15

Multivariate Data Processing in Spectrophotometric Analysis of Complex Chemical Systems

Zoltan Szabadai[2], Vicenţiu Vlaia[2], Ioan Ţăranu[1],
Bogdan-Ovidiu Ţăranu[1], Lavinia Vlaia[2] and Iuliana Popa[1]
[1]*National Institute of Research-Development for Electrochemistry and Condensed Matter,*
[2]*University of Medicine and Pharmacy "Victor Babeş",*
Romania

1. Introduction

There are a great variety of processing the analytical spectroscopy data, especially useful in multicomponent systems [Ewing et al., 1953; Garrido et al., 2004; Lykkesfeld, 2001; Oka et al., 1991; Sánchez & Kowalski, 1986]. These methods essentially are based on different strategies of mathematical strategies including specific formalism of mathematical statistics and of matrix algebra [Garrido et al., 2004; Szabadai, 2005]. The matrix-based methods reffer to quantitative analysis [Bosch-Reigh et al., 1991; Garrido et al., 2008; Li et al., 2011; Lozano et al., 2009; Ruckenbusch et al., 2006; Szabadai, 2005], to determination of the number of independent chemical equilibria in multicomponent systems [Szabadai, 2005] and for correction the action of various perturbing factors such as stray light or backgroud absorption [Burnius, 1959; Fox & Mueller, 1950; Melnick, 1952; Morton & Stubbs, 1946, 1947, 1948; Owen, 1995; Page & Berkovitz, 1943; Szabadai, 2005].

In the present chapter original approaches of matrix treatment of the aforementioned items are presented, with special consideration to the simultaneous assay of compounds in a mixter, to backgruond correction procedures and to the standard addition method in a generalized form.

2. Simultaneous assay of nonreacting compunds in a mixture

The issue of the quantitative analysis of a mixture, when the components do not interact chemically, can be approached, in a rigorous and general manner, with the help of matrix computation [Ewing et al., 1953; Garrido et al., 2004, 2008; Lozano et al., 2009; Lykkesfeld, 2001; Oka et al., 1991; Ruckenbusch et al., 2006; Sánchez & Kowalski, 1986; Szabadai, 2005]. In the case of a mixture with M component, the quantitative determination of the components, one has to measure the absorbance at Λ distinct values of wavelength ($\Lambda > M$). Given a set of N standard solutions ($N > M$ and supposing that, as a rule, each standard solution may contain all of M chemical components of interest in known concentrations), absorbances are to be measured at the same set of wavelengths and in identical conditions as done for standard solutions.

The following notations will be used in what follows: $X_m^n(\lambda)$ represents a quantity X referring to the standard mixture of number n (superscript index), at the individual chemical component of number m (subscript index), measured at the wavelength of number λ (between parentheses). Thus,

c_m^n represents the concentration of the component of number m in the standard solution of number "n" ;
c_m represents the concentration of the component of number m in the mixture undergoing the analysis (sample of unknown composition) ;
$A^n(\lambda)$ is the absorbance of the standard mixture of number m measured at the wavelength of number "λ" ;
$A_m(\lambda)$ is the contribution of the pure m-numbered component to the absorbance of the analyzed mixture, registered at the wavelength λ ;
$A(\lambda)$ is the absorbance of the mixture under analysis, measured at the wavelength of number λ ;
$\varepsilon_m(\lambda)$ is the molar absorptivity of the chemical component of number "m", measured at the wavelength of number λ ;
p^n is the weight percent of the spectrum of the standard solution of number n in the spectrum of the mixture under analysis.

If the components of a mixture do not interact chemically and if the absorbances of each component satisfies the Bouguer-Lambert-Beer relation, then the absorbance of the mixture, at each wavelength taken into account, consists of the sum of contributions of the individual absorbent chemical components. The absorbances of the N standard solutions, measured at Λ distinct values of wavelength, may be arranged in matrix form (1).

$$\begin{bmatrix} A^1(1) & \cdots & A^n(1) & \cdots & A^N(1) \\ \vdots & & \vdots & & \vdots \\ A^1(\lambda) & \cdots & A^n(\lambda) & \cdots & A^N(\lambda) \\ \vdots & & \vdots & & \vdots \\ A^1(\Lambda) & \cdots & A^n(\Lambda) & \cdots & A^N(\Lambda) \end{bmatrix} = d \cdot \begin{bmatrix} \varepsilon_1(1) & \cdots & \varepsilon_m(1) & \cdots & \varepsilon_M(1) \\ \vdots & & \vdots & & \vdots \\ \varepsilon_1(\lambda) & \cdots & \varepsilon_m(\lambda) & \cdots & \varepsilon_M(\lambda) \\ \vdots & & \vdots & & \vdots \\ \varepsilon_1(\Lambda) & \cdots & \varepsilon_m(\Lambda) & \cdots & \varepsilon_M(\Lambda) \end{bmatrix} \cdot \begin{bmatrix} c_1^1 & \cdots & c_1^n & \cdots & c_1^N \\ \vdots & & \vdots & & \vdots \\ c_m^1 & \cdots & c_m^n & \cdots & c_m^N \\ \vdots & & \vdots & & \vdots \\ c_M^1 & \cdots & c_M^n & \cdots & c_M^N \end{bmatrix} \quad (1)$$

The left side of the relation includes the matrix of absorbances of the standard solutions and the optical path the radiation has been covered, „d" (i.e. the width of the cell used).

