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Linear regression analysis and its application to the multivariate spectral calibrations for the multiresolution of a ternary mixture of caffeine, paracetamol and metamizol in tablets

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Abstract

The multivariate spectral calibration methods, tri-linear regression-calibration (TLRC) and multi-linear regression-calibration (MLRC) were developed for the multiresolution of a ternary mixture of caffeine (CAF), paracetamol (APAP), metamizol (MET), which have closely overlapped in the spectra. The calibration algorithms were briefly described for the three-component system, CAF–APAP–MET. By using the various synthetic mixtures of three compounds, the validity of the TLRC and MLRC methods was confirmed and applied to the real samples containing the above-mentioned compounds in two different commercial tablet formulations. The TLRC and MLRC methods which are very rapid, easy to apply, yet not expensive, are powerful tools with very simple mathematical contents for multiresolution of the three- or multi-component mixture systems. The data treatments were carried out by the MAPLE V, EXCEL and SPSS 10.0 Softwares. The obtained results were successfully compared with each other as well as with those obtained by other literature methods.

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1. Introduction

The resolution of the mixtures containing two or more different analytes without using a chemical separation and a graphical procedure is one of the main problems of the classical analytical chemis-

try. With the development of chemometric techniques such as classical least-squares, inverse least-squares, partial least-squares and principal component regression, a lot of the problems of the simultaneous analysis of two- or multi-component mixtures have been solved [1–5]. Although these methods are very easy to apply to spectrophotometric [6–10], chromatographic [10] and electrochemical [11] quantitative analysis, they require data processing with powerful softwares as well as

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the manipulation of the abstract vector space and its application to regression analysis.

In the spectrophotometric studies, normal derivative and ratio spectra derivative spectrophotometry have been used for the quantitative resolving of the binary and ternary mixtures [12,13,27–29]. Unfortunately, in some cases these two methods have a great disadvantage: higher derivative process diminishes the peak amplitude and it is difficult to find zero-crossing points. For this reason, the sensitivity of the method decreases. On the other hand, the ratio spectra derivative method leads us to an infinite value of ratio spectra in some cases.

Other spectrophotometric methods such as dual wavelength spectrophotometry [14–17], pH-induced differential spectrophotometry [18–20], multi-component analysis program with multi-wavelength linear regression analysis [21,22] for the simultaneous determination of compounds in mixtures, have been reported in the literature.

Recently, López-de-Alba and co-workers developed the bivariate calibration method for the resolution of two-component mixtures by spectrophotometry [23–26]. This method is based on the use of the four linear regression calibration equations with two calibrations for each component at two-wavelengths selected. The tri-linear regression-calibration (TLRC) and multi-linear regression-calibration (MLRC) methods were developed from the method of López-de-Alba et al. for the multiresolution of three-component mixtures.

Among the various analytical techniques available for the simultaneous determination of caffeine (CAF), paracetamol (APAP), metamizol (MET) and their mixtures with other compounds are spectrophotometry [27–43], HPLC [41–44], and voltammetry [45].

The aim of the presented work is the application of TLRC and MLRC methods to the multiresolution of a ternary mixture containing CAF, APAP and MET without requiring a chemical pre-treatment and a graphical procedure of the overlapping spectra. As an alternative method, CLS method was studied for the quantitative resolution of the mixtures of the subject matter compounds. All of the developed methods were also applied to two

commercial tablet formulations. The obtained results were successfully compared with each other as well as with those obtained by other literature methods.

2. Theoretical basics of methods

A linear regression equation between two variables, concentration and absorbance, for the spectrophotometric determination of the X analyte at λ_i wavelength can be defined by the equation:

$$A_{X_i} = b_{X_i} C_X + a_{X_i} \quad (1)$$

Where, A_{X_i} is the absorbance of the X analyte at λ_i wavelength, C_X is the concentration of the X analyte (the concentration units are $\mu\text{g/ml}$ in the two newly developed methods), b_{X_i} is the slope of the linear regression equation, a_{X_i} is the intercept of the regression model. These intercept values indicate the difference between the ideal and calculated system.

