Mathematical Algorithms Applied to the Multi-linear Regression Functions for the Multicomponent Determination of Pharmaceutical Dosage Form Containing Three-component Mixtures

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In the presence of closely overlapping spectra, the quantitative multiresolution of ternary mixtures of three active compounds paracetamol (PAR), caffeine (CAF) and acetylsalycilic acid (ASP) in tablets, without using pretreatment such as separation step and graphical procedure of spectra was accomplished by the multivariate spectral calibration models, tri-linear regression calibration (TLRC), multi-linear regression calibration (MLRC) and Cramer's rule solution (CRS) of three linear equation functions in the matrix form. In the first two models, TLRC and MLRC are based on the use of the linear regression functions at selected wavelength sets in the spectral region of 210—300 nm. In the case of CRS model, A_i^1 (1%, 1 cm) were used to obtain three linear equation functions and this linear equation system was resolved by the Cramer's rule for the prediction of PAR, CAF and ASP in samples. In the TLRC and CRS models, the selection of the appropriate wavelength set was performed by the Kaiser's technique. The algorithms of these mathematical calibration models were briefly described. The validation of TLRC, MLRC and CRS models was carried out by analyzing various synthetic ternary mixtures and by using the standard addition technique. These three calibration approaches were applied to the analysis of the real pharmaceutical tablets containing PAR, CAF and ASP. The obtained results were statistically compared with each other by using experimental and statistical tests. In the comparison of TLRC and MLRC models to the classical approach, CRS technique, the successful assay results were observed for the quantitative multiresolution of ternary mixture of the subject active compounds.

Key words TLRC model; MLRC model; Cramer's rule; quantitative multiresolution; ternary mixture

Applied methods for the analytical problems have considerable importance in the rapidity, reliability and cost of method for the quantitative analysis of compounds. Most of the new method developments and instrumental advances are focus on the less time and money consumption. In pharmaceutical science the quantitation of the compounds in the mixtures has a big importance and there are several techniques for the quantitation of those compounds including as a well known technique HPLC, derivative spectrophotometry and chemometric techniques. Advances in spectrophotometric methods with various mathematical algorithms allow to a wide application of UV absorption in the drug analysis, biological sample analysis and environmental analysis. Spectrophotometric methods are a rapid and cheap method and suitable for the analysis of raw materials and finished products without sample preparation by the combination of numerical methods using software. Hence, applications of other analytical methods such as dual wavelength spectrophotometry,[1—3] pH-induced differential spectrophotometry[4] and multi-component analysis method aided computer software[5,6] can be given as an example for the analytical studies. All the motioned methods give good results, but they contain some disadvantage in the application to the analytical problems. For example HPLC is a laborious method that requires development of an acceptable method for separation and sometimes it is not possible to resolve the mixture of compounds, and some others contain abstract mathematical knowledge to understand the theory behind it. On the other hand while derivative spectrophotometry provides good results for the resolution of binary mixtures, it has not given successful results in the analysis of ternary or multi-mixtures.

Analytical chemists have been forcing the limit of every applicable mathematical methods ranging from very abstract to a simple one to apply them to solve the analytical problems. The simplest method will make the life easier and will provide faster analysis of compounds.

In recent years the simultaneous analysis of binary mixtures using a numerical method has been carried out.[7—9] The modification of this method was the first time applied to the analysis of ternary mixtures[10] and called as tri linear regression analysis (TLRC). This method also further modified the same method for the three and more component mixtures and called as multi linear regression analysis (MLRC). While TLRC method based on the finding of the best three wavelengths in the spectrum, MLRC method contains multi critical wavelength points in the working wavelength range and both use the linear algebra for matrix form obtained from the linear regression functions.

The quantitation of PAR, ASP and CAF in the same ternary mixture has been done using N-PLS chemometric method[11] but this method contains various disadvantage including complex and abstract mathematical treatments and special software. The proposed TLRC and MLRC models are very powerful tools having the simple mathematical content which can be applied in every laboratory for simultaneous quantitative multiresolution of complex mixtures containing two or more active compounds.

This paper describes the application of TLRC, MLRC and another alternative method CRS model to the simultaneous determination of PAR, CAF and ASP in the pharmaceutical tablets. Under optimization conditions the applied numerical

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methods provide considerable resolving power, sensitivity, rapidity, and low cost for the quantitative analysis, quality control and routine analysis of subject compounds in the pharmaceutical tablets.

Methodology The basic principles of TLRC and MLRC model can be explained starting from simple linear regression function. A linear regression function between two variables, concentration and absorbance, for the spectrophotometric determination of the *X* analyte at λ_i wavelength can be defined by the equation:

$$A_{X_i}=b_{X_i}C_X+a_{X_i} \tag{1}$$

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 μg/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.