It may be allowed that the absorbance of the sample, measured at the same set of wavelengths as in the case of standard solutions, consists of the weighted contributions of the standard solutions. The contribution weight of each standard solution to the absorbance of the sample depends on the concentration of the chemical components in the sample under analysis and in the individual standard solutions. This is expressed, in matrix form, according to relation (2).

$$\begin{bmatrix} A(1) \\ \vdots \\ A(\lambda) \\ \vdots \\ A(\Lambda) \end{bmatrix} = \begin{bmatrix} A^1(1) & \cdots & A^n(1) & \cdots & A^N(1) \\ \vdots & & \vdots & & \vdots \\ A^1(\lambda) & \cdots & A^n(\lambda) & \cdots & A^N(\lambda) \\ \vdots & & \vdots & & \vdots \\ A^1(\Lambda) & \cdots & A^n(\Lambda) & \cdots & A^N(\Lambda) \end{bmatrix} \cdot \begin{bmatrix} p^1 \\ \vdots \\ p^n \\ \vdots \\ p^N \end{bmatrix} \tag{2}$$

In what follows, bold characters are used for denoting matrices: the matrix of the absorbances of the sample will be denoted by **A**, the matrix of the absorbances of the standard solutions by $\mathbf{A_{st}}$, the matrix of the concentrations of chemical components in the analysed sample and in the standard solutions by **C** and $\mathbf{C_{st}}$ respectively, the matrix of the molar absorptivities by **E** and the matrix of the contribution weight of the standard solutions, generating the absorbance of the sample, by **P**. In order to comprehend more easily the matrix formalism, the symbol of matrices is followed (between right brackets) by the specification of the number of rows and columns in the respective matrix. Therefore, matrix $\mathbf{A_{st}}$, made up of Λ rows and N columns, is denoted as follows : $\mathbf{A_{st}}[\Lambda,N]$. Relations (1) and (2) are equivalent to matrix expressions (3) and (4).

$$\mathbf{A_{st}}[\Lambda,N] = d\cdot\mathbf{E}[\Lambda,M]\cdot\mathbf{C_{st}}[M,N] \tag{3}$$

$$\mathbf{A}[\Lambda,1] = \mathbf{A_{st}}[\Lambda,N]\cdot\mathbf{P}[N,1] = d\cdot\mathbf{E}[\Lambda,M]\cdot\mathbf{C}[M,1] \tag{4}$$

Relations (1) and (2) may be written in a condensed matrix form (5).

$$\frac{1}{d}\cdot\mathbf{A}[\Lambda,1] = \frac{1}{d}\cdot\mathbf{A_{st}}[\Lambda,N]\cdot\mathbf{P}[N,1] = \mathbf{E}[\Lambda,M]\cdot\mathbf{C_{st}}[M,N]\cdot\mathbf{P}[N,1] \tag{5}$$

The product matrix $\mathbf{A_{st}}[\Lambda,N]\cdot\mathbf{P}[N,1]$ consisting of Λ rows and one column may be presented in the shortened form $(\mathbf{A_{st}}\cdot\mathbf{P})[\Lambda,1]$.

Practically, the aim is to calculate the elements of matrix **C**[M,1]. In most of the real situations, the molar absorptivities of the chemical components under analysis are not known (especially not for a set of different wavelengths). For this reason, the spectrophotometric analysis is conditioned by the spectrophotometric study of a number of standard solutions, where the concentrations of the chemical components of interest are known**.** The matrix formalism presented allows for the standard solutions used to contain several chemical components (basically, each of the N standard solutions may contain all the M chemical components at known concentrations). In particular cases, it may happen (but it is not mandatory) that each standard solution contains only one chemical component (different from the other chemical components present in the other standard solutions); in this case the matrix $\mathbf{C_{st}}$ of the concentrations in standard solutions is square (has the same number of rows and columns) and diagonal (i.e. the c_m^n elements are null when m and n are different). In this particular case, the number of standard solutions is identical to the number of chemical components of analytical interest.

After the spectrophotometric measurements are accomplished, the elements of matrices $\mathbf{A_{st}}[\Lambda,N]$, **A**[Λ,1] and $\mathbf{C_{st}}[M,N]$ are known, and the further aim is to calculate the elements of

matrix **C**[M,1]. These matrices satisfy relations (6) and (7). In what follows, the desired result is to eliminate matrix **E**[Λ,M] from these two matrix relations and to explicit the resulting relation in relation to matrix **C**[M,1].

$$\mathbf{A}_{st}[\Lambda,N] = d\cdot\mathbf{E}[\Lambda,M]\cdot\mathbf{C}_{st}[M,N] \tag{6}$$

$$\mathbf{A}[\Lambda,1] = d\cdot\mathbf{E}[\Lambda,M]\cdot\mathbf{C}[M,1] \tag{7}$$

In order to solve the above system of equation in relation to matrix **C**[M,1], both members of equation (6) are multiplied on the right by the transpose of matrix $\mathbf{C}_{st}$[M,N].

$$\mathbf{A}_{st}[\Lambda,N]\cdot\mathbf{C}_{st}^{T}[N,M] = d\cdot\mathbf{E}[\Lambda,M]\cdot\mathbf{C}_{st}[M,N]\cdot\mathbf{C}_{st}^{T}[N,M] \tag{8}$$

The product $\mathbf{C}_{st}$[M,N] $\mathbf{C}_{st}^{T}$[N,M] is a MxM square matrix represented, according to the adopted notations, as $(\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})$ [M,M]. If the determinant of this matrix is not zero (i.e. if the set of wavelengths was selected suitably for relevant absorbance values), then the product matrix has an inverse, represented as $(\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})^{-1}$[M,M], with the property expressed by (9).

$$(C_{st}\cdot C_{st}^{T})^{-1}[M,M]\cdot(C_{st}\cdot C_{st}^{T})\,[M,M] = (C_{st}\cdot C_{st}^{T})\,[M,M]\cdot(C_{st}\cdot C_{st}^{T})^{-1}[M,M] = I[M,M] \tag{9}$$

