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Multivariate spectral calibration technique based on regression analysis for the quantitative multiresolution of a ternary mixture containing paracetamol, ascorbic acid and acetylsalicylic acid in effervescent tablets

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Multivariate spectral calibration techniques based on regression analysis were established for the quantitative multiresolution of a ternary mixture containing parecetamol (PAR) ascorbic acid (AA) and acetylsalicylic acid (ASP) having closely overlapping spectra. The mathematical algorithms of multivariate spectral calibrations as namely tri-linear regression calibration (TLRC) and multi-linear regression calibration (MLRC) are based on the use of the linear regression equations at a three-wavelength set and a ten-wavelength set in the range of 215–305 nm. These calibration techniques do not require any chemical pre-treatment and a graphical procedure of the overlapping spectra. The mathematical content of TLRC and MLRC approaches were briefly formulated for the quantitative analysis of three- or multi-component mixtures. The applicability of the formulated calibration models were tested by analysing the various synthetic ternary mixtures consisting of these active compounds and then these models were applied to real pharmaceutical formulations. It was observed that TLRC and MLRC models give a successful quantitative multiresolution. The experimental results of these techniques were compared with each other as well as with those obtained by literature methods.

1. Introduction

In these days numerical popular methods namely principle component regression (PCR) and partial least-squares (PLS) have been widely used for the resolution of the complex mixtures but these methods use highly abstract theory and need complicated and special software. Derivative spectrophotometry provides good results for the resolution of binary mixtures as well, but has not given successful results in the analysis of ternary or multi-mixtures.

Recently the simultaneous analysis of binary mixtures was carried out by the bivariate calibration method (López-de-Alba et al. 1997; López-de-Alba et al. 1997). Another new method, which is called tri-linear regression calibration (TLRC) technique, was developed from this method for the quantitative analysis of binary and ternary mixtures (Dinc 2003; Üstündağ and Dinç 2003). In a further step the TLRC model was modified and generalized as multi-linear-regression calibration (MLRC) for the multiresolution of three-component and multi-component mixtures. The MLRC algorithm applies linear algebra to linear regression equations obtained from the critical wavelength at multipoint sets in the working wavelength range.

For the determination of paracelamol (PAR), ascorbic acid (AA) and acetylsalicylic acid (ASP) in their mixtures with other different active compounds several analytical methods including double divisor-ratio spectra derivative and ratio spectra-zero crossing methods (Dinç 1999), planar chromatography (Franeta et al. 2001), second derivative spectrophotometry (Kokot and Burda 1998), PLS calibration (Marcelo and Ronei J. P. in press), HPLC (Franeta et al. 2002) and near-infrared spectroscopy (NIR) (Merckle and Kovar 1998) have been reported in the literature.

The aim of this work was to develop simple and rapid TLRC and MLRC mathematical methods for the simultaneous determination of PAR, AA, and ASP in effervescent tablets.

2. Investigations, results and discussion

2.1. Methodology

The basis of TLRC and MLRC models briefly explained for the understanding of reader starting from simple linear regression analysis.

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:

$$A_Xi = b_{Xi}C_X + a_{Xi} \quad (1)$$

where, A_{Xi} is the absorbance of the X analyte at λ_i wavelength, C_X is the concentration of the analyte X (the concentration units are μg/mL in the two newly developed methods), b_{Xi} is the slope of the linear regression equa-

tion, a_{Xi} is the intercept of the regression model. These intercept values indicate the difference between the ideal and calculated system.

2.1.1. TLRC model

The TLRC model (Dinç 2003) contains the application of matrix mathematics to three linear regression equations at three wavelength points selected by Kaiser's method (Massart 1988). The mathematical algorithm of TLRC is explained in the following steps.

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:

$$\begin{aligned} A_{mix_1} &= b_{X_1}C_X + b_{Y_1}C_Y + b_{Y_1}C_Z + a_{XYZ_1} \\ A_{mix_2} &= b_{X_2}C_X + b_{Y_2}C_Y + b_{Y_2}C_Z + a_{XYZ_2} \\ A_{mix_3} &= b_{X_3}C_X + b_{Y_3}C_Y + b_{Y_3}C_Z + a_{XYZ_3} \end{aligned} \quad (2)$$

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_1} 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 $a_{XYZ_3} = a_{X_3} + a_{Y_3} + a_{Z_3}$).