TLRC Technique TLRC model requires the application of matrix mathematics to three linear regression equations at three wavelength points selected by Kaiser's technique.[12] 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 (λ_i=1, 2 and 3), the following equations can be written for a three-component analysis:

$$\begin{aligned}
A_{\text{mix}_1}&=b_{X_1}C_X+b_{Y_1}C_Y+b_{Z_1}C_Z+a_{XYZ_1}\\
A_{\text{mix}_2}&=b_{X_2}C_X+b_{Y_2}C_Y+b_{Z_2}C_Z+a_{XYZ_2}\\
A_{\text{mix}_3}&=b_{X_3}C_X+b_{Y_3}C_Y+b_{Z_3}C_Z+a_{XYZ_3}
\end{aligned} \tag{2}$$

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; a_{XYZ_1} and 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 $a_{XYZ_3}=a_{X_3}+a_{Y_3}+a_{Z_3}$).

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

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

If the absorbance matrix, $A_{\text{mix}_{1,2\text{ and }3}}$, and the intercept matrix, $a_{XYZ_{1,2\text{ and }3}}$ are matrices in the same size, then the difference $A_{\text{mix}}-a_{XYZ}$ 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:

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

or, more simply:

$$(A_{\text{mix}}-a_{XYZ})_{3\times1}=K_{3\times3}\cdot C_{3\times1} \tag{5}$$

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

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

In this case, for the calculation of the concentration of the analytes, *X*, *Y* and *Z* in ternary mixture, the matrix, $(A_{\text{mix}}-a_{XYZ})_{3\times1}$, is multiplied by the inverse $(K^{-1})_{3\times3}$ of the matrix $K_{3\times3}$ and it can be written as:

$$C_{3\times1}=(K^{-1})_{3\times3}\cdot(A_{\text{mix}}-a_{XYZ})_{3\times1} \tag{7}$$

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

As explained here, the proposed 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 technique[12] was applied to the selection of the optimum wavelength set in order to provide the best sensitivity and selectivity in the application of the mathematical 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 functions of the individual analytes, *X*, *Y* and *Z* at three selected wavelengths (1, 2 and 3) are considered as the sensitivity parameter.[8—10] 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 nine linear regression functions having three linear regression lines for each compound at three selected wavelengths in our case.

MLRC Technique MLRC algorithm[10] for the quantitative analysis of ternary or multi mixtures is based on the application of linear algebra to linear regression function at a multipoint set of selected wavelengths in the working spectral range. 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 (λ_i=1, 2, …, *n*), the following set of functions can be written for a three-component analysis:

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

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); $b_{X_1}, b_{X_2}, \ldots, b_{X_n}$, $b_{Y_1}, b_{Y_2}, \ldots, b_{Y_N}$ and $b_{Z_1}, b_{Z_2}, \ldots, b_{Z_n}$ are the slopes of *n* linear regression functions 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 functions 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 (8) can be formulated as:

$$\begin{bmatrix} A_{mix_1} \\ A_{mix_2} \\ \cdots \\ \cdots \\ A_{mix_n} \end{bmatrix} = \begin{bmatrix} b_{X_1} b_{Y_1} b_{Z_1} \\ b_{X_2} b_{Y_2} b_{Z_2} \\ \cdots \cdots \cdots \\ \cdots \cdots \cdots \\ b_{X_n} b_{Y_n} b_{Z_n} \end{bmatrix} \cdot \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} + \begin{bmatrix} a_{XYZ_1} \\ a_{XYZ_2} \\ \cdots \\ \cdots \\ a_{XYZ_n} \end{bmatrix} \tag{9}$$

which can be simplified to

$$\begin{bmatrix} A_{mix_1} - a_{XYZ_1} \\ A_{mix_2} - a_{XYZ_2} \\ \cdots \quad \cdots \\ \cdots \quad \cdots \\ A_{mix_n} - a_{XYZ_n} \end{bmatrix} = \begin{bmatrix} b_{X_1} b_{Y_1} b_{Z_1} \\ b_{X_2} b_{Y_2} b_{Z_2} \\ \cdots \cdots \cdots \\ \cdots \cdots \cdots \\ b_{X_n} b_{Y_n} b_{Z_n} \end{bmatrix} \cdot \begin{bmatrix} C_X \\ C_Y \\ C_Z \end{bmatrix} \tag{10}$$

in a compact form

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

As explained in the above TLRC model. The matrix of the slope values is called the matrix K:

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

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

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

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

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

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 multi-component mixture system containing n compounds.

Cramer's Rule Solution Absorptivity A^1_1 (1%, 1 cm) values were calculated by using the absorbances measured at 229 nm (λ_1), 248 nm (λ_2) and 272 nm (λ_3) for zero-order spectra for each of the compounds in the ternary mixture. By using A^1_1 values, a system of equations with three unknowns can be written for three compounds in the ternary mixture, as follows:

$$\begin{aligned} &A = \alpha l C \quad \text{(path length } l \text{ is equal to 1)} \\ &A_1 = \alpha_1 C_1 + \beta_1 C_2 + \gamma_1 C_3 \\ &A_2 = \alpha_2 C_1 + \beta_2 C_2 + \gamma_2 C_3 \\ &A_3 = \alpha_3 C_1 + \beta_3 C_2 + \gamma_3 C_3 \end{aligned} \tag{15}$$

where A_1, A_2 and A_3 denotes the absorbances of solutions of mixtures of PAR, ASP and CAF, and α, β and γ represent the values of A^1_1 values calculated for PAR, ASP and CAF, respectively, at λ_1, λ_2 and λ_3. C_1, C_2 and C_3 are the concentrations of PAR, ASP and CAF, respectively, in g/100 ml. The subscripts 1, 2 and 3 refer to λ_1 (229 nm), λ_2 (248 nm) and λ_3 (272 nm), respectively.