In relation (9) **I**[M,M] is the unit matrix of order M. The elements of this matrix situated on the main diagonal are equal to the unity, and all its other elements are null. The multiplication operation of any matrix by the unit matrix (of the corresponding order) leaves the matrix unchanged. Consequently, after multiplying the equation (8) on the right by $(\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})^{-1}$[M,M], the resulting relation is (10).

$$\mathbf{A}_{st}[\Lambda,N]\cdot\mathbf{C}_{st}^{T}[N,M]\cdot(\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})^{-1}[M,M] = d\cdot\mathbf{E}[\Lambda,M]\cdot\mathbf{I}[M,M] = d\cdot\mathbf{E}[\Lambda,M] \tag{10}$$

In what follows, both members of equation (7) are multiplied on the left by the transpose of matrix **E**[Λ,M] , namely by $\mathbf{E}^{T}$[M,Λ]; the result is (11).

$$\mathbf{E}^{T}[M,\Lambda]\cdot\mathbf{A}[\Lambda,1] = d\cdot\mathbf{E}^{T}[M,\Lambda]\cdot\mathbf{E}[\Lambda,M]\cdot\mathbf{C}[M,1] \tag{11}$$

The product $\mathbf{E}^{T}$[M,Λ]·**E**[Λ,M] = $(\mathbf{E}^{T}\cdot\mathbf{E})$[M,M] in the expression (11) is a square matrix allowing an inverse, $(\mathbf{E}^{T}\cdot\mathbf{E})^{-1}$[M,M], provided that the product matrix is not singular (its determinant is different fron zero). By multiplying equation (11) on the left by matrix $(\mathbf{E}^{T}\cdot\mathbf{E})^{-1}$[M,M], the expression (12) is obtained. This expresses explicitly the seeked column matrix **C**[M,1] of the concentrations of components in the analysed mixture.

$$(\mathbf{E}^{T}\cdot\mathbf{E})^{-1}[M,M]\cdot\mathbf{E}^{T}[M,\Lambda]\cdot\mathbf{A}[\Lambda,1] = d\cdot\mathbf{C}[M,1] \tag{12}$$

Matrix **E**[Λ,M], occuring in expression (4.43), can be calculated with relation (10).

In order to express the matrix of concentrations **C**[M,1] only in relation to quantities resulting directly from spectrophotometric measurements (the elements of matrix **A**[Λ,1]) and in relation to known quantities (the elements of matrix $\mathbf{C}_{et}$[M,N]), the matrix **E**[Λ,M] has to be eliminated from relations (10) and (12).

The transpose of matrix $\mathbf{E}[\Lambda,M]$, namely matrix $\mathbf{E}^{T}[M,\Lambda]$, is expressed from relation (10) :

$$d\cdot \mathbf{E}^{T}[M,\Lambda] = (\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})^{-1}[M,M]\cdot\mathbf{C}_{st}[M,N]\cdot\mathbf{A}_{st}^{T}[N,\Lambda] \tag{13}$$

whereas the inverse matrix of the product of matrices $\mathbf{E}[\Lambda,M]$ and $\mathbf{E}^{T}[M,\Lambda]$ is expressed from (10) and (13):

$$(\mathbf{E}^{T}\mathbf{E})^{-1}[M,M] = d^{2}\cdot(\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})[M,M]\cdot(\mathbf{C}_{st}[\mathbf{M,N}]\cdot\mathbf{A}_{st}^{T}[N,\Lambda]\cdot\mathbf{A}_{st}[\Lambda,N]\cdot \tag{14}$$

$$\cdot\mathbf{C}_{st}^{T}[N,M])^{-1}\cdot(\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})^{-1}[M,M]$$

By replacing expressions (13) and (14) in (12), and taking into consideration relation (15),

$$(\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})^{-1}[M,M]\cdot(\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})[M,M] = \mathbf{I}[M,M] \tag{15}$$

the expression (16) is obtained. This presents, in an explicit form, the matrix of unknown concentrations.

$$\mathbf{C}[M,1] = (\mathbf{C}_{st}\cdot\mathbf{C}_{st}^{T})[M,M]\cdot(\mathbf{C}_{st}[\mathbf{M,N}]\cdot\mathbf{A}_{st}^{T}[N,\Lambda]\cdot\mathbf{A}_{st}[\Lambda,N]\cdot \tag{16}$$

$$\mathbf{C}_{st}^{T}[N,M])^{-1}\cdot\mathbf{C}_{st}[M,N]\cdot\mathbf{A}_{st}^{T}[N,\Lambda]\cdot\mathbf{A}[\Lambda,1]$$

In relation (16) the optical pathway (d) no longer appears if the absorbances of standards $_{st}[\Lambda,N]$ and the absorbances of the sample $\mathbf{A}[\Lambda,1]$ are measured at the same cell thickness.

Relation (10) allows to obtain the elements of matrix $\mathbf{E}[\Lambda,M]$ as well, values which are proportional to the absorbances of the pure components measured at the selected Λ wavelengths. Relation (10) allows thus to obtain the spectrum of the M individual components. This is important if a sufficiently large number of standard solutions are available with known concentrations of components, but individual components are not available for recording their individual spectra.