2.1. Tri-linear regression-calibration (TLRC)

If the absorbance values of a mixture of three analytes (X, Y and Z) are measured at a three-wavelength set ($\lambda_i = 1, 2$ and 3), the following equations can be written for a three-component analysis:

$$A_{\text{mix}_1} = b_{X_1} C_X + b_{Y_1} C_Y + b_{Z_1} C_Z + a_{XYZ_1}$$

$$A_{\text{mix}_2} = b_{X_2} C_X + b_{Y_2} C_Y + b_{Z_2} C_Z + a_{XYZ_2}$$

$$A_{\text{mix}_3} = b_{X_3} C_X + b_{Y_3} C_Y + b_{Z_3} C_Z + a_{XYZ_3} \quad (2)$$

where A_{mix_1} , A_{mix_2} and A_{mix_3} represent the absorbances of the mixture of X, Y and Z analytes at the three-wavelength set, $b_{X_{1,2 \text{ and } 3}}$, $b_{Y_{1,2 \text{ and } 3}}$ and $b_{Z_{1,2 \text{ and } 3}}$ are the slopes of linear regression equations of X, Y and Z, respectively; and a_{XYZ_1} , a_{XYZ_2} and a_{XYZ_3} are the sums of intercepts of linear regression equations at the three-wavelengths ($a_{XYZ_1} = a_{X_1} + a_{Y_1} + a_{Z_1}$, $a_{XYZ_2} = a_{X_2} + a_{Y_2} + a_{Z_2}$ and $a_{XYZ_3} = a_{X_3} + a_{Y_3} + a_{Z_3}$).

Eq. (2) can be formulated in matrix notation as:

$$\begin{bmatrix} A_{\text{mix}_1} \\ A_{\text{mix}_2} \\ A_{\text{mix}_3} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ b_{X_3} & b_{Y_3} & b_{Z_3} \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} + \begin{bmatrix} a_{XYZ_1} \\ a_{XYZ_2} \\ a_{XYZ_3} \end{bmatrix} \quad (3)$$

If the absorbance matrix, $\mathbf{A}_{\text{mix}_{1, 2 \text{ and } 3}}$, and the intercept matrix, $\mathbf{a}_{XYZ_{1, 2 \text{ and } 3}}$ are matrices in the same size, then the difference $\mathbf{A}_{\text{mix}} - \mathbf{a}_{XYZ}$ is the matrix obtained by subtracting the entries of \mathbf{a}_{XYZ} from the corresponding entries of \mathbf{A}_{mix} . According to this procedure, the following equation can be written as:

$$\begin{bmatrix} A_{\text{mix}_1} - a_{XYZ_1} \\ A_{\text{mix}_2} - a_{XYZ_2} \\ A_{\text{mix}_3} - a_{XYZ_3} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ b_{X_3} & b_{Y_3} & b_{Z_3} \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} \quad (4)$$

or, more simply:

$$(\mathbf{A}_{\text{mix}} - \mathbf{a}_{XYZ})_{3 \times 1} = \mathbf{K}_{3 \times 3} \cdot \mathbf{C}_{3 \times 1} \quad (4a)$$

The matrix, \mathbf{b} , corresponding to the slope values of linear regression equations is called the matrix, \mathbf{K} :

$$\mathbf{K} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ b_{X_3} & b_{Y_3} & b_{Z_3} \end{bmatrix} \quad (5)$$

In this case, for the calculation of the concentration of the analytes, X, Y and Z in ternary mixture, the matrix, $(\mathbf{A}_{\text{mix}} - \mathbf{a}_{XYZ})_{3 \times 1}$, is multiplied by the inverse $(\mathbf{K}^{-1})_{3 \times 3}$ of the matrix $\mathbf{K}_{3 \times 3}$ and it can be written as:

$$\mathbf{C}_{3 \times 1} = (\mathbf{K}^{-1})_{3 \times 3} * (\mathbf{A}_{\text{mix}} - \mathbf{a}_{XYZ})_{3 \times 1} \quad (6)$$

This procedure is the mathematical basis of the TLRC method for multi-component analysis.

As explained here, the developed calibration model can be applied easily to the multiresolution of the three-component mixtures. The choice of optimum wavelength set plays an important role for the application of this numerical method to a multi-mixture analysis. For this reason, Kasier's method [46] was applied to the selection of the optimum wavelength set in order to provide the best sensitivity and selectivity in the application of the method.

The sensitivity matrix \mathbf{K} (square matrix) Eq. (5) is formed by taking every three-pairs of pre-selected wavelengths for ternary mixtures.

The matrix \mathbf{K} of the slope values obtained in the linear regression equations of the individual analytes, X, Y and Z at three selected wavelengths (1, 2 and 3) is considered as the sensitivity parameter [23–26]. The sensitivity parameter is used for comparing different three-wavelength sets. The sensitivity of a multi-component analysis is defined as the absolute value of the determinant of the sensitivity matrix \mathbf{K} . For this reason, the determinant values of the matrix \mathbf{K} corresponding to different three-wavelength sets are calculated for the selection of the working wavelength set. The calculated maximum determinant value permits to decide the optimum wavelength set. The method is based on the nine linear regression equations with three linear regression lines for each compound at three selected wavelengths.

