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Spectrophotometric multicomponent analysis of a mixture of metamizol, acetaminophen and caffeine in pharmaceutical formulations by two chemometric techniques

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Abstract

Inverse least squares (ILS) and factor-based (principal component analysis (PCA)) techniques were proposed for the spectrophotometric multicomponent analysis of a ternary mixture consisting of metamizol, acetaminophen and caffeine, without prior separation. In these chemometric techniques, the measurements of the absorbance values were realized in the spectral range from 225 to 285 nm in the intervals of $\Delta\lambda = 5$ nm at the 13 wavelengths in the zero-order spectra of the different ternary mixtures of these active ingredients in 0.1 M HCl. The prepared calibrations of both techniques using the absorbance data and concentration matrix data sets were used to predict the concentration of the unknown concentrations of metamizol acetaminophen and caffeine in their ternary mixture. The 'MAPLE V' software was used for the numerical calculations. Mean recoveries and relative standard deviations for ILS and PCA techniques were found to be 99.8 and 1.68%, 99.9 and 1.66% for caffeine, 99.8 and 1.84%, 100.4 and 2.85% for metamizol, and 99.7 and 1.04%, 99.6 and 1.34% for acetaminophen, respectively, for the first and second techniques. The techniques were successfully applied to two pharmaceutical formulations marketed in Turkey and results were compared with a new high-performance liquid chromatography method.

Keywords: Caffeine; Metamizol; Acetaminophen; Chemometry; HPLC; Pharmaceutical preparations

1. Introduction

Although there are lots of works on the determination of metamizol, acetaminophen and caffeine, including spectrophotometry [1–14], gas chromatography [15,16], high-performance liquid chromatography (HPLC) [17–21] and voltamme-

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try and polarography [22,23], we could not find any chemometric study on the ternary mixture of these drugs in the literature.

Today, the chemometric calibration techniques such as inverse least squares (ILS) and factor-based (principal component analysis (PCA)) has frequently been used in the spectrophotometric multicomponent analysis of the drugs, without any prior separation [24–29]. Chemometric calibration techniques can be summarized as multiple linear regression (MLR) (classical least squares and inverse least squares calibrations), principal component regression and partial least regression techniques.

In this study, ILS and PCA techniques were proposed for the spectrophotometric multicomponent analysis of two pharmaceutical formulations and synthetic ternary mixtures consisting of metamizol, acetaminophen and caffeine. The proposed calibration techniques were tested for synthetic mixtures of metamizol, acetaminophen and caffeine. The numerical calculations were realized using the 'MAPLE V' software. The results of these techniques were compared with each other.

2. Experimental

2.1. Methods

2.1.1. Inverse least squares

ILS is an application of MLR to the inverse expression of the Beer–Lambert Law of spectroscopy:

$$C = P \times A$$

This equation can be written as a linear equation system:

$$C_1 = P_{11}A_1 + P_{12}A_2 + \ldots + P_{1w}A_w$$

$$C_2 = P_{21}A_1 + P_{22}A_2 + \ldots + P_{2w}A_w$$

$$C_3 = P_{31}A_1 + P_{32}A_2 + \ldots + P_{3w}A_w$$

$$\ldots$$

$$C_c = P_{c1}A_1 + P_{w2}A_2 + \ldots + P_{cw}A_w$$

where A_w is the absorbance at the wth wavelength, P_{cw} is the calibration coefficient for the cth component at the wth wavelength, and C_c is the concentration of the cth component

2.1.2. Principal component analysis

This model-building procedure has two steps. The first step is the determination of the eigenvectors or factors for an absorbance data matrix. The second step of PCA uses MLR to regress the concentration data matrix. This procedure can be expressed as:

$$\mathbf{A}_{\text{proj}} = \mathbf{V}_c^{\text{T}}\mathbf{A}$$

where $\mathbf{A}_{\text{proj}}$ is the matrix containing the new coordinates (the projections), $\mathbf{A}$ is the original training set absorbances matrix, $\mathbf{V}_c^{\text{T}}$ is the matrix containing the basis vectors, one column for each factor retained.

$$\mathbf{C} = F\mathbf{A}_{\text{proj}}$$

where F is the calibration coefficient for the obtained linear equation system.