A particular case of the above reasoning is that with each of standard solutions contain only one dissolved chemical component (other than those present in the other standard solutions), so N = M. In this case notation S refers to their common value (N = M = S). Consequently, matrix $\mathbf{C}_{st}[S,S]$ of the concentrations of components in standard solutions is square and diagonal (only elements on the matrix main diagonal differ from zero) (17)).

$$\mathbf{C}_{st}[S,S] = \begin{bmatrix} c_1^1 & & & \\ & \ddots & & 0 \\ & & c_s^s & \\ 0 & & & \ddots \\ & & & & c_S^S \end{bmatrix} \tag{17}$$

If the entry data (the absorbance readings at the selected wavelengths and the concentrations of the standard solutions) do not form sets of relevant data, then singular matrices may be obtained when processing the data (whose determinant is null), namely

matrices which do not admit an inverse. In order to avoid this failure, the condition $\Lambda \geq N \geq M$ is imposed. This is the necessary (but no sufficient) condition to avoid the apparition of singular matrices. The necessity of the condition above results after inspecting the relations (10) and (12). In relation (10) the inverse of a matrix $(\mathbf{C_{st}} \cdot \mathbf{C_{st}^T})^{-1}[M,M]$ appears calculated from matrix $\mathbf{C_{st}}[M,N]$. Consequently, the matrix of the concentrations of the standard solutions must have higher – or at least equal – rank to the number M of chemical components in the sample. The necessary (but sufficient) condition for this requirement is $N \geq M$. In relation (12) the inverse of a matrix $(\mathbf{E^T} \cdot \mathbf{E})^{-1}[M,M]$, is calculated from the matrix of molar absorptivities, $\mathbf{E}[\Lambda,M]$. The necessary (but not sufficient) condition of the non-singularity of matrix $(\mathbf{E^T} \cdot \mathbf{E})^{-1}[M,M]$ is the compliance of inegality $\Lambda \geq N$. The two necessary conditions for avoiding matrix singularity are expressed in the united form $\Lambda \geq N \geq M$. Also in order to avoid singularity in relation (10), the appropriate choice of concentrations of standard solutions is imposed, so that in matrix $\mathbf{C_{st}}[M,N]$ both rows and columns should be linearly independent. Otherwise expressed, it is essential that there should not be any significant intercorrelation neither between different columns nor between different rows of the matrix of standard concentrations (in algebraic terms, the concentrations in standard solutions must form a complete basis in the linear M-dimensional field). In other words, the spectra of individual chemical components should differ significantly in the spectral field chosen for analysis (more precisely, for the selected set of wavelengths). The relevance of the choice of the wavelength set, from the point of view of the above-mentioned facts, can be tested by calculating the eigenvalues of the square and symmetric matrix $\mathbf{A^T_{st}}[N,\Lambda] \cdot \mathbf{A_{st}}[\Lambda,N]$. If one eigenvalue of this matrix is null (or very close to the null value), the selection of the wavelength set is not adequate for the intended analysis. The selection of another wavelength set is therefore necessary. The general issue of row (or column) intercorrelation is solved in linear algaebra by taking into consideration the issue of eigenvalues and eigenvectors. However, the complete and rigorous mathematical treatment of the issue of basis vectors in linear algaebra goes beyond the purpose of the present work.

2.1 Example

Let be N = 5 standard solutions containing M = 3 components of known concentrations. The concentrations, expressed in mg/l, are included in matrix $\mathbf{C_{st}}[3,5]$. As illustrated by this matrix, each of the 5 standard solutions contains (in different and known concentrations) all three dissolved chemical components.

$$\mathbf{C_{st}}[3,5] = \begin{bmatrix} 2.50 & 4.25 & 1.25 & 0.85 & 2.22 \\ 3.00 & 1.00 & 1.62 & 1.15 & 3.36 \\ 4.00 & 0.80 & 5.00 & 4.45 & 0.82 \end{bmatrix} \; ; \; \mathbf{C^T_{st}}[5,3] = \begin{bmatrix} 2.50 & 3.00 & 4.00 \\ 4.25 & 1.00 & 0.80 \\ 1.25 & 1.62 & 5.00 \\ 0.85 & 1.15 & 4.45 \\ 2.22 & 3.36 & 0.82 \end{bmatrix}$$

The matrix $(\mathbf{C_{st}} \cdot \mathbf{C_{st}^T})[3,3]$ resulting after multiplication and the eigenvalues of the product matrix ($\mathbf{EV}[3,1]$) are illustrated below:

$$(\mathbf{C_{st}} \cdot \mathbf{C_{st}^T})[3,3] = \begin{bmatrix} 31.5259 & 22.2117 & 25.2529 \\ 22.2117 & 25.2365 & 28.7727 \\ 25.2529 & 28.7727 & 62.1149 \end{bmatrix} \; ; \; {}_{\mathbf{E}}\mathbf{V}[3,1] = \begin{bmatrix} 4.915255 \\ 18.974022 \\ 94.988023 \end{bmatrix}$$

All three eigenvalues are different from zero (taking into account the concentration values and the precision in expressing concentration values), so the rank of the matrix $\mathbf{C_{st}}[3,5]$ is 3. In other words, the set of concentration values allows to determine quantitatively all three chemical components in their mixture (provided that the wavelength set at which the absorbance values are going to be measured is chosen correctly).

The situation would differ if the matrix of concentrations of the standard solutions contained the following values:

$$\mathbf{C_{st}}[3,5] = \begin{bmatrix} 2.50 & 4.25 & 1.25 & 0.85 & 2.22 \\ 3.00 & 1.00 & 1.62 & 1.15 & 3.36 \\ 5.50 & 5.25 & 2.87 & 2.00 & 5.58 \end{bmatrix} \; ; \; \mathbf{C_{st}^T}[5,3] = \begin{bmatrix} 2.50 & 3.00 & 5.50 \\ 4.25 & 1.00 & 5.25 \\ 1.25 & 1.62 & 2.87 \\ 0.85 & 1.15 & 2.00 \\ 2.22 & 3.36 & 5.58 \end{bmatrix}$$

In this situation, the product $(\mathbf{C_{st}} \cdot \mathbf{C_{st}^T})[3,3]$ of the two matrices has other eigenvalues.