2.2. Multi-linear regression-calibration (MLRC)

If the absorbance values of a mixture of three analytes (X, Y and Z) are measured at n wavelengths ($\lambda_i = 1, 2, \dots, n$), the following set of equations can be written for a three-component analysis:

$$\begin{aligned} A_{\text{mix}_1} &= b_{X_1} C_X + b_{Y_1} C_Y + b_{Z_1} C_Z + a_{XYZ_1} \\ A_{\text{mix}_2} &= b_{X_2} C_X + b_{Y_2} C_Y + b_{Z_2} C_Z + a_{XYZ_2} \\ &\dots \\ &\dots \\ A_{\text{mix}_n} &= b_{X_n} C_X + b_{Y_n} C_Y + b_{Z_n} C_Z + a_{XYZ_n} \end{aligned} \quad (7)$$

where $A_{\text{mix}_1}, A_{\text{mix}_2}, \dots,$ and A_{mix_n} are the absorbances of the mixture of X, Y and Z analytes at selected wavelengths (from λ_1 to λ_n); $b_{X_1}, b_{X_2}, \dots, b_{X_n}, b_{Y_1}, b_{Y_2}, \dots, b_{Y_n}$ and $b_{Z_1}, b_{Z_2}, \dots, b_{Z_n}$ are the slopes of n linear regression equations of X, Y and Z, corresponding to selected wavelengths, respectively; and $a_{XYZ_1}, a_{XYZ_2}, \dots$ and a_{XYZ_n} are the sum of intercepts of linear regression equations at n wavelengths ($a_{XYZ_1} = a_{X_1} + a_{Y_1} + a_{Z_1}$, $a_{XYZ_2} = a_{X_2} + a_{Y_2} + a_{Z_2}$ and $a_{XYZ_n} = a_{X_n} + a_{Y_n} + a_{Z_n}$).

In the matrix terms, the above multi-equation system (7) can be formulated as:

$$\begin{bmatrix} A_{\text{mix}_1} \\ A_{\text{mix}_2} \\ \dots \\ A_{\text{mix}_n} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ \dots & \dots & \dots \\ b_{X_n} & b_{Y_n} & b_{Z_n} \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} + \begin{bmatrix} a_{XYZ_1} \\ a_{XYZ_2} \\ \dots \\ a_{XYZ_n} \end{bmatrix} \quad (8)$$

which can be simplified to

$$\begin{bmatrix} A_{\text{mix}_1} - a_{XYZ_1} \\ A_{\text{mix}_2} - a_{XYZ_2} \\ \dots \\ A_{\text{mix}_n} - a_{XYZ_n} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ \dots & \dots & \dots \\ b_{X_n} & b_{Y_n} & b_{Z_n} \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} \quad (9)$$

in a compact form

$$(\mathbf{A}_{\text{mix}} - \mathbf{a}_{XYZ})_{n \times 1} = \mathbf{K}_{n \times 3}^* \mathbf{C}_{3 \times 1} \quad (10)$$

As explained in the above calibration method, the matrix of the slope values is called the matrix \mathbf{K} :

$$\mathbf{K}_{n \times 3} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ \dots & \dots & \dots \\ b_{X_n} & b_{Y_n} & b_{Z_n} \end{bmatrix} \quad (11)$$

The matrices, $(\mathbf{A}_{\text{mix}} - \mathbf{a})_{n \times 1}$ and $\mathbf{K}_{n \times 3}$, are multiplied by the transpose $(\mathbf{K}')_{3 \times n}$ of the matrix $\mathbf{K}_{n \times 3}$ and it can be written as:

$$(\mathbf{K}')_{3 \times n} (\mathbf{A}_{\text{mix}} - \mathbf{a})_{n \times 1} = (\mathbf{K}')_{3 \times n} \mathbf{K}_{n \times 3}^* \mathbf{C}_{3 \times 1} \quad (12)$$

The concentration of the X, Y and Z compounds in ternary mixture can be calculated by using the following formula:

$$\mathbf{C}_{3 \times 1} = [(\mathbf{K}')_{3 \times n} \mathbf{K}_{n \times 3}]_{3 \times 3}^{-1} * [(\mathbf{K}')_{3 \times n} (\mathbf{A}_{\text{mix}} - \mathbf{a}_{XYZ})_{n \times 1}] \quad (13)$$

In this case, the MLRC model contains the use of linear algebra, also known as matrix mathematics. This calibration model can be applied to the multiresolution of multi-component mixture system containing n compounds.

