation procedure. The chemometric calibrations were prepared by measuring the absorbances values at the 15 wavelengths in the spectral region 215–285 nm and by using a training set of the mixtures of both drugs in 0.1 M HCl. The obtained chemometric calibrations were used for the estimation of acetaminophen and caffeine in samples. The numerical calculations were performed with the 'MAPLE V' software. By applying two techniques to synthetic mixtures, the mean recoveries and the relative standard deviations in the CLS and PCR techniques were found as 99.5 and 1.29, 99.7 and 1.00% for acetaminophen and 99.9 and 1.92, 100.0 and 1.178% for caffeine, respectively. Our results were compared with those obtained previously by one of us considering HPLC method as a reference method. These two methods were successfully applied to a pharmaceutical tablet formulation of two drugs.

Keywords: Spectrophotometric approach; Classical least-squares; Principal component regression techniques; Acetaminophen; Caffeine

1. Introduction

One of the fundamental problems of analytical chemistry is the simultaneous determination of compounds in samples. The development of chemometric techniques, namely CLS, ILS (inverse least-squares), PCR and PLS (partial least-squares) has solved a lot of the problems of the simultaneous analysis of multi-mixtures containing two or more compounds [1–3].

Comparing to other conventional methods, chemometric techniques do not imply any pretreatment such as the separation procedure in HPLC, a derivation of spectrum in derivative spectrophometry and a division of the spectrum in ratio spectra derivative spectrophotometry. For other drugs, the application of these new chemometric techniques to drug analysis was reported in the literature [4–6].

The quantitative determination of active ingredients in pharmaceutical formulations containing caffeine and acetaminophen with other active compounds, using various methods as spectrophometry [4–14], chromatography [7,15], TLC [16] and titrimetric determination [17] has been performed in pharmaceutical formulation.

In this study, two chemometric techniques were applied to the chemometric analysis of the synthetic mixtures and tablets containing acetaminophen and caffeine with overlapping spectra. The results given by the applying techniques to the tablets were compared with the HPLC methods (as a reference method).

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2. Experimental

2.1. Instruments

The absorbance measurements were performed by using a Shimadzu UV-160 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 Office Jet Pro 1150C.

2.2. Pharmaceutical preparation

A commercial tablet preparation (Remidon® tablet, Deva Pharm. Ind., Turkey, Batch no. 706-1770) was assayed. Its declared content was as follows: 65 mg caffeine and 500 mg acetaminophen per tablet. Caffeine and acetaminophen were obtained as a donation of Deva Pharm. Ind., Turkey.

2.3. Standard solutions

Stock solutions of caffeine and acetaminophen of 100 mg were prepared in 100 ml volumetric flasks within 0.1 M HCl. The training set containing 0–40 μg/ml caffeine and 0–40 μg/ml acetaminophen and the synthetic mixture solutions consisting of 4–40 μg/ml caffeine and 8–40 μg/ml acetaminophen in 25 ml volumetric flasks were prepared by using the above stock solutions.

2.4. Analysis of tablet formulation

Twenty tablet were accurately weighed and powdered in a mortar. An amount of the powder equivalent to a tablet was dissolved in 0.1 M HCl in 100 ml-calibrated flasks. After 30 min of shaking, the solution was filtrated and the residue was washed three times with 10 ml of solvent, then the volume was completed to 100 ml with 0.1 M HCl. The resulting solution was diluted to 1:150 in a 100 ml-calibrated flasks. Both techniques were applied to the prepared sample solutions.