2.1.3. HPLC method

The chromatogram of three compounds were plotted and stored in the computer. The detector responses were measured in terms of peak area. The data was processed using Borwin software.

Separation was carried out at ambient temperature on a Nucleosil 100-5 C_{18} (250×4.6 μm) column (Macherey-Nagel, Germany) and the mobile phase consisted of water–methanol (18:82, v/v). The flow rate was set at 1.0 ml min^{-1} with 10 μl as injection volume. The photometric detection was performed at 254 nm.

2.2. Apparatus

A Shimadzu 1601 PC 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 for all the ab-

sorbance measurements and the treatment of data was made by means of MAPLE V software.

2.3. Pharmaceutical preparations

Two commercial preparations; REMIDON® tablet (Deva Pharm. Ind., Turkey; batch number 4122415, containing 200 mg metamizol (MET), 200 mg acetaminophen (ACE) and 50 mg caffeine (CAF) per tablet) and PIROSAL® tablet (Saba Pharm. Ind., Turkey; batch number containing 220 mg MET, 160 mg ACE and 30 mg CAF per tablet) were analyzed.

Acetaminophen, caffeine and metamizole were kindly donated by Deva Pharm. Ind. and Saba Pharm. Ind. (Turkey).

2.4. Standard solutions

The 100 mg/100 ml solutions of CAF, MET and ACE in 0.1 M HCl were prepared and used in all the procedures as standard solutions.

2.5. Sample preparation

Twenty tablets were accurately weighed and powdered in a mortar. An amount of the tablet mass equivalent to one tablet content was dissolved in 60 ml of 0.1 M. After 30 min of mechanical shaking, the solution was filtered in a 100 ml volumetric flask. The residue was washed three times with 10 ml solvent then the volume was completed to 100 ml with the same solvent (solution 1). Solution 1 was diluted 1:60 with the same solvent. All the spectrophotometric methods and the HPLC method were applied to the latest diluted solution.

3. Results and discussion

3.1. Chemometric methods

Fig. 1 shows the absorption spectra for MET, ACE, CAF and their ternary mixture in 0.1 M