$$(\mathbf{C_{st}} \cdot \mathbf{C_{st}^T})[3,3] = \begin{bmatrix} 31.5259 & 22.2117 & 53.7376 \\ 22.2117 & 25.2365 & 47.4482 \\ 53.7376 & 47.4482 & 101.1858 \end{bmatrix}$$

$${}_{\mathbf{E}}\mathbf{V}[3,1] = \begin{bmatrix} 5.966037 \\ 2.6393 \cdot 10^{-14} \\ 151.982163 \end{bmatrix}$$

In this case the rank of matrix $\mathbf{C_{st}}[3,5]$ is only two because the second element in the column matrix of eigenvalues ($\mathbf{EV}[3,1]$) is a lot smaller than the elements of the initial matrix and a lot smaller than the estimated accepted errors in expressing the standard concentrations. Consequently, even if a number of N = 5 standard solutions were used (with the considered concentrations), the concentrations of the three components in their mixture cannot be determined (irrespective of the wavelengths set chosen for measuring the absorbances), because the values of the concentrations of the standard solution have not been chosen properly.

2.2 Example

For numeric illustration of the spectrophotometric data processing with matrix formalism, the measurement data obtained analyzing the mixture of salicylic acid, caffeine and acetaminophen will be further presented [Szabadai, 2005]. The number of standard solutions is N = 5 and each standard solution contains all three components (in known concentrations). Table 1 contains absorbance values for the 5 standard solutions (A_{st}) and for the mixture of three substances (A), registered at the same set of 18 wavelengths. Table 1 also presents the known concentrations of the three components in the five standard solutions (elements of matrix $\mathbf{C_{st}}[3,5]$), i.e. M = 3, N = 5, Λ = 18. The matrix of concentrations

of the standard solutions $\mathbf{C_{st}}[3,5]$, the matrix of absorbances of the standard solutions $\mathbf{A_{et}}[18,5]$ and the matrix of absorbances of the sample $\mathbf{A}[18,1]$ have the following forms:

Table 1

		Standard solution 1	Standard solution 2	Standard solution 3	Standard solution 4	Standard solution 5	Mixture (sample)
Absorbance values for different wavelengths (cell thickness d = 1 cm)		1.167	0.456	1.179	1.011	0.565	0.581
		1.192	0.435	1.257	1.048	0.513	0.566
		1.169	0.377	1.288	1.123	0.439	0.515
		1.109	0.290	1.265	1.109	0.374	0.443
		1.020	0.244	1.154	1.010	0.362	0.395
		0.932	0.228	1.000	0.867	0.402	0.370
		0.822	0.218	0.799	0.679	0.462	0.350
		0.747	0.217	0.645	0.534	0.524	0.336
		0.714	0.233	0.548	0.440	0.585	0.347
		0.654	0.232	0.487	0.388	0.552	0.329
		0.509	0.209	0.379	0.302	0.422	0.276
		0.295	0.167	0.228	0.183	0.225	0.195
		0.152	0.133	0.115	0.092	0.110	0.130
		0.089	0.100	0.060	0.046	0.069	0.095
		0.049	0.058	0.032	0.025	0.038	0.048
		0.030	0.026	0.023	0.018	0.021	0.030
		0.022	0.013	0.019	0.016	0.015	0.011
		0.020	0.009	0.018	0.015	0.013	0.010
Concentrations of components (mg/l)	Salicylic acid	2.50	4.25	1.25	0.85	2.22	
	Caffeine	3.00	1.00	1.62	1.15	3.36	
	Acetaminophen	4.00	0.80	5.00	4.45	0.82	

$$\mathbf{C_{st}}[3,5] = \begin{bmatrix} 2.50 & 4.25 & 1.25 & 0.85 & 2.22 \\ 3.00 & 1.00 & 1.62 & 1.15 & 3.36 \\ 4.00 & 0.80 & 5.00 & 4.45 & 0.82 \end{bmatrix}$$

$$\mathbf{A_{st}}[18,5] = \begin{bmatrix} 1.167 & 0.456 & 1.179 & 1.011 & 0.565 \\ 1.192 & 0.435 & 1.257 & 1.087 & 0.513 \\ 1.169 & 0.377 & 1.288 & 1.123 & 0.439 \\ 1.109 & 0.290 & 1.265 & 1.109 & 0.374 \\ 1.020 & 0.244 & 1.154 & 1.010 & 0.362 \\ 0.932 & 0.228 & 1.000 & 0.867 & 0.402 \\ 0.822 & 0.218 & 0.799 & 0.679 & 0.462 \\ 0.747 & 0.217 & 0.645 & 0.534 & 0.524 \\ 0.714 & 0.233 & 0.548 & 0.440 & 0.585 \\ 0.654 & 0.232 & 0.487 & 0.388 & 0.552 \\ 0.509 & 0.209 & 0.379 & 0.302 & 0.422 \\ 0.295 & 0.167 & 0.228 & 0.183 & 0.225 \\ 0.152 & 0.133 & 0.115 & 0.092 & 0.110 \\ 0.089 & 0.100 & 0.060 & 0.046 & 0.069 \\ 0.049 & 0.058 & 0.032 & 0.025 & 0.038 \\ 0.030 & 0.026 & 0.023 & 0.018 & 0.021 \\ 0.022 & 0.013 & 0.019 & 0.016 & 0.015 \\ 0.020 & 0.009 & 0.018 & 0.015 & 0.013 \end{bmatrix} ; \quad \mathbf{A}[18,1] = \begin{bmatrix} 0.581 \\ 0.566 \\ 0.515 \\ 0.443 \\ 0.395 \\ 0.370 \\ 0.350 \\ 0.336 \\ 0.347 \\ 0.329 \\ 0.276 \\ 0.195 \\ 0.130 \\ 0.095 \\ 0.048 \\ 0.030 \\ 0.011 \\ 0.010 \end{bmatrix}$$