3. Results and discussion

3.1. Methods

3.1.1. Classical least-squares

CLS is involved the application of multiple linear regression (MLR) to the classical expression of the Beer–Lambert law of spectroscopy given by

$$A = K \times C \tag{1}$$

The above matrix equation can be rewritten as a linear equation system:

$$\begin{aligned} A_1 &= K_{11}C_1 + K_{12}C_2 + \cdots + K_{1c}C_c \\ A_2 &= K_{21}C_1 + K_{22}C_2 + \cdots + K_{2c}C_c \\ A_3 &= K_{31}C_1 + K_{32}C_2 + \cdots + K_{3c}C_c \\ &\vdots \\ A_w &= K_{w1}C_1 + K_{w2}C_2 + \cdots + K_{wc}C_c \end{aligned} \tag{2}$$

where A_w represents the absorbance at the wth wavelength, K_{wc} is the calibration coefficient corresponding to the cth component at the wth wavelength, whilst C_c is the concentration of the cth component.

3.1.2. Principal component regression

This method contains two steps. The first one deals with the calculation of the eigenvectors corresponding to the centred absorbance data matrix as well as the second one uses MLR to regress the concentration data matrix. The mathematical formulation of the method is given by

$$A_{\text{proj}} = V_{\text{c}}^{\text{T}} A \tag{3}$$

where A_{proj} represents the matrix containing the new coordinates (the projections), V_{c}^{T} represents the matrix containing the basis vectors, one column for each factor retained, whilst A denotes the original training set absorbance matrix. If we know the matrix A_{proj} we find, after some simple calculations, the unknown concentration matrix using the following formula

$$C = FA_{\text{proj}} \tag{4}$$

Here F represents the calibration coefficient for the obtained linear equation system.

Fig. 1 displays the absorption spectra of caffeine, acetaminophen and their binary mixture in 0.1 M HCl. For both techniques, the absorbances data were obtained by measurements between 215 and 285 nm with the intervals of $\Delta\lambda = 5$ nm at the 15 wavelengths in the zero order spectra for the different binary mixtures of the mentioned drugs in 0.1 M HCl. A calibration for each technique was obtained by using a training set consisting of the standard synthetic mixtures of both drugs in possible compositions (Table 1) and its absorbance data. The chemometric calibrations of two techniques were used to predict the unknown concentrations of acetaminophen and caffeine in the samples. The numerical calculations were performed by using the powerful MAPLE V software.

In order to test the proposed techniques for the sets of synthetic mixtures containing two drugs in variable combination, the mean recoveries and the relative standard deviations for classical least-squares and principal component regression were found as 99.5% and 1.29%, 99.7% and 1.00% for acetaminophen and 99.9% and 1.92%, 100.0% and 1.27% for caffeine, respectively (Table 2).