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

$$\begin{vmatrix} A_1 \\ A_2 \\ A_3 \end{vmatrix} = \begin{vmatrix} \alpha_1 & \beta_1 & \gamma_1 \\ \alpha_2 & \beta_2 & \gamma_2 \\ \alpha_3 & \beta_3 & \gamma_3 \end{vmatrix} \cdot \begin{vmatrix} C_1 \\ C_2 \\ C_3 \end{vmatrix} \quad \text{or} \quad A = E \cdot C \tag{16}$$

This matrix can be easily solved by means of Cramer's rule and the concentration of each active compound in the ternary mixture was determined by solving the following operations:

$$C_1 = \frac{\begin{vmatrix} A_1 & \beta_1 & \gamma_1 \\ A_2 & \beta_2 & \gamma_2 \\ A_3 & \beta_3 & \gamma_3 \end{vmatrix}}{\begin{vmatrix} \alpha_1 & \beta_1 & \gamma_1 \\ \alpha_2 & \beta_2 & \gamma_2 \\ \alpha_3 & \beta_3 & \gamma_3 \end{vmatrix}} \tag{17}$$

The concentration of the other active compounds, C_2 and C_3, are computed in the same way.

Experimental

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 were used to record the absorption spectra. The application of Kaiser's technique, the regression and statistical analysis were achieved by using the *MATLAP* 6.5 and *EXCEL* softwares.

Commercial Tablet Formulation A commercial tablet formulation (EXCEDRINE® MIGRANE coated tablets produced by Bristol-Myers Squibb Co., U.S.A., and Batch no. 306532) contains 250 mg PAR, 250 mg ASP and 65 mg CAF per tablet. The pharmaceutical tablets of their active compounds were analyzed using the multivariate spectral calibration models.

PAR, ASP and CAF were kindly donated from Turkish Pharmaceutical Industrial firms.

Standard Solutions Stock solutions containing 50 mg/100 ml PAR, ASP and CAF were prepared in 0.1 M HCl. A standard series of the solutions containing 5—25 μg/ml for PAR, ASP and 4—28 μg/ml for CAF was obtained from the stock solutions. A validation set consisting of 15 synthetic mixture solutions in the concentration range of 5—20 μg/ml PAR, ASP and 4—28 μg/ml CAF was prepared by using the same stock solutions. All the solutions were prepared freshly and protected from light.

Analysis of Tablet Twenty 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. After dissolution process prepared solutions were filtered with 0.2 μm disposable membrane filter (Sartorious, minisart, ϕ=0.20 μm) by using an injector. The final solution was diluted to the working concentration range for the application of the three optimized mathematical calibration models.

Result and Discussion

Figure 1 shows the absorption spectra of three active compounds PAR, CAF and ASP ranging from 210 to 300 nm. The spectra are strongly overlapped in this spectral range and this makes impossible the quantitative analysis of these three compounds using the classical analytical methods based on the regular absorption measurements. In this study two mathematical models TLRC and MLRC were utilized for the multi mixture analysis and third one (GRS) used as an alternative method.

For the construction of TLRC and MLRC methods the standard series of solutions were prepared in the concentration range of 4—28 μg/ml for CAF and 5—25 μg/ml for PAR and ASP in 0.1 M HCl. The absorption spectra of prepared solutions were recorded over the wavelength range of 210—300 nm and then used for the construction of TLRC and MLRC. The construction of models based on the use of the linear regression functions was done by applying simple

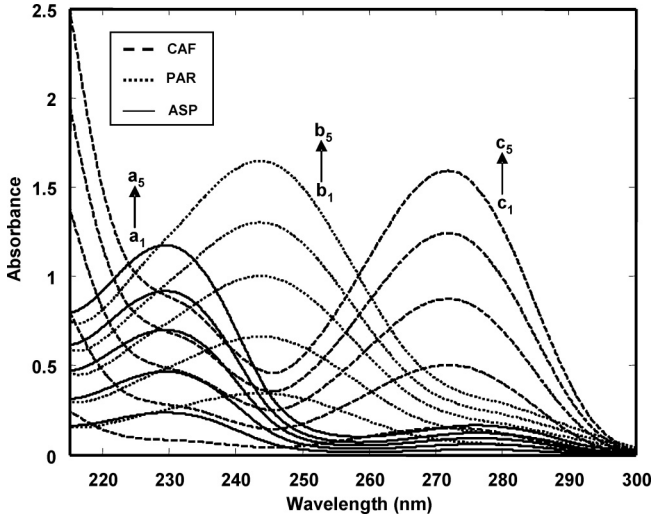

Fig. 1. Absorption Spectra of PAR (·····), ASP (——) in the Concentration Range of (a_1=b_1) 5 μg/ml, (a_2=b_2) 10 μg/ml, (a_3=b_3) 15 μg/ml, (a_4=b_4) 20 μg/ml, and (a_5=b_5) 25 μg/ml, and CAF (- - - - -) in the Concentration of (c_1) 4 μg/ml, (c_2) 10 μg/ml, (c_3) 16 μg/ml, (c_4) 22 μg/ml and (c_5) 28 μg/ml in 0.1 M HCl

mathematical algorithms given in the methodology section. The validation of these methods was carried out by analyzing the synthetic mixture solutions of PAR, CAF and ASP prepared in the same concentration range with the standard series.