Equation (2) can be formulated in matrix notation as:

$$\begin{bmatrix} A_{mix_1} \\ A_{mix_2} \\ A_{mix_3} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ b_{X_3} & b_{Y_3} & b_{Z_3} \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} + \begin{bmatrix} a_{XYZ_1} \\ a_{XYZ_2} \\ a_{XYZ_3} \end{bmatrix} \quad (3)$$

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

$$\begin{bmatrix} A_{mix_1} - a_{XYZ_1} \\ A_{mix_2} - a_{XYZ_2} \\ A_{mix_3} - a_{XYZ_3} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ b_{X_3} & b_{Y_3} & b_{Z_3} \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} \quad (4)$$

or, more simply:

$$(A_{mix} - a_{XYZ})_{3\times 1} = K_{3\times 3} \cdot C_{3\times 1} \quad (5)$$

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

$$K = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ b_{X_3} & b_{Y_3} & b_{Z_3} \end{bmatrix} \quad (6)$$

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

$$C_{3\times 1} = (K^{-1})_{3\times 3} \cdot (A_{mix} - a_{XYZ})_{3\times 1} \quad (7)$$

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

As explained here, the developed calibration model can easily be applied to the multiresolution of three-component mixtures. The choice of the 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 (Massart 1988) 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 matrices K (square matrix) in eq. (6) are formed by taking every three-pairs of pre-selected wavelengths for ternary mixtures.

The matrices K of the slope values obtained from the linear regression equations of the individual analytes, X, Y and Z at three selected wavelengths (1, 2 and 3) are considered as the sensitivity parameter (López-de-Alba et al. 1997a, b, 2002; Dinc 2003, Üstündağ and Dinç 2003). The sensitivity parameter is used for comparing different three-wavelength sets. The sensitivity of a multicomponent analysis is defined as the absolute value of the determinant of the sensitivity matrix K. For this reason, the determinant values of the matrices 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 ten linear regression equations with three linear regression lines for each compound at three selected wavelengths in our case.

2.1.2. MLRC model

The MLRC algorithm (Dinç 2003) for the quantitative analysis of ternary or multi mixtures is based on the application of linear algebra to linear regression equations at a multipoint set of selected wavelengths in the working spectral range. A MLRC algorithm contains the following steps.

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

$$\begin{aligned} A_{mix_1} &= b_{X_1}C_X + b_{Y_1}C_Y + b_{Z_1}C_Z + a_{XYZ_1} \\ A_{mix_1} &= b_{X_2}C_X + b_{Y_2}C_Y + b_{Z_2}C_Z + a_{XYZ_2} \\ \ldots\ldots &\quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots \\ \ldots\ldots &\quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots \\ A_{mix_n} &= b_{X_n}C_X + b_{Y_n}C_Y + b_{Z_n}C_Z + a_{XYZ_n} \end{aligned} \quad (8)$$

Where A_{mix_1}, A_{mix_2}, ..., 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}, ..., b_{X_n}, b_{Y_1}, b_{Y_2}, ... b_{Y_n} and b_{Z_1}, b_{Z_2}, ..., 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}, ... 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 matrix terms, the above multi-equation system (8) can be formulated as:

$$\begin{bmatrix} A_{mix_1} \\ A_{mix_2} \\ \ldots\ldots \\ \ldots\ldots \\ A_{mix_n} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ \ldots\ldots & \ldots\ldots & \ldots\ldots \\ \ldots\ldots & \ldots\ldots & \ldots\ldots \\ b_{X_n} & b_{Y_n} & b_{Z_n} \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} + \begin{bmatrix} a_{XYZ_1} \\ a_{XYZ_2} \\ \ldots\ldots \\ \ldots\ldots \\ a_{XYZ_n} \end{bmatrix} \quad (9)$$

which can be simplified to

$$\begin{bmatrix} A_{mix_1} - a_{XYZ_1} \\ A_{mix_2} - a_{XYZ_2} \\ \ldots\ldots \quad \ldots\ldots \\ \ldots\ldots \quad \ldots\ldots \\ A_{mix_3} - a_{XYZ_n} \end{bmatrix} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ \ldots\ldots & \ldots\ldots & \ldots\ldots \\ \ldots\ldots & \ldots\ldots & \ldots\ldots \\ b_{X_n} & b_{Y_n} & b_{Z_n} \end{bmatrix} * \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} \quad (10)$$

in a compact form

$$(A_{mix} - a_{XYZ})_{n\times 1} = K_{n\times 3} \times C_{3\times 1} \quad (11)$$