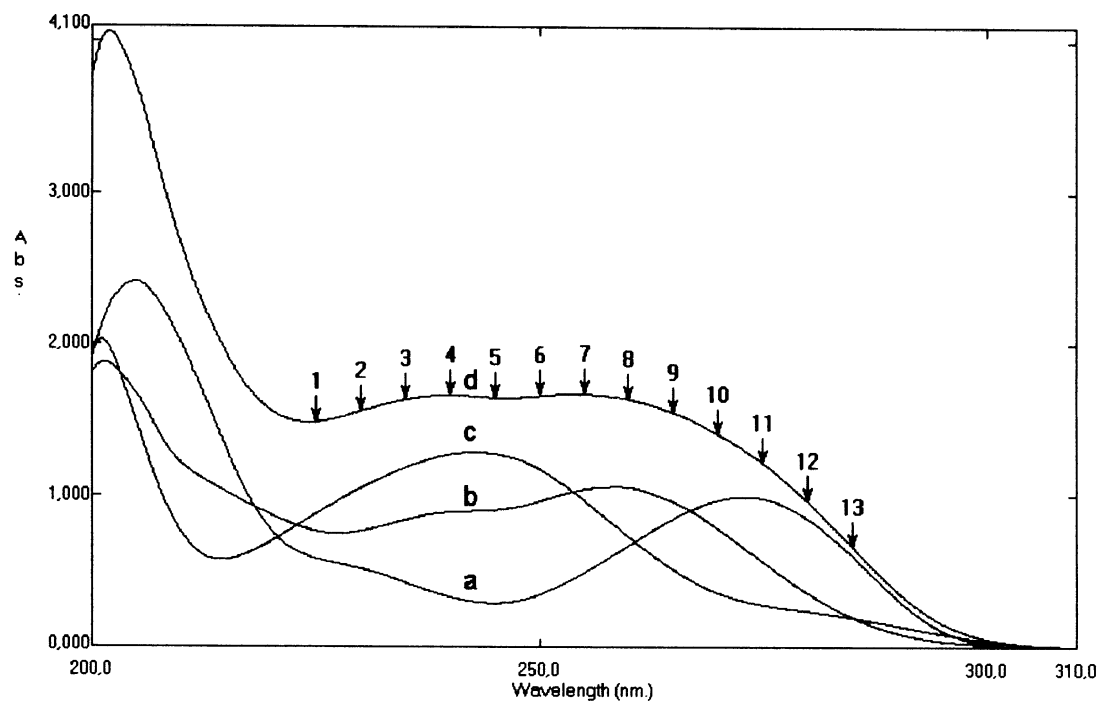

Fig. 1. Absorption spectra of (a) 20 μg ml^{-1} caffeine, (b) 40 μg ml^{-1} metamizol, (c) 20 μg ml^{-1} acetamiophen and (d) their mixtures. (*i*-numbered arrows correspond to λ_i).

Table 1
Composition of the training set in chemometric methods

Standard number	Caffeine (μg ml^{-1})	Metamizol (μg ml^{-1})	Acetaminophen (μg ml^{-1})
1	4.0	20.0	16.0
2	12.0	20.0	16.0
3	20.0	20.0	16.0
4	32.0	20.0	16.0
5	48.0	20.0	16.0
6	16.0	12.0	16.0
7	16.0	20.0	16.0
8	16.0	32.0	16.0
9	16.0	40.0	16.0
10	16.0	56.0	16.0
11	16.0	20.0	8.0
12	16.0	20.0	16.0
13	16.0	20.0	20.0
14	16.0	20.0	32.0
15	16.0	20.0	40.0

HCl. In chemometric techniques, the measurement of the absorbance values were realized in the spectral range 225.0–285.0 nm in the intervals of $\Delta\lambda = 5$ nm at the 13 wavelengths in the zero-order spectra of the different binary mixtures in 0.1 M HCl. The prepared calibrations of both techniques using the absorbance data sets and concentration matrix data sets were used to predict the concentration of the unknown values of MET, ACE and CAF in their ternary mixture. In the procedure, the set consisted of 15 samples that included all possible combinations at each of five concentration levels (Table 1).

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 by the following equation:

$$\mathrm{SEP} = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{\mathrm{added}} - C_i^{\mathrm{found}})}{n}}$$

where C_i^{added} is the added concentration of drug, $C_i^{\mathrm{found}}$is the predicted concentration of drug, and n is the total number of synthetic mixtures.

To test the proposed techniques, the sets of synthetic mixtures containing the three drugs in variable compositions were prepared. The results obtained in the application of ILS and PCA to the same ternary mixture are indicated in Tables 2 and 3. The errors of prediction (SEP) were found completely acceptable in the ILS and PCA methods (0.38 and 0.28% for CAF, 0.52 and 0.42% for MET, and 0.16 and 0.21% for ACE) respectively (Table 4).

In Table 4, r is defined as the correlation between constituent concentrations and shows the absorbance effects relating to the constituent of interest. The r values obtained in the methods close to 1 mean no interference was coming from the other constituents in this set of synthetic mixtures.

Another value is the standard error of calibration (SEC) and the calculation of this value was realized using following equation:

$$\mathrm{SEC} = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{\mathrm{added}} - C_i^{\mathrm{found}})^2}{n-p-1}}$$

where C_i^{added} is the added concentration of drug, $C_i^{\mathrm{found}}$is the predicted concentration of drug, n is the total number of synthetic mixtures, and p is the number of components in the mixtures.

In the proposed techniques, the sets of synthetic mixtures containing these three drugs in variable compositions as already mentioned were prepared. The results obtained in the application of

ILS and PCA to the same ternary mixture are indicated in Tables 2 and 3. The errors of calibration (SEC) were also found acceptable in the ILS and PCA methods (0.44 and 0.33% for CAF, 0.61 and 0.49% for MET, and 0.19 and 0.25% for ACE, respectively) (Table 4).