TLRC Technique This method based on the selection of critical wavelengths in the working spectral range of three active compounds. For three compounds, eighteen wavelength points were selected and each wavelength point was represented by a linear regression function. The calculated linear regression functions and their statistical results were shown in Table 1. The obtained results were reliable for the construction of TLRC model.

The sensitivity matrices indicated in Table 2 were created by the slope values of the linear regression functions for each compound in the ternary mixture of PAR, CAF and ASP. According to the wavelength selection technique of Kaiser,[13] the best sensitivity for the construction of TLRC model was obtained from the absolute values of the determinant of the sensitivity matrices. In this treatment, different 816 three-pairs of the sensitivity matrices were possible for the selection of optimum three-wavelength set. This result was calculated by using the following formula (18):

$$C_n^p = \frac{p!}{(p-n)!n!} \tag{18}$$

where C_n^p is the number of three pair of sensitivity matrices, p is the number of wavelengths and n is the number of components. The results of the computing process corresponding to the determinant value of sensitivity matrices in different 816 three-pair combinations contain 3-dimensional structure as shown in Fig. 2. An optimum three-wavelength set having the highest determinant value of the sensitivity matrices which correspond to six maximum was found as 229, 248 and 272 nm for the TLRC model. The individual linear regression functions for each compound at this selected three-wavelength set were presented in Table 1. The following sets of functions were created for the TLRC technique.

Table 1. Linear Regression Analysis and Its Statistical Results at Eighteen Wavelengths

λ	ASP					CAF					PAR				
	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)
222	A=0.0396C −0.0020	0.9996	0.0101	0.0006	0.0096	A=0.0468C −0.0753	1.0000	0.0045	0.0002	0.0047	A=0.0353C −0.0062	0.9998	0.0065	0.0004	0.0062
224	A=0.0426C −0.0020	0.9997	0.0101	0.0006	0.0096	A=0.0405C −0.0658	1.0000	0.0036	0.0002	0.0038	A=0.0387C −0.0076	0.9998	0.0067	0.0004	0.0064
226	A=0.0449C −0.0016	0.9997	0.0102	0.0006	0.0098	A=0.0370C −0.0605	1.0000	0.0029	0.0002	0.0030	A=0.0421C −0.0085	0.9998	0.0071	0.0004	0.0067
229	A=0.0466C −0.0010	0.9997	0.0104	0.0006	0.0099	A=0.0342C −0.0562	1.0000	0.0024	0.0001	0.0025	A=0.0470C −0.0102	0.9998	0.0088	0.0005	0.0084
232	A=0.0455C −0.0011	0.9997	0.0099	0.0006	0.0095	A=0.0321C −0.0528	1.0000	0.0019	0.0001	0.0020	A=0.0516C −0.0125	0.9998	0.0095	0.0006	0.0090
235	A=0.0411C −0.0015	0.9997	0.0088	0.0005	0.0084	A=0.0289C −0.0469	1.0000	0.0019	0.0001	0.0020	A=0.0566C −0.0143	0.9998	0.0101	0.0006	0.0096
240	A=0.0280C −0.0041	0.9996	0.0075	0.0005	0.0071	A=0.0217C −0.0359	1.0000	0.0010	0.0001	0.0011	A=0.0631C −0.0170	0.9998	0.0114	0.0007	0.0109
244	A=0.0161C −0.0058	0.9993	0.0057	0.0003	0.0054	A=0.0178C −0.0300	1.0000	0.0007	0.0000	0.0007	A=0.0649C −0.0177	0.9998	0.0120	0.0007	0.0114
248	A=0.0086C −0.0078	0.9975	0.0059	0.0004	0.0056	A=0.0186C −0.0317	1.0000	0.0008	0.0000	0.0009	A=0.0620C −0.0177	0.9998	0.0110	0.0007	0.0105
251	A=0.0060C −0.0083	0.9953	0.0056	0.0003	0.0053	A=0.0225C −0.0376	1.0000	0.0016	0.0001	0.0016	A=0.0572C −0.0165	0.9998	0.0100	0.0006	0.0096
254	A=0.0047C −0.0083	0.9933	0.0052	0.0003	0.0050	A=0.0281C −0.0457	1.0000	0.0022	0.0001	0.0023	A=0.0506C −0.0154	0.9998	0.0087	0.0005	0.0083
258	A=0.0041C −0.0083	0.9921	0.0050	0.0003	0.0048	A=0.0370C −0.0594	1.0000	0.0033	0.0002	0.0034	A=0.0406C −0.0111	0.9999	0.0065	0.0004	0.0062
262	A=0.0044C −0.0083	0.9935	0.0048	0.0003	0.0045	A=0.0466C −0.0747	1.0000	0.0048	0.0003	0.0051	A=0.0307C −0.0065	0.9999	0.0050	0.0003	0.0048
266	A=0.0051C −0.0081	0.9956	0.0046	0.0003	0.0044	A=0.0550C −0.0865	0.9999	0.0065	0.0004	0.0068	A=0.0223C −0.0034	0.9999	0.0032	0.0002	0.0030
270	A=0.0060C −0.0080	0.9969	0.0045	0.0003	0.0043	A=0.0598C −0.0936	0.9999	0.0064	0.0004	0.0067	A=0.0169C −0.0013	0.9998	0.0029	0.0002	0.0027
272	A=0.0063C −0.0078	0.9973	0.0045	0.0003	0.0043	A=0.0603C −0.0937	0.9999	0.0069	0.0004	0.0072	A=0.0152C −0.0008	0.9999	0.0024	0.0001	0.0023
276	A=0.0067C −0.0075	0.9977	0.0043	0.0003	0.0041	A=0.0570C −0.0885	0.9999	0.0066	0.0004	0.0069	A=0.0129C −0.0000	0.9999	0.0018	0.0001	0.0018
280	A=0.0063C −0.0074	0.9977	0.0041	0.0002	0.0039	A=0.0482C −0.0763	0.9999	0.0056	0.0003	0.0059	A=0.0115C −0.0006	0.9999	0.0016	0.0001	0.0015