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

$$K_{n\times 3} = \begin{bmatrix} b_{X_1} & b_{Y_1} & b_{Z_1} \\ b_{X_2} & b_{Y_2} & b_{Z_2} \\ \cdots\cdots & \cdots\cdots & \cdots\cdots \\ \cdots\cdots & \cdots\cdots & \cdots\cdots \\ b_{X_n} & b_{Y_n} & b_{Z_n} \end{bmatrix} \quad (12)$$

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

$$(K')_{3\times n}(A_{mix} - a)_{n\times 1} = (K')_{3\times n} K_{n\times 3} \cdot C_{3\times 1} \quad (13)$$

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

$$C_{3\times 1} = \left[(K')_{3\times n} K_{n\times 3}\right]^{-1}_{3\times 3} \cdot \left[(K')_{3\times n}(A_{mix} - a_{XYZ})_{n\times 1}\right] \quad (14)$$

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 multicomponent mixture system containing n compounds.

2.2. Results and discussion

The absorption spectra of the investigated compounds PAR, AA and ASP in 0.1 M HCl were strongly overlapped in the spectral range from 215 nm to 305 nm as shown in the Fig. Because of these overlapping spectra, the simultaneous quantitative analysis of PAR, AA and ASP in their mixtures is not possible by classical analytical methods such as the method of direct absorbance measurement and derivative spectrophotometry. In this study, two methods TLRC and MLRC were developed to the quantitative multiresolution of the ternary mixtures and effervescent tablets containing PAR, AA and ASP.

After optimization of the experimental conditions the standard series of solutions of PAR, AA and ASP in the concentration range of 4–20 μg/mL in 0.1 M HCl were prepared for the construction of TLRC and MLRC. The absorption spectra of the prepared standard series were recorded over the wavelength range 215–305 nm against a blank (0.1 M HCl). Then multivariate TLRC and MLRC models were built by means of the mathematical algorithms described in sections 2.1.1 and 2.1.2. The validation of these methods was carried out by analysing the synthetic mixture solutions of PAR, AA and ASP prepared in the working range of the individual compounds.

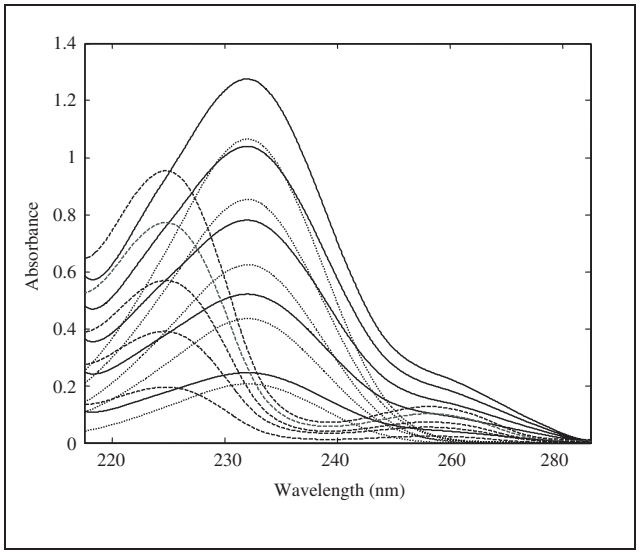

Fig.: Absorption spectra of: 4 μg/mL, 8 μg/mL, 12 μg/mL, 16 μg/mL, 20 μg/mL (–) PAR, (. . . .) AA and (- - - -) ASP in 0.1 M HCl

2.2.1. Multivariate TLRC method

In this method, ten critical wavelength points in the working spectral range were selected for the ternary mixtures of the active compounds. Using the above standard series for each compound in the mixtures, ten linear regression equations were constructed with the absorbance values at ten wavelength points.

The calculated linear regression equations and their statistical results were summarised in Table 1. The results of linear regression analysis were found to be reliable for the construction of the TLRC model.