Table 2
Results obtained for the determination of CAF, MET and ACE in different synthetic mixtures using the ILS technique

Added (μg)			Found (μg)			Recovery (%)		
CAF	MET	ACE	CAF	MET	ACE	CAF	MET	ACE
4.0	20.0	16.0	4.0	20.0	15.9	100.0	100.0	99.4
12.0	20.0	16.0	12.0	20.1	15.8	100.0	100.5	98.8
20.0	20.0	16.0	20.0	12.0	16.0	100.0	100.0	100.0
32.0	20.0	16.0	32.5	19.7	16.1	101.6	98.5	100.6
48.0	20.0	16.0	47.9	19.5	16.0	99.8	97.5	100.0
16.0	12.0	16.0	15.9	12.0	15.8	99.4	100.0	98.8
16.0	20.0	16.0	16.0	20.7	16.2	100.0	103.5	101.3
16.0	32.0	16.0	15.5	31.3	15.6	96.8	97.8	97.5
16.0	40.0	16.0	15.5	41.0	16.0	96.8	102.5	100.0
16.0	56.0	16.0	16.3	55.8	16.1	101.9	99.6	100.6
16.0	20.0	8.0	15.8	19.8	8.1	98.8	99.0	101.3
16.0	20.0	16.0	16.1	19.7	15.9	100.6	98.5	99.4
16.0	20.0	20.0	16.5	20.5	19.7	103.1	102.5	98.5
16.0	20.0	32.0	15.8	19.5	32.0	98.8	97.5	100.0
16.0	20.0	40.0	16.0	20.0	40.0	100.0	100.0	100.0
$\bar{x}$						99.8	99.8	99.7
RSD						1.68	1.84	1.04

RSD: relative standard deviation.

Table 3
Results obtained for the determination of CAF, MET and ACE in different synthetic mixtures using the PCA technique

Added (μg)			Found (μg)			Recovery (%)		
CAF	MET	ACE	CAF	MET	ACE	CAF	MET	ACE
4.0	20.0	16.0	4.0	20.1	16.0	100.0	100.5	99.4
12.0	20.0	16.0	12.0	20.2	15.9	100.0	101.0	98.8
20.0	20.0	16.0	20.1	19.9	15.8	100.5	99.5	99.4
32.0	20.0	16.0	32.4	20.1	15.9	101.3	100.5	98.8
48.0	20.0	16.0	47.8	19.7	16.1	99.6	98.5	99.4
16.0	12.0	16.0	15.8	11.9	16.0	98.8	99.2	100.0
16.0	20.0	16.0	15.9	20.6	15.8	99.4	103.4	98.8
16.0	32.0	16.0	15.6	31.0	16.5	97.5	96.9	103.1
16.0	40.0	16.0	16.4	40.8	15.6	102.5	102.0	97.5
16.0	56.0	16.0	15.9	55.8	16.0	99.4	99.6	100.0
16.0	20.0	8.0	15.7	19.7	8.1	98.1	98.5	101.3
16.0	20.0	16.0	16.0	20.1	15.9	100.0	100.5	99.4
16.0	20.0	20.0	16.6	20.4	19.6	103.8	102.0	98.8
16.0	20.0	32.0	15.7	19.6	32.1	98.1	98.0	100.3
16.0	20.0	40.0	16.0	20.0	39.9	100.0	100.0	99.8
$\bar{x}$						99.9	100.5	99.4
RSD						1.66	1.30	1.33

Table 4
Summary of statistics in PCA and ILS methods for CAF, MET and ACE in the mixture

	SEP		SEC		r		Intercept		Slope	
	PCA	ILS	PCA	ILS	PCA	ILS	PCA	ILS	PCA	ILS
CAF	0.28	0.38	0.33	0.44	0.9996	0.9993	1.39×10^{-2}	1.89×10^{-2}	0.99	0.97
MET	0.42	0.52	0.49	0.61	0.9992	0.9988	2.21×10^{-2}	2.01×10^{-2}	0.99	0.98
ACE	0.21	0.16	0.25	0.19	0.9995	0.9998	2.48×10^{-2}	2.61×10^{-2}	0.98	0.99

The slope and intercept refer to the regression of the estimated determination values on the actual values. A good method will produce slope and intercept values of approximately 1.0 and 0.0, respectively. Values calculated in the ILS and PCA methods for the determination of CAF, MET and ACE in the mixture were found satisfactory (0.97–0.99 and $< 2.61 \times 10^{-2}$ for the slope and intercept, respectively; Table 4)

Mean recoveries and relative standard deviations for the ILS and PCA techniques were found to be 99.8 and 1.68%, 99.9 and 1.66% for CAF, 99.8 and 1.84%, 100.5 and 1.30% for MET, and 99.7 and 1.04%, 99.6 and 1.33% for ACE, respectively (Tables 2 and 3).