After performing the matrix operations in relation (16), the elements of matrix **C**[3,1] are obtained. They represent the concentrations, expressed in mg/l, of the three components of interest (salicylic acid, caffeine and paracetamol) in the analysed sample.

$$\mathbf{C}[3,1] = \begin{bmatrix} 3.538 \\ 1.553 \\ 1.381 \end{bmatrix}$$

3. Generalization of the 3-point method to correct backgroud absorption

Before dealing generally with the issue of foreign components in the sample (components which cannot be found in standard solutions) – which may cause deviations from the hypothesis according to which the sample spectrum is formed by adding (with different weights) the spectra of standard solutions – the quantitative analysis method and the baseline correction algorithm suggested by *Morton* and *Stubbs* [Burnius, 1959; Ewing et al., 1953; Fox & Mueller, 1950; Melnick et al., 1952; Morton & Stubbs, 1946, 1947, 1948; Owen, 1995; Page & Berkovitz, 1943; Szabadai, 2005;] (also known as "3-point method") will be presented.

The *Morton – Stubbs* method takes into account that the sample often contains – besides the chemical substance of interest – other foreign absorbent chemical components. If the chemical removal of these foreign components is difficult, the elimination (or at least the minimisation) of their contribution to the final result of the analysis by correcting the absorbance read could be a confortable solution. Accordind to the original form of the *Morton* and *Stubbs* method [Morton & Stubbs, 1946, 1947, 1948], it is possible to eliminate the disturbing effect of a foreign component only in the case in which the absorption of the disturbing component, manifested in the spectral field taken into consideration, does not present a maximum of absorption, but appears as a baseline absorption, dependent on the wavelength according to a linear function, which overlaps the absorption spectrum of the chemical component of interest.

The absorption spectrum of the component of intereset is deformed because of the background absorption (linearly dependent on the wavelength), and the effect of this deformation is eliminated through the special method of processing the measured absorbance values. According to the original *Morton – Stubbs* formalism, it is essential to determinate the absorbance of the sample at at least three wavelengths [Morton & Stubbs, 1946]. The wavelengths values involved are selected as follows: the wavelength used (λ_{max}) is the one at which the standard solution of the substance of interest (where the disturbing component is not present) presents a local absorbance maximum and another two wavelengths ((λ_1 and λ_2 , λ_{max} being between these wavelengths) at which the substace of interest presents equal molar absorptivities (Á(1) = Á(2)). Figure 1 represents the spectrum of the standard solution by dotted line whereas the spectrum of the mixture, where the quantitative determination of the substances of interest is intended, is represented by a continuous line. The absorbance values corresponding to the three wavelengths selected (λ_1 , λ_2 and λ_{max}) are denoted as A(1), A(2) and A(max) in the spectrum of the sample and as Á(1), Á(2) and Á(max) in the spectrum of the pure (standard) component. The purpose is to calculate quantity Á(max) (namely the absorbance associated with the substance of interest but without the backgroud absorbance) from the measured values A(1), A(2) and A(max). The absorbance Á(max) is obtained by subtracting from the measured value A(max) the value denoted by x + y in Figure 1.

$$\acute{A}(max) = A(max) - (x + y) \tag{18}$$

The value x is expressed from the similarity of two triangles chosen conveniently:

$$\frac{\lambda_2 - \lambda_1}{\lambda_2 - \lambda_{max}} = \frac{A(1) - A(2)}{x}; \quad x = \frac{\lambda_2 - \lambda \max}{\lambda_2 - \lambda_1} \cdot \left[A(1) - A(2) \right] \tag{19}$$

For calculating the value y in expression (18), the ratio of the absorbances Á(max) and Á(2) is needed, which can be determined from the spectrum of the standard solution. When elaborating an analytical method in order to determine a certain substance of interest, in a standardized work method, the ratio of the absorbances Á(max) and Á(2) once determined, it can be used for subsequent analyses, provided that analyses should be performed strictly in unchanged conditions (in the same solvent, at the same pH, the same temperature, with the same slit program of the spectrophotometer, preferably the same type of spectrophotometer as the one used for determining the above mentioned ratio). Let be denoted the aforementioned ratio as ρ:

$$\rho = \frac{A'(\max)}{A'(2)} \tag{20}$$

In possession of the ratio ρ, the value y is obtained from relation (18) and (21).

$$\acute{A}(2) = A(2) - y \tag{21}$$

After dividing member by member relations (18) and (21), results:

$$\rho = \frac{A'(\max)}{A'(2)} = \frac{A(\max) - (x+y)}{A(2) - y} \;\; ; \;\; y = \frac{\rho \cdot A(2) - A(\max) + x}{\rho - 1} \tag{22}$$

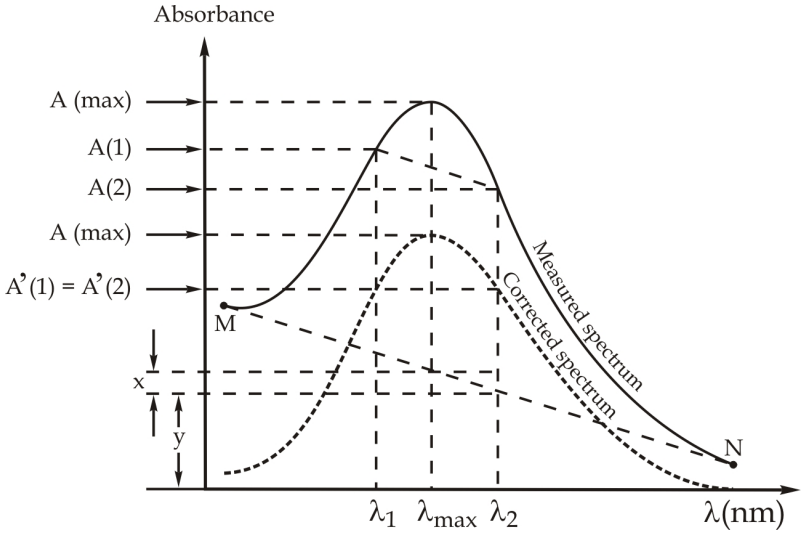

Fig. 1. Illustration of the *Morton – Stubbs* method

After replacing the expressions x and y in the latter relation, relation (23) results. It expresses the absorbance associated to the component of interest A′(max), which lacks baseline absorption.