The sensitivity matrices indicated in Table 2 were created by the slope values of the linear regression analysis for each compound in the ternary mixture of PAR, AA and ASP. According to the wavelength selection method of Kaiser (Kaiser 1972), the best sensitivity for construction of the TLRC model was obtained from the absolute values of the determinant of the sensitivity matrices. In this treatment, different 120 three-pairs of the sensitivity matrices were possible for the selection of optimum three-wavelength set. This result was calculated according to the following formula:

$$C^p_n = \frac{p!}{(p-n)!n!} \quad (15)$$

where C^p_n is the number of three pair of sensitivity matrices, p! is the wavenumbers and n is the number of components. So that the results of the computing process contain 3-dimensional spaces in great volume, the diagonal sensitivity values here were not placed. An optimum three-wavelength set having the highest determinant value of the sensitivity matrices was calculated and selected as 229, 247 and 269 nm for the TLRC calibration. The individual linear regression equations for each compound at this selected three-wavelength set were presented in Table 1. The following set of equations were created for the TLRC method.

$$\begin{aligned} \lambda_{229}, A_{mix2} &= 0.04633\, C_{PAR} + 0.03306\, C_{AA} \\ &\quad + 0.04744\, C_{ASP} - 0.00151 \\ \lambda_{247}, A_{mix6} &= 0.06292\, C_{PAR} + 0.05193\, C_{AA} \\ &\quad + 0.00987\, C_{ASP} - 0.00544 \\ \lambda_{263}, A_{mix10} &= 0.02852\, C_{PAR} + 0.01756\, C_{AA} \\ &\quad + 0.00400\, C_{ASP} - 0.00465 \end{aligned} \quad (16)$$

The TLRC algorithm explained in section 2.1.1 was subject to the above equation set by means of linear algebra, also known as matrix mathematics. The constructed TLRC calibration was used for the analysis of the synthetic mixtures and effervescent tablets.

2.2.2. Multivariate MLRC method

The MLRC algorithm using linear algebra contains its application to n-regression equations at n-wavelength set

Table 1: Linear regression analysis and its statistical results at ten wavelengths

Wavelength	PAR					AA					ASP				
	Regression equation	r	SE(b)	SE(a)	SE(r)	Regression equation	r	SE(b)	SE(a)	SE(r)	Regression equation	r	SE(b)	SE(a)	SE(r)
225	$A = 0.0398C - 0.0016$	0.9995	0.0007	0.0093	0.0088	$A = 0.0264C - 0.0069$	0.9990	0.0007	0.0090	0.0086	$A = 0.0445C + 0.0081$	0.9998	0.0005	0.0068	0.0065
229	$A = 0.0463C - 0.0020$	0.9995	0.0008	0.0107	0.0102	$A = 0.0331C - 0.0060$	0.9993	0.0007	0.0098	0.0093	$A = 0.0474C + 0.0064$	0.9998	0.0005	0.0064	0.0061
233	$A = 0.0526C - 0.0021$	0.9996	0.0008	0.0111	0.0105	$A = 0.0402C - 0.0053$	0.9995	0.0007	0.0098	0.0093	$A = 0.0453C + 0.0039$	0.9999	0.0004	0.0054	0.0051
237	$A = 0.0589C - 0.0023$	0.9997	0.0009	0.0115	0.0109	$A = 0.0471C - 0.0049$	0.9996	0.0008	0.0100	0.0095	$A = 0.0375C + 0.0012$	0.9999	0.0003	0.0045	0.0043
243	$A = 0.0643C - 0.0010$	0.9996	0.0010	0.0132	0.0126	$A = 0.0532C - 0.0041$	0.9996	0.0008	0.0111	0.0105	$A = 0.0192C - 0.0016$	0.9999	0.0002	0.0025	0.0024
247	$A = 0.0629C - 0.0004$	0.9996	0.0010	0.0131	0.0125	$A = 0.0519C - 0.0035$	0.9997	0.0008	0.0105	0.0100	$A = 0.0099C - 0.0016$	0.9993	0.0002	0.0028	0.0027
251	$A = 0.0572C - 0.0005$	0.9996	0.0009	0.0118	0.0113	$A = 0.0460C - 0.0030$	0.9996	0.0007	0.0094	0.0090	$A = 0.0056C - 0.0007$	0.9969	0.0003	0.0034	0.0032
255	$A = 0.0484C - 0.0007$	0.9996	0.0008	0.0109	0.0104	$A = 0.0372C - 0.0032$	0.9995	0.0007	0.0087	0.0083	$A = 0.0039C - 0.0002$	0.9929	0.0003	0.0036	0.0034
259	$A = 0.0382C - 0.0008$	0.9995	0.0007	0.0089	0.0085	$A = 0.0271C - 0.0031$	0.9993	0.0006	0.0075	0.0072	$A = 0.0036C + 0.0000$	0.9910	0.0003	0.0037	0.0035
263	$A = 0.02850C - 0.0017$	0.9995	0.0005	0.0068	0.0065	$A = 0.0176C - 0.0029$	0,9990	0.0005	0.0060	0.0058	$A = 0.0040C - 0.0001$	0.9926	0.0003	0.0037	0.0036