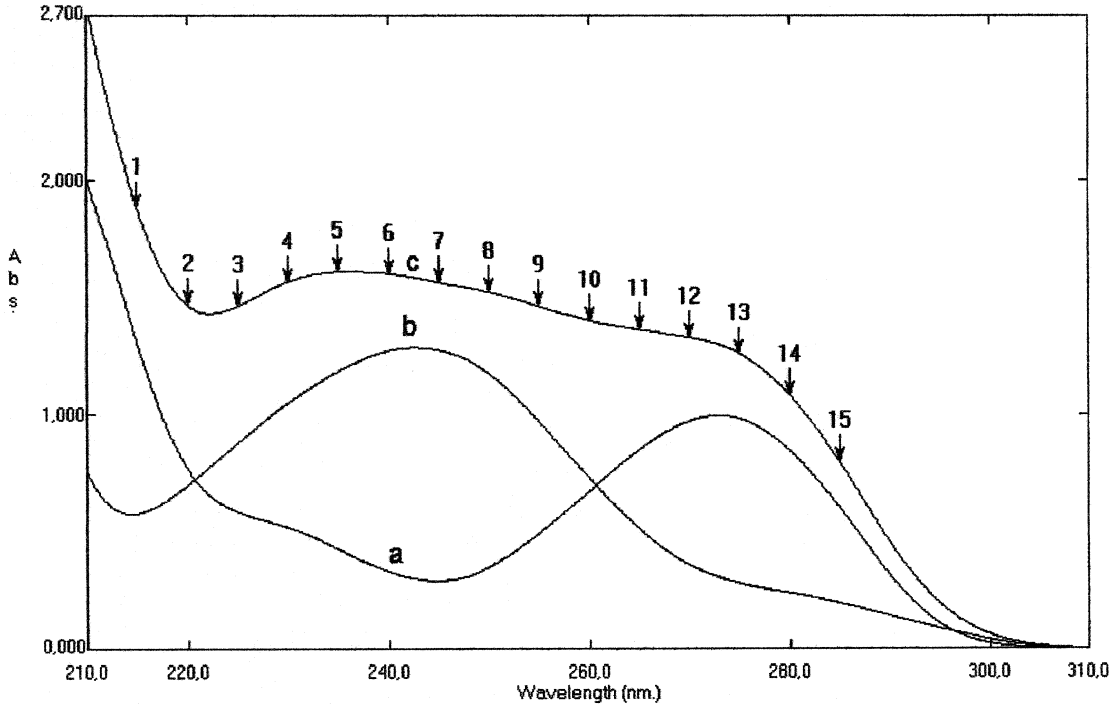

Fig. 1. Absorption spectra of: (a) 20 μg/ml caffeine; (b) 20 μg/ml acetaminophen; and (c) their mixture in 0.1 M HCl (1 2 15 ↓, ↓, ..., ↓ corresponding to $\lambda_1, \lambda_2, \ldots, \lambda_{15}$ (from 215 to 285 nm)).

3.2. Statistical parameters

Some statistical calculations were expressed for the evaluation of the experimental errors by applying the CLS and PCR techniques to the synthetic binary mixtures of both drugs as illustrated below.

The predictive applicability of a model can be defined in various ways. The most general expression is the standard error of prediction (SEP) which is given the following formula:

$$\mathrm{SEP} = \sqrt{\frac{\sum_{i=1}^{n} (C_i^{\mathrm{Added}} - C_i^{\mathrm{Found}})^2}{n}} \quad (5)$$

where C_i^{Added} is the added concentration of drug, C_i^{Found} is the found concentration of drug and n is the total number of the synthetic mixtures. The SEP for CLS and PCR techniques were obtained and presented in Table 3.

Another significant parameter is the standard error of calibration denoted by SEC and having the following expression:

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

where p is the number of components in the samples. The values of SEC corresponding to the CLS and PCR were calculated and the results are presented in Table 3.

The statistical parameters such as slope, intercept, correlation coefficient between the added and found concentrations of drugs in the mixtures are given in Table 3.

All the computed statistical values indicated us that both proposed techniques are suitable for the simultaneous determination of both drugs in the samples.

Table 4 displays the experimental results obtained by CLS and PCR techniques as well as other literature methods. In order to compare the performances of the investigated chemometric techniques according to the HPLC method [7] for the same tablet formulation we applied both Student's t-test and Snedecor's F-test. Since the calculated or the experimental t-values did not exceed the theoretical values, we observed that

Table 1
Composition of the training set of standard synthetic mixtures of two drugs

Standard no.	Acetaminophen (μg/ml)	Caffeine (μg/ml)
1	8.0	4.0
2	12.0	8.0
3	16.0	12.0
4	20.0	20.0
5	20.0	28.0
6	32.0	32.0
7	40.0	00.0
8	00.0	40.0
9	12.0	12.0
10	16.0	20.0

Table 2
Results obtained for acetaminophen and caffeine in different synthetic mixtures by using the classical least-squares and the principal component regression techniques