$$A'(\max) = A(\max) - \frac{\lambda_2 - \lambda_{\max}}{\lambda_2 - \lambda_1} \cdot [A(1) - A(2)] - \\ - \frac{\rho \cdot A(2) - A(\max) + \frac{\lambda_2 - \lambda_{\max}}{\lambda_2 - \lambda_1} \cdot [A(1) - A(2)]}{\rho - 1} \tag{23}$$

The liniarity of the background absorption in a large spectral field is not always satisfied. In the case of wide absorption bands it is recommended to measure the absorbance of the sample at several wavelengths; in these cases however, the processing of the absorbance values measured requires more elaborated mathematical methods.

As it can be noticed, the *Morton – Stubbs* formalism allows the presence in the spectrum of the sample a linear background (a linear foreign spectrum in relation to the wavelength) which cannot be put down to any component of the standard solutions, ensuring corrected results (sample concentrations of the components of interest).

The original algorithm may be extended to ensure the obtention of corrected results in the case in which the sample spectrum contains, besides the chemical components represented in the standard spectra, a G degree polynomial baseline in relation to the wavelength. The spectrum of the sample is thus considered to consist of the spectra of the standard solutions

and of the background spectrum, the latter being approximated to an adequate G degree polynomial (relation 24).

$$A(\lambda_i) = \sum_{k=1}^{K} p_k \cdot A_k(\lambda_i) + \sum_{g=0}^{G} q_g \cdot \lambda_i^g \quad ; \quad (i = 1,2,\ldots,N) \tag{24}$$

The purpose is to calculate the contribution weight p_k of each standard solution to the spectrum of the sample, namely the coefficients p_k (k = 1 , 2 , . . . , K). In the ideal case, when the spectrum of the sample does not contain a foreign baseline, but only the components represented in standard solutions, the coefficients q_g (g = 0 , 1 , 2 , . . . , G) are all null. Because of inherent measurement errors these coefficients are not null, but if the polynomial (25) is positive and has small values (for all wavelengths λ_i selected) in relation to the measured absorbances, the approach of the issue is correct and there are still chances to remove, by calculation, the effect of the polynomial backgroud (G degree) from the spectrum of the sample on the results. On the contrary, if the polynomial (25) has a high value or a negative one, even for one wavelength (one i value), the foreign backgroud cannot be approximated to a G degree polynomial form, and forcing the algorithm might lead to an erroneous result.

$$P(i) = \sum_{g=0}^{G} q_g \cdot \lambda_i^g \tag{25}$$

Obviously, the highest the G degree of the polynomial (25) which corrects the foreign backgroud in the spectrum of the sample, The more flexible the correction algorithm of a real backgroud absorption, but the more wavelengths should be selected where the absorbance readings are performed (in other words the inegality N > G + K + 1 is imposed in practice in order to obtain, from the measured absorbance values, a supra-determined system of equations).

For the statistical processing of the set of N absorbance values obtained for the sample and N×K absorbance values for the K standard solutions, the function (26) is defined imposing that for the values p_k (k = 1 , 2 , . . . , K) and the values q_g (g = 0 , 1 , . . . , G), which ensure the best global correspondence between the measured absorbances of the sample and the absorbances approximated with the relation (24), the function $F(p_k,q_g)$ should present a local minimum. The condition formulated is equivalent cancel the partial derivatives of the function (26) calculated in relation to p_k (k = 1 , 2 , . . . , . . . , K) and q_g (g = 0 , 1 , . . . , G). The cancellation of partial derivatives in (26) represents the necessary (but sufficient) condition for a local minimum of the function (26).

$$\begin{gathered} F(p_k,q_g) = \sum_{i=1}^{N} \left[A(\lambda_i) - \sum_{k=1}^{K} p_k \cdot A_k(\lambda_i) - \sum_{g=0}^{G} q_g \cdot \lambda^g \right]^2 = \min \\ \frac{\partial F(p_k,q_g)}{\partial q_{g^*}} = 0 \quad ; \quad \frac{\partial F(p_k,q_g)}{\partial p_{k^*}} = 0 \\ (k^* = 1,2,\ldots,K \quad ; \quad g^* = 0,1,2,\ldots,G) \end{gathered} \tag{26}$$

After derivation and equalization the derivatives to zero, a system of K + G + 1 linear equations is obtained, having the same number of unknowns (27).

$$\begin{gathered}\sum_{g=0}^{G} q_g \cdot \sum_{i=1}^{N} \lambda_i^g \cdot \lambda_i^{g^*} + \sum_{k=1}^{K} p_k \cdot \sum_{i=1}^{N} A_k(\lambda_i) \cdot \lambda_i^{g^*} = \sum_{i=1}^{N} A(\lambda_i) \cdot \lambda_i^{g^*} \\ (g^* = 0,1,2,\ldots,G) \\ \sum_{g=0}^{G} q_g \cdot \sum_{i=1}^{N} \lambda_i^g \cdot A_{k^*}(\lambda_i) + \sum_{k=1}^{K} p_k \cdot \sum_{i=1}^{N} A_k(\lambda_i) \cdot A_{k^*}(\lambda_i) = \sum_{i=1}^{N} A(\lambda_i) \cdot A_{k^*}(\lambda_i) \\ (k^* = 1,2,\ldots,K)\end{gathered} \tag{27}$$

In order to express the wavelength (and its different powers) any unit of measure can be used, provided that the same unit of measure is used in all equations and for all wavelengths.