A = absorbance of the analysed compound
r = correlation coefficient of regression equation
SE(b) = standard error of slop
SE(b) = standard error of intercept
SE(r) = standard error of correlation coefficient
C = concentration of the analysed compound

Table 2: The obtained sensitivity values of PAR, AA and ASP using single-component regression analysis at nine-wavelengths

Wavelength (nm)	$b_{PAR} \times 10^{-3}$	$b_{AA} \times 10^{-3}$	$b_{ASP} \times 10^{-3}$
225	39.8	26.4	44.5
229	46.3	33.1	47.4
233	52.6	40.2	45.3
237	58.9	47.1	37.5
243	64.3	53.2	19.2
247	62.9	51.9	9.9
251	57.2	46.0	5.6
255	48.4	37.2	3.9
259	38.2	27.1	3.6
263	28.5	17.6	4.0

in the working spectral range. This MLRC approach is analogous to the TLRC model, but MLRC uses a n-wavelength procedure instead of three-wavelengths. In our case a ten-wavelength set was chosen for the linear regression analysis between each compound's standard series and its absorbance values. For this procedure, the ten-wavelengths set (225, 229, 233, 237, 243, 247, 251, 255, 259 and 263 nm), at the critical points, which correspond to the maximum, shoulder and minimum in the spectral range 215–305 nm were selected for the construction of the linear regression equations of PAR, AA and ASP in the ternary mixture. Ten linear regression equations for each compound were obtained by measuring the absorbance values at this ten-wavelengths set (Table 1). The equation set (17) obtained from Table 1 was arranged as:

$$\begin{aligned}
\lambda_{225}, A_{mix1} &= 0.03982\, C_{PAR} + 0.02637\, C_{AA} \\
&\quad + 0.04446\, C_{ASP} - 0.00035 \\
\lambda_{229}, A_{mix2} &= 0.04633\, C_{PAR} + 0.03306\, C_{AA} \\
&\quad + 0.04744\, C_{ASP} - 0.00151 \\
\lambda_{233}, A_{mix3} &= 0.05264\, C_{PAR} + 0.04015\, C_{AA} \\
&\quad + 0.04535\, C_{ASP} - 0.00355 \\
\lambda_{237}, A_{mix4} &= 0.05892\, C_{PAR} + 0.04715\, C_{AA} \\
&\quad + 0.03750\, C_{ASP} - 0.00606 \\
\lambda_{243}, A_{mix5} &= 0.06432\, C_{PAR} + 0.05322\, C_{AA} \\
&\quad + 0.01920\, C_{ASP} - 0.00673 \qquad (17) \\
\lambda_{247}, A_{mix6} &= 0.06292\, C_{PAR} + 0.05193\, C_{AA} \\
&\quad + 0.00987\, C_{ASP} - 0.00544 \\
\lambda_{251}, A_{mix7} &= 0.05716\, C_{PAR} + 0.04603\, C_{AA} \\
&\quad + 0.00556\, C_{ASP} - 0.00422 \\
\lambda_{255}, A_{mix8} &= 0.04838\, C_{PAR} + 0.03720\, C_{AA} \\
&\quad + 0.00394\, C_{ASP} - 0.00410 \\
\lambda_{259}, A_{mix9} &= 0.03819\, C_{PAR} + 0.02714\, C_{AA} \\
&\quad + 0.00359\, C_{ASP} - 0.00392 \\
\lambda_{263}, A_{mix10} &= 0.02852\, C_{PAR} + 0.01756\, C_{AA} \\
&\quad + 0.00400\, C_{ASP} - 0.00465
\end{aligned}$$

The MLRC algorithm explained in section 2.1.2 was applied to the above equation set and the obtained MLRC model was used for the quantitative multiresolution of PAR, AA and ASP in ternary mixtures and effervescent tablets.