		CLS				PCR			
AMP	CAF	AMP		CAF		AMP		CAF	
Added (μg)		Found (μg)	Recovery (%)	Found (μg)	Recovery (%)	Found (μg)	Recovery (%)	Found (μg)	Recovery (%)
8.0	4.0	8.2	102.5	4.0	100.0	8.1	101.3	3.9	97.5
12.0	4.0	11.7	97.5	3.9	97.5	11.9	99.2	4.1	102.5
16.0	4.0	15.8	98.8	3.9	97.5	16.2	101.3	4.0	100.0
20.0	4.0	20.1	100.5	4.1	102.5	19.9	99.5	3.9	97.5
32.0	4.0	31.5	98.4	3.9	97.5	31.6	98.8	4.1	102.5
40.0	4.0	39.6	99.0	4.0	100.0	39.6	99.0	3.9	97.5
32.0	4.0	31.9	98.7	4.1	102.5	31.9	98.7	4.0	100.0
32.0	12.0	31.5	98.4	12.3	102.5	31.5	98.4	12.1	100.8
32.0	20.0	31.9	99.7	19.8	99.0	32.1	100.3	20.3	101.5
32.0	28.0	31.8	99.4	28.3	101.1	32.1	100.3	28.2	100.7
32.0	32.0	32.0	100.0	31.7	99.1	31.9	99.7	32.1	100.3
32.0	40.0	32.1	100.3	39.9	99.8	31.5	98.4	39.8	99.5
	$\bar{X}$ =	99.5	$\bar{X}$ =	99.9	$\bar{X}$ =	99.7	$\bar{X}$ =	100.0	
	RSD =	1.29	RSD =	1.92	RSD =	1.00	RSD =	1.27	

AMP, acetaminophen; CAF, caffeine; RSD, relative standard deviation.

Table 3
Statistical parameters of the CLS and PCR methods with absorption spectra in acid medium for the mixtures

	SEP		SEC		*R*		Intercept		Slope	
Components	CLS	PCR	CLS	PCR	CLS	PCR	CLS	PCR	CLS	PCR
Acetaminophen	0.32	0.63	0.36	0.73	0.9997	0.9999	8.8×10^{-2}	2.1×10^{-2}	0.99	0.98
Caffeine	0.63	0.45	0.73	0.52	0.9999	0.9995	4.3×10^{-2}	1.7×10^{-2}	0.98	0.98

Table 4
Results obtained for the pharmaceutical samples (mg per tablet) by using the two chemometric techniques

Comp.	CLS	PCR	Vierordt's method [a]	Ratio spectra derivative spectrophotometry [a]		HPLC [a]
Acetaminophen (mean ± SD)	495.8 ± 1.9	497.1 ± 1.2	501.1 ± 0.6	500.2 ± 1		500.6 ± 0.7
$t_{calculated}$	0.738	0.359	0.112	0.153	$t_{theoretical}$	2.26
$F_{calculated}$	2.714	0.736	0.473	0.376	$F_{theoretical}$	3.18 ($P = 0.05$)
Caffeine (mean ± SD)	63.9 ± 1.8	64.2 ± 1.1	64.9 ± 0.4	65.4 ± 0.7		65.2 ± 0.6
$t_{calculated}$	0.217	0.235	0.108	0.142	$t_{theoretical}$	2.26
$F_{calculated}$	3.000	1.833	0.447	0.865	$F_{theoretical}$	3.18 ($P = 0.05$)

Results obtained are an average of ten experiments for each technique.
[a] The results obtained in Ref. [7].

there was no significant difference between the results of the methods (Table 4).

4. Conclusions

Although the zero-order spectra of two drugs overlap in the spectral region 210–310 nm (Fig. 1), the CLS and PCR techniques gave successful results for the chemometric determination of both drugs in the synthetic mixture and tablets.

CLS and PCR techniques without using a prior procedure such as separation step, derivation and division of the spectra, were found to be more simple, precise and less expensive than the traditional methods described in literature.

These techniques are very easy to apply, they require only data processing with powerful software as well as the manipulation of the abstract vector space and its application to regression analysis.

The results obtained in this paper strongly encourage us to apply these techniques for a routine analysis of two drugs in pharmaceutical preparation.

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