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

Table 2. The Obtained Sensitivity Values of ASP, CAF and PAR Using Single-Component Regression Analysis at Eighteen Wavelengths

Wavelength (nm)	$B_{ASP}\times10^{-3}$	$B_{CAF}\times10^{-3}$	$B_{PAR}\times10^{-3}$
222	39.6	46.8	35.3
224	42.6	40.5	38.7
226	44.9	37.0	42.1
229	46.6	34.2	47.0
232	45.5	32.1	51.6
235	41.1	28.9	56.6
240	28.0	21.7	63.1
244	16.1	17.8	64.9
248	8.6	18.6	62.0
251	6.0	22.5	57.2
254	4.7	28.1	50.6
258	4.1	37.0	40.6
262	4.4	46.6	30.7
266	5.1	55.0	22.3
270	6.0	59.8	16.9
272	6.3	60.3	15.2
276	6.7	57.0	12.9
280	6.3	48.2	11.5

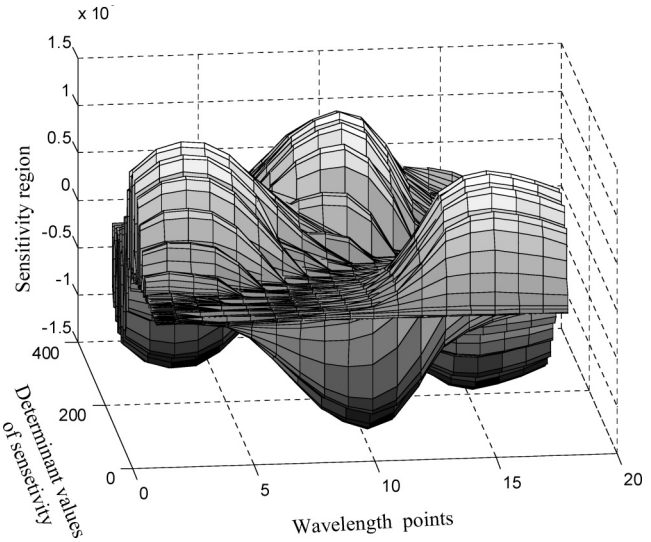

Fig. 2. An Optimum Three-Wavelength Set Corresponding to Six Highest Determinant Values Obtained from 816 Sensitivity Matrices in 3-Dimensional Space by Using the Kaiser's Technique

Six maximum indicates the negative and positive determinant values of sensitivity matrices which correspond to the wavelengths 229, 248 and 272.

$$\begin{aligned}
&\lambda_{229}, A_{mix_1}=0.0466\cdot C_{ASP}+0.0470\cdot C_{PAR}+0.0342\cdot C_{CAF}-0.0470\\
&\lambda_{248}, A_{mix_3}=0.0086\cdot C_{ASP}+0.0620\cdot C_{PAR}+0.0186\cdot C_{CAF}-0.0218\\
&\lambda_{272}, A_{mix_3}=0.0063\cdot C_{ASP}+0.0152\cdot C_{PAR}+0.0603\cdot C_{CAF}-0.1007
\end{aligned} \quad (19)$$