Table 3: Recoveries obtained for the determination of PAR, AA and ASP in different synthetic mixtures by TLRC technique

No:	Added (mg/mL)			Found (mg/mL)			Recovery (%)		
	PAR	AA	ASP	PAR	AA	ASP	PAR	AA	ASP
1	4	18	18	4.05	17.40	17.69	101.3	96.6	98.3
2	8	18	18	8.28	17.60	17.89	103.5	97.8	99.4
3	12	18	18	12.03	17.66	17.66	100.3	98.1	98.1
4	16	18	18	16.24	17.39	17.84	101.5	96.6	99.1
5	20	18	18	19.96	17.92	17.72	99.8	99.6	98.4
6	12	4	18	12.42	3.80	17.91	103.5	95.0	99.5
7	12	8	18	12.64	7.50	17.87	105.3	93.8	99.3
8	12	12	18	12.83	11.30	18.01	106.9	94.2	100.0
9	12	16	18	12.72	15.20	17.88	106.0	95.0	99.3
10	12	20	18	12.53	19.10	17.76	104.5	95.5	98.7
11	12	18	4	12.13	17.76	3.85	101.1	98.6	96.2
12	12	18	8	12.19	17.61	7.85	101.6	97.9	98.1
13	12	18	12	12.50	17.25	11.93	104.2	95.8	99.4
14	12	18	16	12.43	17.31	15.83	103.6	96.1	98.9
15	12	18	20	12.19	17.37	19.82	101.6	96.5	99.1
						Mean	103.0	96.5	98.8
						RSD	2.02	1.67	0.89

RSD = relative standard deviation

Table 4: Recoveries obtained for the determination of PAR, AA and ASP in different synthetic mixtures by MLRC technique

No:	Added (mg/mL)			Found (mg/mL)			Recovery (%)		
	PAR	AA	ASP	PAR	AA	ASP	PAR	AA	ASP
1	4	18	18	3.82	18.13	17.81	95.5	100.7	99.0
2	8	18	18	7.76	18.24	17.96	97.0	101.3	99.8
3	12	18	18	11.27	18.58	17.82	93.9	103.2	99.0
4	16	18	18	15.42	18.43	17.90	96.4	102.4	99.4
5	20	18	18	19.44	18.47	17.90	97.2	102.6	99.4
6	12	4	18	11.98	3.84	17.96	99.8	95.9	99.8
7	12	8	18	12.02	7.81	17.95	100.2	97.7	99.7
8	12	12	18	12.54	11.44	18.05	104.5	95.3	100.3
9	12	16	18	11.92	16.20	18.02	99.4	101.2	100.1
10	12	20	18	11.99	19.80	17.77	99.9	99.0	98.7
11	12	18	4	11.55	18.47	3.96	96.2	102.6	99.0
12	12	18	8	11.65	18.25	7.95	97.1	101.4	99.4
13	12	18	12	11.81	18.14	12.01	98.4	100.8	100.1
14	12	18	16	11.87	18.00	15.93	98.9	100.0	99.6
15	12	18	20	11.83	17.82	19.84	98.6	99.0	99.2
						Mean	98.2	100.2	99.5
						RSD	2.47	2.32	0.45

2.2.3. Validation of the developed methods

Beer's law for TLRC and MLRC models based on the use of individual regression equation was valid in the concentration range of 4–20 μg/mL for PAR, AA and ASP in the ternary mixture.

The ability and validation of TLRC and MLRC approaches were checked by the multiresolution of the synthetic mixtures containing various concentrations of subject three compounds. Results of the means, recoveries and the relative standard deviations of the methods were computed and indicated in Tables 3 and 4. We observed that both calibration models gave satisfactory results in the case of overlapping spectra of PAR, AA and ASP according to the recovery study. In the prediction step the standard error of calibrations (SEP) were found to be as 0.4760 for PAR, 0.6881 for AA and 0.1895 for ASP using TLRC and 0.3631 for PAR, 0.3275 for AA and 0.1107 for ASP using MLRC according to the difference between added and predicted concentrations. The SEP values of MLRC were obtained smaller than those obtained for TLRC. The SEP values indicate that the MLRC in determinations gave better performance than TLRC for the quantitative resolution of ternary-mixtures of compounds PAR, AA and ASP.