The linearity range was 4–48 μg ml^{-1} for CAF, 12–56 μg ml^{-1} for MET and 8–40 μg ml^{-1} for ACE in both chemometric methods.

Comparison of the spectra of CAF, MET and ACE in standard and drug formulation solutions showed that the wavelength of maximum absorbances in the zero-order spectra did not changed. It has been decided that excipients placed in the commercial preparations selected (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil, magnesium stearate, sodium lauryl sulfate) did not interfere the quantitation of CAF, MET and ACE in these methods.

3.2. HPLC method

We developed a new HPLC method for the simultaneous analysis of ternary mixture of CAF, MET and ACE, and this was used as a reference method. On a Nucleosil C_{18} column, several mobile phase systems and different internal standards (IS) were tested for separation and determination of the drugs, and water–methanol (18:82, v/v) was found suitable as the mobile phase and ceterimide was found suitable as IS for this purpose. At a flow rate of 1.0 ml min^{-1}, retention times for IS, ACE, CAF and MET were 1.96, 2.84, 3.38 and 4.21 s, respectively (Fig. 2). UV detection was at 254 nm. In the method, the ratio of the peak areas of analytes to IS were plotted versus the concentrations of ACE, CAF and MET. In this case, a straight line was obtained. By using these calibration graphs, the amount of ACE, CAF and MET was determined in the samples containing these drugs.

As seen in Table 5, to determine the validity and applicability of this HPLC method, recovery studies were performed by analysing synthetic mixtures of ACE, CAF and MET prepared in different ratios. The mean recoveries and relative standard deviations for ACE, CAF and MET were found as 99.7 and 1.04%, 99.5 and 1.30%, and 99.9 and 1.75%, respectively (Table 5)

In the method, the regression equations and correlation coefficients are presented in Table 6 for the determination of ACE, CAF and MET in their ternary mixture. Linearity ranges were found as 1–28 μg ml^{-1} for CAF, 2–28 μg ml^{-1} for MET, and 4–32 μg ml^{-1} for ACE in this method.

A summary of the assay results for commercial preparations are presented in Table 7. The results of two chemometric methods and also the HPLC method we developed for the same commercial formulation were compared by Student's t-test. The calculated (experimental) t-values did not exceed the tabulated (theoretical) values in the test, indicating that there was no significant difference between the methods compared (Table 7).

4. Conclusion

Two new chemometric methods in spectrophotometric analysis, ILS and PCA, were proposed for the simultaneous determination of ACE, CAF and MET in their ternary mixture. These techniques were applied with great success to two commercial pharmaceutical preparations (tablets). The assay results obtained using these chemometric methods were also compared with the HPLC method proposed in this work and a good coincidence was observed. Although the HPLC method

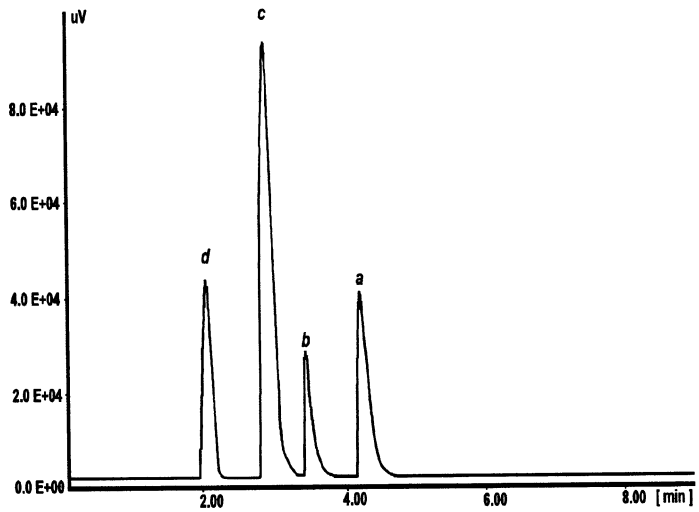

Fig. 2. Typical chromatograms of (a) metamizol, (b) caffeine, (c) acetaminophen and (d) cetrimide as internal standard (IS).