2.3. CLS method

In this approach, the method is based on the use of the absorptivity values at the selected wavelengths for the spectrophotometric quantitative analysis

multi-component mixture system containing n compounds [1,5]. Absorptivity, A_1^1 (1%, 1 cm), values of three compounds, X, Y and Z are calculated by using the absorbances measured at the selected wavelengths in the zero-order spectra for each of the compounds in ternary mixture. By using A_1^1 values, a system of equations with n unknowns were written for the compounds in the ternary mixture, as follows:

$$\begin{aligned} A_1 &= \alpha_1 C_X + \beta_1 C_Y + \gamma_1 C_Z \\ A_2 &= \alpha_2 C_X + \beta_2 C_Y + \gamma_2 C_Z \\ \dots & \\ A_n &= \alpha_n C_X + \beta_n C_Y + \gamma_n C_Z \end{aligned} \quad (14)$$

where A_1, A_2, \dots and A_n represent the absorbances of solution of mixtures of X, Y and Z, $\alpha_1, 2, \dots$ and $n, \beta_1, 2, \dots$ and n and $\gamma_1, 2, \dots$ and n denote the A_1^1 values calculated for X, Y and Z, respectively, at $\lambda_1, \lambda_2, \dots$ and λ_n . C_X, C_Y and C_Z are the concentration of X, Y and Z, respectively, in g/100 ml. The subscripts 1, 2, \dots and n refer to at $\lambda_1, \lambda_2, \dots$ and λ_n , respectively.

Matrix notation greatly simplifies matters and easily solves the system of equations with three unknowns, as shown below:

$$\begin{bmatrix} A_1 \\ A_2 \\ \dots \\ A_n \end{bmatrix} = \begin{bmatrix} \alpha_1 & \beta_1 & \gamma_1 \\ \alpha_2 & \beta_2 & \gamma_2 \\ \dots & \dots & \dots \\ \alpha_n & \beta_n & \gamma_n \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} \quad \text{or} \quad (15)$$

$$\mathbf{A} = \mathbf{E} * \mathbf{C}$$

Using the similar procedure described in Section 2.2, this matrix was solved, and it was determined, the concentration of X, Y and Z in their mixture.

3. Experimental

3.1. Instruments

A Shimadzu UV-160 double beam UV-Vis spectrophotometer possessing a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a HP DeskJet 600 printer were used to record the absorption spectra. The application of Kaiser's method, the regression

and statistical analysis were achieved by using the MAPLE V, EXCEL and SPSS 10.0 softwares, respectively.

3.2. Commercial tablet formulation

Two commercial tablet formulations (REMIDON[®] tablets produced by Deva Pharm., Turkey, Batch no. 306 1582, consisting of 50 mg of CAF, 200 mg APAP and 200 mg MET per tablet and PIROSAL[®] tablets produced by Saba Pharm., Turkey, Batch no. 48, consisting of 30 mg CAF, 160 mg APAP and 220 mg MET per tablet) were investigated.

Deva Pharm. Ind. and Saba Pharm. Ind. kindly donated the active compounds.

3.3. Standard solutions

Stock solutions containing 100 mg/100 ml CAF, APAP and MET were prepared in 0.1 M HCl. A standard series of the solutions containing 4–40 µg/ml CAF, 8–40 µg/ml APAP and 12–48 µg/ml MET were obtained from the stock solutions. A validation set consisting of 15 synthetic mixture solutions in the concentration range of 4–40 µg/ml CAF, 8–40 µg/ml APAP and 12–48 µg/ml MET was prepared by using the same stock solutions. All the solutions were prepared freshly and protected from light.

4. Tablet analysis procedure

Twenty tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in 0.1 M HCl in a 100 ml calibrated flask with the aid of mechanical shaking for 20 min. This solution was filtered into a 100 ml calibrated flask through Whatman No. 42 filter paper. The residue was washed three times with 0.1 M HCl. An analogous procedure was applied to both Remidon[®] (I) and Pirosoal[®] (II) tablets. (I) and (II) solutions were diluted 1:100 and 1:62.5 with the same solvent. The analysis of the sample solutions was carried out by using TLRC, MLRC and CLS methods.

5. Results and discussion

The individual spectra of CAF ($\lambda_{\max} = 272.6$ nm), PAR ($\lambda_{\max} = 242.7$ nm), MET ($\lambda_{\max} = 258.4$ nm) and their mixture spectrum were observed in the spectral region 220–320 nm as indicated in Fig. 1. Since the spectra of three compounds overlaps in the working wavelength range, it is not possible to determine simultaneously CAF, APAP and MET in their mixture by conventional spectrophotometric methods. In order to solve this problem, the two methods (TLRC and MLRC) were applied to the multiresolution of the three-component mixture system of the subject matter compounds. As an alternative, the CLS method was used to solve the problem. For this purpose, the standard series of solutions of CAF (4–40 µg/ml), APAP (8–40 µg/ml), and MET (12–48 µg/ml) in 0.1 M HCl were prepared. Their absorption spectra were recorded over the wavelength range 220–320 nm against a blanc (0.1 M HCl). In order to validate the method, the synthetic mixture solutions of CAF, APAP and MET were prepared according to the working range of the individual compounds.