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

MLRC Technique The MLRC algorithm using linear algebra contains its application to *n*-linear regression functions at *n*-wavelength set in the working spectral range. This MLRC approach is analogous to TLRC model, but MLRC uses *n*-wavelength procedure instead of three-wavelengths. The MLRC does not require the Kaiser's technique for the selection of wavelength set. In our case an eighteen-wavelength set was chosen for the linear regression analysis between each compound's standard series and its absorbance values. For this procedure, the eighteen-wavelengths set (222, 224, 226, 229, 232, 235, 240, 244, 248, 251, 254, 258, 262, 266, 270, 272, 276, 280 nm) at the critical points, which correspond to the maximum, shoulder and minimum in the spectral range 210—300 nm were selected for the construction of the linear regression of PAR, CAF and ASP in the ternary mixture. Eighteen linear regression functions for each compound were obtained by measuring the absorbance values at this eighteen-wavelengths set (see Table 1). The set of functions (20) obtained from Table 1 were arranged as:

$$\begin{aligned}
&\lambda_{222}, A_{mix_1}=0.0396\cdot C_{ASP}+0.0353\cdot C_{PAR}+0.0468\cdot C_{CAF}-0.0711\\
&\lambda_{224}, A_{mix_2}=0.0426\cdot C_{ASP}+0.0387\cdot C_{PAR}+0.0405\cdot C_{CAF}-0.0602\\
&\lambda_{226}, A_{mix_3}=0.0449\cdot C_{ASP}+0.0421\cdot C_{PAR}+0.0370\cdot C_{CAF}-0.0535\\
&\lambda_{229}, A_{mix_4}=0.0466\cdot C_{ASP}+0.0470\cdot C_{PAR}+0.0342\cdot C_{CAF}-0.0470\\
&\lambda_{232}, A_{mix_5}=0.0455\cdot C_{ASP}+0.0516\cdot C_{PAR}+0.0321\cdot C_{CAF}-0.0414\\
&\lambda_{235}, A_{mix_6}=0.0411\cdot C_{ASP}+0.0566\cdot C_{PAR}+0.0289\cdot C_{CAF}-0.0342\\
&\lambda_{240}, A_{mix_7}=0.0280\cdot C_{ASP}+0.0631\cdot C_{PAR}+0.0217\cdot C_{CAF}-0.0231\\
&\lambda_{244}, A_{mix_8}=0.0161\cdot C_{ASP}+0.0649\cdot C_{PAR}+0.0178\cdot C_{CAF}-0.0182\\
&\lambda_{248}, A_{mix_9}=0.0086\cdot C_{ASP}+0.0620\cdot C_{PAR}+0.0186\cdot C_{CAF}-0.0218\\
&\lambda_{251}, A_{mix_{10}}=0.0060\cdot C_{ASP}+0.0572\cdot C_{PAR}+0.0225\cdot C_{CAF}-0.0295\\
&\lambda_{254}, A_{mix_{11}}=0.0047\cdot C_{ASP}+0.0506\cdot C_{PAR}+0.0281\cdot C_{CAF}-0.0386\\
&\lambda_{258}, A_{mix_{12}}=0.0041\cdot C_{ASP}+0.0406\cdot C_{PAR}+0.0370\cdot C_{CAF}-0.0567\\
&\lambda_{262}, A_{mix_{13}}=0.0044\cdot C_{ASP}+0.0307\cdot C_{PAR}+0.0466\cdot C_{CAF}-0.0765\\
&\lambda_{266}, A_{mix_{14}}=0.0051\cdot C_{ASP}+0.0223\cdot C_{PAR}+0.0550\cdot C_{CAF}-0.0912\\
&\lambda_{270}, A_{mix_{15}}=0.0060\cdot C_{ASP}+0.0169\cdot C_{PAR}+0.0598\cdot C_{CAF}-0.1003\\
&\lambda_{272}, A_{mix_{16}}=0.0063\cdot C_{ASP}+0.0152\cdot C_{PAR}+0.0603\cdot C_{CAF}-0.1007\\
&\lambda_{276}, A_{mix_{17}}=0.0067\cdot C_{ASP}+0.0129\cdot C_{PAR}+0.0570\cdot C_{CAF}-0.0960\\
&\lambda_{280}, A_{mix_{18}}=0.0063\cdot C_{ASP}+0.0115\cdot C_{PAR}+0.0482\cdot C_{CAF}-0.0842
\end{aligned} \quad (20)$$

The MLRC algorithm described in previous section was applied to the above function set and the obtained MLRC model was used for the quantitative multiresolution of PAR, CAF and ASP in ternary mixtures and tablets.

Cramer's Rule Solution Technique As in the TLRC model, this calibration model was constructed by using the absorptivity A_1^1 (1%, 1 cm) values corresponding to the calculated maximum determinant values at three wavelength set for 229, 248 and 272 nm. In the region of 210—300 nm, an optimum three wavelength set of the absorption spectra of three active compounds are λ_1 (229) for PAR, λ_2 (248) for ASP and λ_3 (272) for CAF. By using the matrix calculation with Cramer's rule technique, the determination of the three compounds is possible for direct measurements of absorbances at 229, 248 and 272 nm in the zero-order spectra. In the previous section the matrix resolution has been explained and the parameters are shown in Table 3. As it is seen in the calculations, Beer's Law was valid in the concentration range 4—28 μg/ml for CAF, 5—25 μg/ml for PAR and 5—25 μg/ml for ASP. The validation of this technique was performed by the analysis of the synthetic mixtures prepared by mixing known amounts of active compounds. This was explained in detail in the following sub-section.