Table 5
Results obtained for the determination of CAF, MET and ACE in different synthetic mixtures using the HPLC method

Added (μg)			Found (μg)			Recovery (%)		
CAF	MET	ACE	CAF	MET	ACE	CAF	MET	ACE
4.0	16.0	4.0	4.0	15.8	4.0	100.0	98.8	100.0
4.0	16.0	8.0	4.0	16.0	8.0	100.0	100.0	100.0
4.0	16.0	16.0	4.0	15.9	15.9	100.0	99.4	98.8
4.0	16.0	24.0	4.0	15.8	23.9	100.0	98.8	99.6
4.0	16.0	32.0	4.1	16.1	32.1	102.5	100.6	100.3
4.0	2.0	20.0	4.0	2.0	19.9	100.0	100.0	99.5
4.0	8.0	20.0	4.0	7.9	19.9	100.0	98.8	99.5
4.0	16.0	20.0	4.0	15.9	20.0	100.0	99.4	100.0
4.0	20.0	20.0	3.9	21.0	19.9	97.5	105.0	99.5
4.0	28.0	20.0	3.9	27.2	20.0	97.5	99.6	100.0
1.0	16.0	20.0	1.0	15.8	19.9	100.0	99.0	99.5
4.0	16.0	20.0	3.9	16.0	20.4	97.5	98.5	100.2
12.0	16.0	20.0	11.9	15.9	20.0	99.2	102.5	100.0
20.0	16.0	20.0	19.6	16.2	19.9	98.0	97.5	99.5
28.0	16.0	20.0	28.2	16.1	20.0	100.7	100.0	100.0
$\bar{x}$						99.5	99.9	99.7
RSD						1.30	1.75	1.04

Table 6
Regression results for CAF, MET and ACE in the HPLC method

λ (nm)	Component	Linearity range ($\mu g\ ml^{-1}$)	Regression, a (S.E.)	Equation, b (S.E.)	Regression coefficient (r)
254	CAF	1–28	7.4×10^{-3} (1.3×10^{-4})	4.7×10^{-4} (3.8×10^{-5})	0.9996
254	ACE	4–32	5.9×10^{-2} (2.8×10^{-3})	1.9×10^{-3} (4.7×10^{-4})	0.9997
254	MET	2–28	2.8×10^{-3} (5.1×10^{-4})	3.0×10^{-3} (5.0×10^{-4})	0.9990

a, Slope; b, intercept; S.E., standard error.

Table 7
Results obtained for the assay of commercial pharmaceutical preparations using two chemometric techniques (mg/tablet)

Method	ACE (mean ± S.D.)		MET (mean ± S.D.)		CAF (mean ± S.D.)	
	I	II	I	II	I	II
ILS	159.4 ± 2.2	204.1 ± 3.2	219.5 ± 1.8	200.9 ± 2.1	29.5 ± 0.8	51.0 ± 1.2
PCA	161.4 ± 2.6	198.1 ± 2.8	222.1 ± 3.1	202.6 ± 2.9	30.0 ± 0.7	51.5 ± 1.0
HPLC	161.0 ± 3.3	200.1 ± 4.2	221.5 ± 1.1	201.4 ± 2.0	29.9 ± 0.6	51.0 ± 1.0

Data presented as I: Pirosal® tablet; II: Remidon® tablet. Obtained results are average of ten tablets for both techniques. S.D., standard deviation. Theoretical value for t at $P = 0.05$ level is 2.26.

is more specific than the chemometric spectrophotometric methods, HPLC methods need expensive equipment and materials such as columns and HPLC grade solvents. Chemometric methods are less expensive methods and they do not require sophisticated instrumentation and any prior separation step. This can be considered a superiority of these chemometric techniques over HPLC. But they need software for resolution and determination of active ingredients in the mixtures. The chemometric methods proposed are very powerful methods for the simultaneous analysis of multicomponent mixtures in which the spectra of the active compounds overlap each other and also, by the fact that zero-order spectra is enough for the analysis, there is no need for the spectrophotometer to have any other modes such as derivation and ratio spectra. These three methods described in the text, ILS and PCA, and HPLC, were found to be suitable for the routine analysis of ingredients in the two different pharmaceutical formulations containing the ternary mixture of CAF, ACE and MET marketed in Turkey.

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