5.1. TLRC method

As an application of this method, nine-wavelength points were considered for the ternary mixture systems. Nine linear regression equations were obtained by using the absorbances measured at nine-wavelengths against the concentrations of standard solution for each compound.

The highest values for the regression coefficients (r) were obtained for all regression equations. The linear regression analysis and its results were presented in Table 1.

The slope values obtained from the linear regression analysis for each compound in the ternary mixture of CAF, APAP and MET were used to create the sensitivity matrices (Table 2). According to Kaiser's method (46), the absolute values of the determinant of the sensitivity matrices were used to find the best sensitivity for the application of TLRC model. In this procedure, it was possible to calculate the different 84 three-pairs of the sensitivity matrices for the selection of

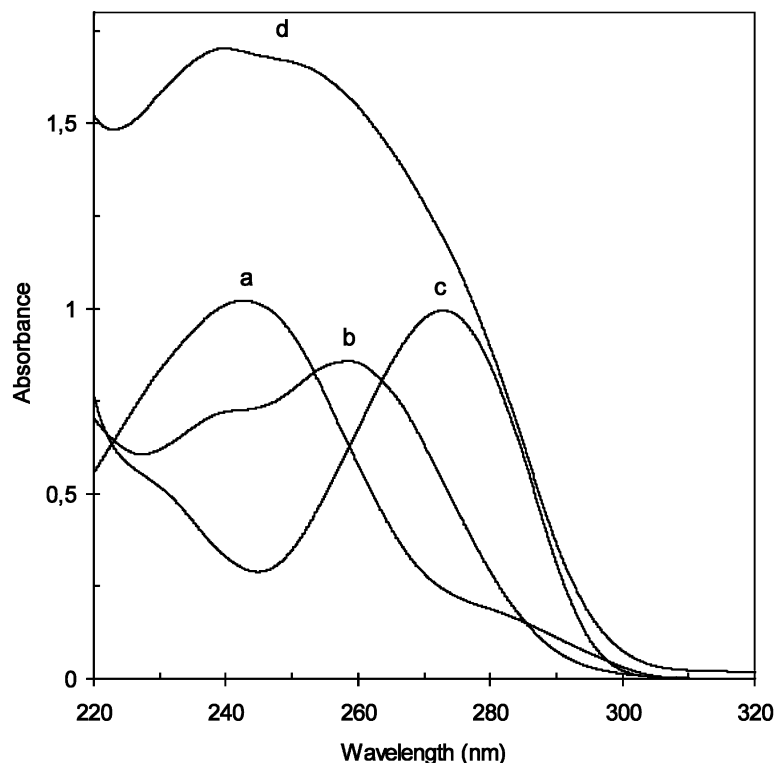


Fig. 1. Absorption spectra of: (a) 20 µg/ml APAP; (b) 32 µg/ml MET; (c) 20 µg/ml CAF; and (d) their mixture in 0.1 M HCl.

optimum three-wavelength set. So that the computing process contains 3-dimensional spaces in great volume, the diagonal sensitivity values here were not placed. An optimum three-wavelength set, which gives the highest determinant value of

the sensitivity matrices, was selected as 242, 262 and 277 nm for the calibration modelled. At this selected three-wavelength set, the individual linear regression equations for each compound were can be seen in Table 1. The following set of equations