Validation of the Optimized Calibration Models The TLRC and MLRC and CRS models based on the use of individual regression functions obeyed Beer's Law was valid in the concentration range of 4—28 μg/ml for CAF and 5—25 μg/ml for ASP and PAR in the ternary mixture.

The validation of TLRC, MLRC and CRS approaches

Table 3. Absorptivity A_1^1 (%1, 1 cm) Values Calculated for PAR, ASP and CAF at the Optimum Three-Wavelength Set Determined by Kaiser's Technique

λ (nm)	ASP			CAF			PAR		
	α_1	α_2	α_3	β_1	β_2	β_3	γ_1	γ_2	γ_3
λ_1=229	465.42	—	—	287.09	—	—	479.30	—	—
λ_2=248	—	80.10	—	—	155.17	—	—	636.66	—
λ_3=272	—	—	56.81	—	—	511.39	—	—	152.55
Linearity range		5—25			4—28			5—25	

Table 4. Recoveries Obtained for the Determination of PAR, CAF and ASP in Different Synthetic Mixtures by Proposed Mathematical Calibration Techniques

	Added (μg/ml)			Recovery (%)								
				TLRC			MLRC			CRS		
No:	CAF	PAR	ASP	PAR	ASP	CAF	PAR	ASP	CAF	PAR	ASP	CAF
1	5.2	5.0	20.0	98.5	97.5	97.9	98.7	97.4	97.4	97.9	103.5	101.7
2	5.2	10.0	20.0	99.2	99.5	99.7	99.4	99.4	99.5	98.7	102.6	100.2
3	5.2	15.0	20.0	100.2	100.9	100.9	100.5	100.9	100.8	99.7	103.1	100.2
4	5.2	20.0	20.0	99.3	103.6	99.4	99.5	100.6	99.1	99.0	102.4	98.2
5	5.2	25.0	20.0	99.3	101.0	100.2	99.5	101.1	100.2	99.1	102.5	98.7
6	5.2	20.0	5.0	97.6	102.1	100.4	98.5	102.9	100.2	100.6	105.8	98.6
7	5.2	20.0	10.0	98.9	103.4	99.8	99.6	100.0	99.6	99.7	105.8	98.2
8	5.2	20.0	15.0	99.3	99.7	99.9	99.8	103.8	99.7	99.4	105.1	98.5
9	5.2	20.0	20.0	99.1	98.5	99.6	99.2	98.6	99.5	98.8	102.8	98.4
10	5.2	20.0	25.0	99.4	98.5	99.4	99.2	98.0	99.4	98.9	105.4	98.4
11	4.0	20.0	20.0	99.0	102.7	99.7	99.5	100.3	99.5	98.7	105.9	98.5
12	10.0	20.0	20.0	98.7	100.2	99.1	98.9	100.3	99.0	98.5	101.4	98.0
13	16.0	20.0	20.0	98.2	101.3	100.0	98.9	101.3	99.8	98.1	101.5	99.0
14	22.0	20.0	20.0	99.8	98.4	100.2	99.4	98.4	100.2	99.8	100.8	99.3
15	28.0	20.0	20.0	97.4	98.3	100.0	98.1	98.4	99.9	97.5	101.4	99.3
			Mean:	98.9	100.4	99.7	99.2	100.1	99.6	98.9	103.3	99.0
			RSD	0.76	1.97	0.69	0.6	1.8	0.8	0.8	1.7	1.0

SD=standard deviation. RSD=relative standard deviation.

were checked by the quantitative analysis of the synthetic mixtures containing various concentrations of subject three compounds. Results of the means, recoveries and the relative standard deviations of the mathematical calibration models were computed and indicated in Table 4. The results showed that the calibration models gave satisfactory results in the case of overlapping spectra of PAR, CAF and ASP according to the recovery study.

In the prediction step the standard error of calibrations (SEP) were found to be as 0.2306 for ASP, 0.1917 for CAF and 0.0917 for PAR using TLRC, 0.1793 for ASP, 0.1844 for CAF and 0.1074 for PAR using MLRC and 02736 for ASP, 02360 for CAF and 02762 for PAR using CRS according to the difference between added and predicted concentrations. The SEP values of MLRC were obtained smaller than those obtained for TLRC and CRS. The SEP values indicate that the MLRC in determinations gave better performance than TLRC for the quantitative resolution of ternary-mixtures of active compounds ASP, CAF and PAR.

For the analytical validation of the proposed numerical calibration models, TLRC, MLRC and GRS, the standard addition method was applied. The findings of methods showed that three methods give precise and reliable results. In application of standard addition technique to tablets, the statistical results for the TLRC, MLRC and CRS were calculated and indicated in Table 5 for six replicate. The results also confirm the precision and accuracy of the proposed mathematical calibration models and the excipients in tablets do not interfere in the analysis of the active compounds.