Moreover, TLRC and MLRC models were validated by the standard addition method. The mean percentage recoveries and their standard deviation for the TLRC and MLRC were found as $104.0\% \pm 2.5$, $99.1\% \pm 2.40$ for PAR and $97.3\% \pm 1.81$, $101.3\% \pm 2.3$ for AA, $98.5\% \pm 1.1$, $99.8\% \pm 0.85$ for ASP, respectively, for five replicates. The results also confirm the precision and accuracy of the proposed calibration methods and the excipients in tablets do not interfere with the analysis of the active compounds.

2.2.4. Analysis of effervescent tablets

The results obtained by applying the two mathematical approaches TLRC and MLRC to commercial effervescent tablets are shown in Table 5. A good coincidence was ob-

Table 5: Results obtained with pharmaceutical dosage forms by the developed calibration techniques

	mg/tablet					
	TLRC			MLRC		
Parameter	PAR	AA	ASP	PAR	AA	ASP
Mean:	211.1	312.3	307.2	203.5	299.9	306.8
Standard deviation	2.17	3.41	4.40	3.98	4.74	4.67
Relative standard deviation	1.03	1.06	1.43	1.96	1.58	1.52
Standard error	0.97	1.97	2.54	2.30	2.74	2.69
Confidencial limit (P = 0.005)	1.96	3.97	5.12	4.63	5.51	5.43

Label claim = 200 mg PAR, 300 mg AA and 300 mg ASP per tablet

served between the experimental results and the label claim of the commercial preparations. The numerical values of all the statistical parameters calculated (Table 5) are acceptable determination limit.

2.3. Conclusion

In this work, TLRC and MLRC models based on linear regression analysis were developed and applied to the quantitative multiresolution of ternary mixtures and effervescent tablets containing PAR, AA and ASP without any pre-treatment and a graphical procedure in the presence of very closely overlapping spectra. In fact, the traditional method for the simultaneous analysis of complex mixtures needs a priori separation step and chromatographic techniques presents relatively high costs and time consumption. TLRC and MLRC using special mathematical algorithms based on linear algebra can be considered suitable methods for a precise, accurate, rapid and less expensive determination of our compounds which is a clear advantage over other spectrophotometric methods in the quantitative resolution of ternary mixtures (Dinç 1999). The results also showed that these methods are powerful tools with very simple mathematical content while being more reliable than other spectrophotometric methods.

Finally, the mathematical TLRC and MLRC models can be applied to routine analysis in quality control of multicomponent mixtures and commercial pharmaceutical preparations containing the subject compounds.

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 and EXCEL softwares.

3.2. Commercial tablet formulation

A commercial effervescent tablet formulation (AFEBRYL® effervescent tablet produced by Laboratories SBM Farmaceutica N. V., Belgium, Batch no. B01) contains 200 mg > PAR, 300 mg AA and 300 mg ASP per tablet.

3.3. Standard solutions

PAR, AA and ASP were obtained from a Turkish Pharmaceutical Industrial firm. Stock solutions containing 50 mg/100 mL PAR, AA and ASP were prepared in 0.1 M HCl. A standard series of the solutions containing 4–20 μg/ mL PAR, AA and ASP was obtained from the stock solutions. A validation set consisting of 15 synthetic mixture solutions in the concentration range of 4–20 μg/mL PAR, AA and ASP was prepared by using the same stock solutions. All the solutions were prepared freshly and protected from light.

3.4. Analysis of effervescent tablets

Twenty effervescent tablets were weighted and powdered in a mortar. A tablet amount was transferred to a 100-ml calibrated flask and dissolved in 100 ml 0.1 M HCl and stirred until effervescence tablet particles were completely dissolved. After the dissolution process, the prepared solutions were filtered through a 0.2 μm disposable membrane filter (Sartorious, minisart, $\phi = 0.20$ μm) using an injector. The final solution was diluted to the working range befor application of the developed methods.

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