Table 1
Linear regression analysis and its results at nine-wavelengths

λ_i	CAF		APAP		MET	
	Regression equation	(<i>r</i>)	Regression equation	(<i>r</i>)	Regression equation	(<i>r</i>)
237	$A = 0.0192C - 0.0040$	0.9999	$A = 0.0606C + 0.0123$	0.9999	$A = 0.0222C - 0.0105$	0.9999
242	$A = 0.0153C - 0.0046$	0.9999	$A = 0.0636C + 0.0122$	0.9999	$A = 0.0229C - 0.0098$	0.9998
247	$A = 0.0150C - 0.0042$	0.9999	$A = 0.0618C + 0.0131$	0.9999	$A = 0.0235C - 0.0091$	0.9998
252	$A = 0.0199C - 0.0022$	0.9999	$A = 0.0545C + 0.0115$	0.9999	$A = 0.0254C - 0.0083$	0.9999
257	$A = 0.0279C + 0.0003$	0.9999	$A = 0.0433C + 0.0063$	0.9999	$A = 0.0268C - 0.0074$	0.9999
262	$A = 0.0370C + 0.0028$	0.9999	$A = 0.0316C + 0.0057$	0.9999	$A = 0.0261C - 0.0063$	0.9999
267	$A = 0.0448C + 0.0067$	0.9999	$A = 0.0218C + 0.0050$	0.9999	$A = 0.0228C - 0.0053$	0.9999
272	$A = 0.0488C + 0.0100$	0.9999	$A = 0.0158C + 0.0048$	0.9999	$A = 0.0176C - 0.0037$	0.9999
277	$A = 0.0465C + 0.0095$	0.9999	$A = 0.0128C + 0.0046$	0.9999	$A = 0.0121C - 0.0032$	0.9999

A = absorbance of compound, *r* = correlation coefficient.

Table 2

The obtained sensitivity values of CAF, APAP and MET using single-component regression analysis at nine-wavelengths

Wavelength (nm)	$b_{CAF} \times 10^{-3}$	$b_{MET} \times 10^{-3}$	$b_{APAP} \times 10^{-3}$
237	19.2	22.2	60.6
242	15.3	22.9	63.6
247	15.0	23.5	61.8
252	19.9	25.4	54.5
257	27.9	26.8	43.3
262	37.0	26.1	31.6
267	44.8	22.8	21.8
272	48.8	17.6	15.8
277	46.5	12.1	12.8

b_{CAF} , b_{APAP} and b_{MET} denote the slopes of regression equation of CAF, APAP and MET.

were created for the TLRC method:

$$\begin{aligned} \lambda_1 &= 242, A_{\text{mix}_1} \\ &= 0.0153C_{CAF} + 0.0636C_{APAP} + 0.0229C_{MET} \\ &\quad - 0.0023 \\ \lambda_2 &= 262, A_{\text{mix}_2} \\ &= 0.0370C_{CAF} + 0.0316C_{APAP} + 0.0261C_{MET} \\ &\quad + 0.0023 \\ \lambda_3 &= 277, A_{\text{mix}_3} \\ &= 0.0465C_{CAF} + 0.0128C_{APAP} + 0.0121C_{MET} \\ &\quad + 0.0109 \end{aligned} \quad (16)$$

As described in Section 2.1, the TLRC procedure was achieved by using the linear algebra, also known as matrix mathematics. The constructed calibration was applied to the analysis of the synthetic mixtures and two pharmaceutical tablet formulations.

5.2. MLRC method

This approach is analogous to tri-linear regression calibration, but multi-linear regression calibration contains n -wavelength procedure instead of three-wavelengths. For this reason, the nine-wavelengths set (237, 242, 247, 252, 257, 262, 267, 272, 277) at the critical points, which correspond to the maximum, shoulder and minimum in the spectral range 220–320 nm were selected for the

construction of the individual linear regression for CAF, APAP and MET in the ternary mixture. As indicated in Table 1, nine linear regression equations of CAF, APAP and MET, for each compound, were obtained by measuring the zero-order absorbance values at the wavelengths set. The equation set (17) for the MLRC method were obtained as:

$$\begin{aligned} \lambda_1 &= 237, A_{\text{mix}_1} \\ &= 0.0192C_{CAF} + 0.0606C_{APAP} + 0.0222C_{MET} \\ &\quad - 0.0023 \end{aligned}$$

$$\begin{aligned} \lambda_2 &= 242, A_{\text{mix}_2} \\ &= 0.0153C_{CAF} + 0.0636C_{APAP} + 0.0229C_{MET} \\ &\quad - 0.0022 \end{aligned}$$

$$\begin{aligned} \lambda_3 &= 247, A_{\text{mix}_3} \\ &= 0.0150C_{CAF} + 0.0618C_{APAP} + 0.0235C_{MET} \\ &\quad - 0.0003 \end{aligned}$$

$$\begin{aligned} \lambda_4 &= 252, A_{\text{mix}_4} \\ &= 0.0199C_{CAF} + 0.0545C_{APAP} + 0.0254C_{MET} \\ &\quad + 0.0010 \end{aligned}$$

$$\begin{aligned} \lambda_5 &= 257, A_{\text{mix}_5} \\ &= 0.0279C_{CAF} + 0.0433C_{APAP} + 0.0268C_{MET} \\ &\quad - 0.0008 \end{aligned}$$