In the standard addition technique, we applied a one-way ANOVA test to the experimental results of three calibration models as shown in Table 6. The statistical results with %95 of confidential limit indicate that there is no significant difference among all the proposed mathematical models in respect to tabulated values (critical).

Tablet Analysis Tablet analysis by applying the TLRC, MLRC and CRS methods were conducted and the obtained results were shown in Table 7. The experimental results and label claim of tablets showed good coincidence. The numerical values of all statistical parameters calculated in Table 8 are acceptable determination limits in application of two methods and validation method CRS to the tablets.

The statistical results obtained by comparing TLRC, MLRC and CRS in the quantitative analysis of tablets were summarized in Tables 7 and 8. In this comparison of proposed mathematical approaches, *t*-, *F*- and one-way ANOVA tests were applied to the assay results. The results with %95 of confidential limit indicate that there is no significant difference between three multivariate models in respect to tabulated values (critical).

Table 5. Results of Standard Addition Method Applied to Commercial Tablet Preparation by the Proposed Calibration Techniques

Calibration technique	mg/tablet								
	TLRC			MLRC			CRS		
Parameter	ASP	CAF	PAR	ASP	CAF	PAR	ASP	CAF	PAR
Mean:	250.1	63.9	244.8	249.1	63.8	245.5	248.3	66.7	244.6
Standard deviation	0.50	0.32	0.70	0.88	0.93	0.80	0.50	1.19	1.94
Relative standard deviation	0.20	0.50	0.28	0.35	1.45	0.33	0.20	1.79	0.79
Standard error	0.20	0.13	0.28	0.36	0.38	0.33	0.20	0.49	0.79
Confidential limit (P=0.005)	0.40	0.26	0.56	0.70	0.74	0.64	0.40	0.96	1.55

Label claim (mg): 250 mg PAR, 250 mg ASP and 65 mg CAF per tablet. Results obtained are average of 6 replicate for each method. SE=standard error, CL=confidential limit.

Table 6. The Analysis of Variance (ANOVA) in Application of Three Methods to Commercial Pharmaceutical Preparation

Source of variation	SS			df	MS			*F* test			*F* crit
	PAR	CAF	ASP		PAR	CAF	ASP	PAR	CAF	ASP	
Between groups	2.62	6.20	1.039	2	1.31	3.10	0.52	2.03	3.15	0.38	3.68
Within groups	9.70	14.76	20.34	15	0.65	0.98	1.36				
Total	12.33	20.96	21.38	17							

SS: sum of squares, df: degree of freedom, MS: mean squares.

Table 7. Results Obtained in the Pharmaceutical Dosage Forms by the Proposed Calibration Techniques

Calibration technique	mg/tablet								
	TLRC			MLRC			CRS		
Parameter	ASP	CAF	PAR	ASP	CAF	PAR	ASP	CAF	PAR
Mean	251.4	64.6	246.0	252.1	64.4	242.8	250.5	66.8	245.4
Standard deviation	2.38	1.43	2.06	3.56	0.72	3.73	2.69	0.91	1.81
Relative standard deviation	0.95	2.21	0.84	1.41	1.12	1.54	1.07	1.36	0.74
Standard error	0.79	0.48	0.69	1.19	0.24	1.24	0.90	0.30	0.60
Confidential limit (P=0.05)	1.55	0.93	1.35	2.33	0.47	2.44	1.76	0.59	1.18
t-test (P=0.05)	0.50	0.50	0.02	0.54	0.37	0.01	2.30 (*t*-critical value)		
F-test	1.29	2.01	1.28	1.49	1.94	1.75	3.44 (*F*-critical value)		

Table 8. The Analysis of Variance (ANOVA) in Application of three Methods to Commercial Pharmaceutical Preparation

Source of variation	SS			df	MS			*F*			*F* crit
	PAR	CAF	ASP		PAR	CAF	ASP	PAR	CAF	ASP	
Between groups	11.38	1.24	1.822	2	5.69	0.62	0.91	0.7	0.52	0.28	3.4
Within groups	204.3	28.6	77.91	24	8.51	1.19	3.25				
Total	215.7	29.8	79.73	26							

Conclusions

The TLRC and MLRC models based on the linear regression functions were optimized and applied to the quantitative multiresolution of ternary mixtures containing PAR, ASP and CAF without any pre-treatment and graphical procedure in the presence of very closely overlapped spectra. Another alternative calibration technique CRS was also subjected to the simultaneous analysis of the same ternary mixture analysis. The present traditional methods require a priori separation step as chromatographic method that brings high cost and time consumption for the analysis of tablets. The application of TLRC, MLRC and CRS using special mathematical algorithms based on linear algebra can be considered suitable methods for a precise, accurate, rapid and less expensive determination of subject three compounds in samples. This can be considered as an advantage of new mathematical calibration models TLRC, MLRC and CRS techniques over other spectrophotometric methods for the quantitative resolution of ternary mixtures. Consequently, TLRC, MLRC and CRS models can be applied to the routine analysis, quality control of multi-component mixtures and commercial pharmaceutical preparation containing the subject compounds.

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