$$\begin{aligned} \lambda_6 &= 262, A_{\text{mix}_6} \\ &= 0.0370C_{CAF} + 0.0316C_{APAP} + 0.0261C_{MET} \\ &\quad + 0.0023 \end{aligned}$$

$$\begin{aligned} \lambda_7 &= 267, A_{\text{mix}_7} \\ &= 0.0448C_{CAF} + 0.0218C_{APAP} + 0.0228C_{MET} \\ &\quad + 0.0064 \end{aligned}$$

$$\begin{aligned} \lambda_8 &= 272, A_{\text{mix}_8} \\ &= 0.0488C_{CAF} + 0.0158C_{APAP} + 0.0176C_{MET} \\ &\quad + 0.0110 \end{aligned}$$

$$\begin{aligned} \lambda_9 = 277, A_{\text{mix}_9} \\ = 0.0465C_{\text{CAF}} + 0.0128C_{\text{APAP}} + 0.0121C_{\text{MET}} \\ + 0.0109 \end{aligned} \quad (17)$$

As described in Section 2.2, the MLRC approach was applied to the multiresolution of the ternary mixture and two pharmaceutical dosage forms containing CAF, APAP and MET.

5.3. CLS method

Absorptivity, A_1^1 (1%, 1 cm), values of three compounds, CAF, APAP and MET were calculated by using the absorbances measured at the above mentioned nine wavelengths (see Section 5.2) in the zero-order spectra for each of the compounds in ternary mixture (Table 3). By using A_1^1 values, a system of equations with nine unknowns was written for the compounds in the ternary mixture, as described in Section 2.3. Using the procedure explained in the same section, this matrix was solved, and it was determined the concentration of CAF, APAP and MET in their mixture.

5.4. Validation of the developed methods

In these methods, Beer's law was valid in the concentration range 4–40 $\mu\text{g/ml}$ for CAF, 8–40 $\mu\text{g/ml}$ for APAP and 12–48 $\mu\text{g/ml}$ for MET.

To check the validity of the calibration models, the multiresolution of the synthetic mixtures containing various concentrations of CAF, APAP and MET was carried out by the TLRC, MLRC and CLS methods. Results were summarized in Table

4. The means recoveries and the relative standard deviations of the methods were computed. The obtained results can be considered satisfactory in the case of spectral overlapping between CAF, APAP and MET. Their numerical values were found satisfactory for the validation of all calibration methods.

In addition, the standard addition was also applied to commercial pharmaceutical formulations for the control of the validity of the TLRC, MLRC and CLS calibrations. The mean percentage recoveries and their standard deviation for the TLRC, MLRC and CLS methods were found to be $100.4\% \pm 2.1$, $101.9\% \pm 1.9$ and $99.6\% \pm 1.5$ for CAF, $100.92\% \pm 2.6$, $102.1\% \pm 1.0$ and $99.20\% \pm 1.8$ for APAP, and $101.2\% \pm 2.9$, $101.9\% \pm 2.0$ and $99.0\% \pm 1.6$ for MET, respectively, by using the mean values obtained in two commercial pharmaceutical formulations. It was observed that the results also confirm the precision and accuracy of the proposed calibration methods and the excipients in tablets do not interfere in the analysis of the active compounds.

5.5. Analysis of pharmaceutical tablet formulations

The experimental results of commercial tablet were given in Table 5. The results of all the methods were very close to each other as well as to the label value of commercial tablets. The numerical values of all statistic parameters indicated that the mathematical methods are suitable for the determination of three drugs in the tablet formulation.

To compare the differences between methods, one-way ANOVA test was carried out by using the

Table 3
Absorptivity values calculated for the CLS method

λ_i	237	242	247	252	257	262	267	272	277
CAF	α_1 188.7	α_2 149.2	α_3 146.3	α_4 197.4	α_5 279.9	α_6 373.0	α_7 454.5	α_8 496.0	α_9 472.8
APAP	β_1 613.7	β_2 643.2	β_3 625.8	β_4 551.6	β_5 437.2	β_6 319.9	β_7 221.7	β_8 160.9	β_9 130.9
MET	γ_1 217.4	γ_2 225.1	γ_3 231.1	γ_4 250.9	γ_5 265.4	γ_6 258.1	γ_7 226.2	γ_8 174.5	γ_9 119.6

Table 4

Recoveries obtained for the determination of CAF, APAP and MET in different synthetic mixtures by using the developed methods

Composition of mixture ($\mu\text{g/ml}$)			TLRC			MLRC			CLS		
			Recovery (%)			Recovery (%)			Recovery (%)		
CAF	APAP	MET	CAF	APAP	MET	CAF	APAP	MET	CAF	APAP	MET
16	16	12	99.4	101.4	100.4	99.3	101.3	101.3	98.9	101.4	99.8
16	16	20	98.4	101.1	98.4	98.4	100.9	98.9	98.1	101.4	97.9
16	16	32	99.9	103.9	99.3	100.3	103.8	99.0	100.1	104.8	98.3
16	16	40	100.0	102.6	97.7	100.3	102.8	97.8	100.1	104.1	97.3
16	16	48	100.2	104.1	96.0	100.6	103.8	96.3	100.5	105.0	95.8
16	8	20	99.4	102.0	99.7	99.3	101.4	100.4	99.2	103.8	99.1
16	16	20	99.6	100.6	99.7	99.3	100.3	100.6	99.0	100.8	99.7
16	20	20	98.3	99.9	100.4	98.8	100.2	99.4	98.4	100.3	98.6
16	32	20	99.3	99.9	100.2	99.3	99.5	101.5	98.4	99.0	101.4
16	40	20	103.4	99.7	99.0	105.8	101.2	96.0	104.6	100.5	95.0
4	16	20	105.0	103.9	99.5	105.0	104.1	99.2	110.8	103.9	98.0
12	16	20	101.8	103.4	100.6	102.0	103.7	100.2	102.2	103.9	99.2
19	16	20	105.1	103.0	101.3	105.1	102.9	101.8	104.4	103.6	101.0
32	16	20	100.0	103.0	99.6	100.2	103.4	98.8	99.0	104.8	98.3
40	16	20	99.0	102.6	99.1	99.2	102.9	98.1	97.9	104.8	97.7
Mean =	100.6	102.1	99.4	100.8	102.13	99.27	100.8	102.8	98.5		
RSD =	2.20	1.51	1.31	2.44	1.49	1.76	3.45	1.94	1.74		

SD = standard deviation, RSD = relative standard deviation.

Table 5

Results obtained in the pharmaceutical dosage forms by the developed methods

Methods	CAF		APAP		MET	
	Mean ^a \pm SD ^b		Mean ^a \pm SD ^b		Mean ^a \pm SD ^b	
	I	II	I	II	I	II
TLRC	49.9 \pm 1.0	30.1 \pm 1.2	203.0 \pm 3.1	160.5 \pm 1.1	202.8 \pm 3.5	220.2 \pm 1.2
MLRC	50.0 \pm 1.2	30.6 \pm 0.4	202.0 \pm 3.8	161.1 \pm 1.7	201.3 \pm 2.4	220.1 \pm 1.0
CLS	50.1 \pm 1.1	31.3 \pm 1.1	205.1 \pm 5.9	162.5 \pm 3.3	203.3 \pm 3.1	219.3 \pm 1.4

^a Results obtained are the average of ten experiment for each method.^b SD = standard deviation, I = Remidon[®] tablet, II = Pirosal[®] tablet.

parallel results obtained by applying the three methods to 10 samples for each compound in two tablet formulations. For this purpose, Snedecor's *F*-values were computed and compared with the standard tabulated value ($P = 0.05$). The same computation process was repeated for three compounds. In standard table, for $n_1 = 2$ and $n_2 = 27$ ($P = 0.05$), the *F*-value is 3.35. ANOVA test results were found as 1.55 and 1.36 for CAF, 1.17 and 2.18 for APAP, and 1.22 and 1.51 for

MET. The experimental (calculated) *F*-values did not exceed the *F*-tabulated value in the variance analysis. It was observed that there was no significant difference among the methods.

6. Conclusion

In this presented work, two new approaches were formulated for the multiresolution of the

multi-component mixture systems, CAF–APAP–MET. As an alternative method, CLS method was also used for the same aim. Although the individual spectra of APAP, APAP and CAF overlap in the 220–310 nm wavelength range, the TLRC, MLRC and CLS methods gave successful results for the quantitative multiresolution of the multi-component mixture and two pharmaceutical dosage forms consisting of three compounds. This can be considered as an advantage of two new methods, TLRC and MLRC over alternative methods for the quantitative resolution of the multi-component mixtures. It was observed that the quantitative determination results obtained in the newly developed methods are comparable with the HPLC for the same commercial tablets [41]. Besides, three methods described in this paper do not require a chemical pretreatment such as a priory separation step as used in HPLC, and a graphical procedure such as a derivation and a division of the spectra as in ratio spectra derivative spectrophotometry described in the literature. Results also showed that these methods which are powerful tools with very simple mathematical content are more reliable than other spectrophotometric methods [27].

The results obtained in this paper strongly encourage us to apply these calibration models for a routine analysis, quality control of multi-component mixtures and commercial products containing multi compounds.

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