The generalisation of the *Morton – Stubbs* algorithm for the polynomial correction of the spectrum of the sample can also be presented in a matrix form. The equation system (24), written in a conventional algebraic form, is equivalent to matrix relation (28).

$$\begin{bmatrix} A(\lambda_1) \\ A(\lambda_2) \\ \vdots \\ A(\lambda_i) \\ \vdots \\ A(\lambda_N) \end{bmatrix} = \begin{bmatrix} A_1(\lambda_1) & \cdots & A_k(\lambda_1) & \cdots & A_K(\lambda_1) & 1 & \lambda_1 & \cdots & \lambda_1^g & \cdots & \lambda_1^G \\ A_1(\lambda_2) & \cdots & A_k(\lambda_2) & \cdots & A_K(\lambda_2) & 1 & \lambda_2 & \cdots & \lambda_2^g & \cdots & \lambda_2^G \\ \vdots & & \vdots & & \vdots & \vdots & \vdots & & \vdots & & \vdots \\ A_1(\lambda_i) & \cdots & A_k(\lambda_i) & \cdots & A_K(\lambda_i) & 1 & \lambda_i & \cdots & \lambda_i^g & \cdots & \lambda_i^G \\ \vdots & & \vdots & & \vdots & \vdots & \vdots & & \vdots & & \vdots \\ A_1(\lambda_N) & \cdots & A_k(\lambda_N) & \cdots & A_K(\lambda_N) & 1 & \lambda_N & \cdots & \lambda_N^g & \cdots & \lambda_N^G \end{bmatrix} \cdot \begin{bmatrix} p_1 \\ \vdots \\ p_k \\ \vdots \\ p_K \\ q_0 \\ q_1 \\ \vdots \\ q_g \\ \vdots \\ q_G \end{bmatrix} \tag{28}$$

If the matrix of the absorbance values of the sample (on the left member of the equation (28)) is denoted by **X**[N,1], the first matrix factor on the right member by **Y**[N,K+G+1] and the second matrix factor on the right member by **Z**[K+G+1,1], the equation (28) can have the form (29).

$$\mathbf{X}[N,1] = \mathbf{Y}[N,K+G+1] \cdot \mathbf{Z}[K+G+1,1] \tag{29}$$

The unknowns of interest are found in matrix **Z**[K+G+ 1,1] ; the relative weights are p_k (k = 1, 2 , . . . , K). In order to explain the elements of matrix **Z**, both members of relation (29) are multiplied on the left by the transpose of matrix **Y**.

$$\mathbf{Y}^T[K+G+1,N] \cdot \mathbf{X}[N,1] = \mathbf{Y}^T[K+G+1,N] \cdot \mathbf{Y}[N,K+G+1] \cdot \mathbf{Z}[K+G+1,1] \tag{30}$$

Matrix $(\mathbf{Y}^T \cdot \mathbf{Y}) = \mathbf{Y}^T[K+G+1,N] \cdot \mathbf{Y}[N,K+G+1]$ is square and allows an inverse matrix $(\mathbf{Y}^T \cdot \mathbf{Y})^{-1}[N,N]$ if the associated determinant is not null. By multiplying relation (30) on the left by the inverse matrix, the explicit form of matrix **Z** results.

$$(\mathbf{Y}^T\cdot\mathbf{Y})^{-1}[N,N]\cdot\mathbf{Y}^T\,[K+G+1,N]\cdot\mathbf{X}[N,1] = \mathbf{Z}[K+G+1,1] \quad (31)$$

It is decisively important to determine the correct set of wavelengths at which the absorbance values should be measured in case of a concrete analytical problem. The choice of the optimal wavelength (or wavelengths) is often a difficult issue even in case of a single component of interest. In real samples the component of interest may be accompanied by different other components whithout analytical interest ("the sample ballast"), but which can modify the molar absorptivity of the component of interest and so the sensitivity of the spectral answer of the chemical substance representing the object of the analysis. If one can identify the wavelength value at which the absorption of the sample ballast is negligible and at which the absorption of the component of interest is considerable, the respective wavelength is recommended for the determination. When at this wavelength the component of interest has even a local absorption maximum, this is an additional advantage, because at this wavelength the absorbance value depends in a minimum extent on the possible disorders in setting the wavelengths of the spectrophotometer. In the less fortunate case, where the sample ballast covers the entire spectral field available, more wavelengths are selected in order to determine the component in the sample in order to improve the specificity of the spectral answer in favour of the component of interest.

When the absorption of the component of interest and that of the ballast cannot be separated, a set of wavelengths can often be chosen so that the absorbances measured express the concentration of the component of interest through a multilinear relation (32).

$$c = f(\lambda_1)\cdot A(\lambda_1) + f(\lambda_2)\cdot A(\lambda_2) + \ldots + f(\lambda_i)\cdot A(\lambda_i) + \ldots + f(\lambda_N)\cdot A(\lambda_N) \quad (32)$$

The aim is to determine numerically the coefficients $f(\lambda_i)$; (i = 1 , 2 , . . . , N) for each wavelength in the spectral field considered (it is considered that the entire spectrum consists of N absorbance values associated to N discrete wavelength values) to calculate according to (32) the concentration of the chemical substance of interest in different samples, containing a different and unpredictable ballast. This purpose can sometimes be accomplished, sometimes not, according to the ballast variability of the analysed samples.

If there is any chance to determine a set of coefficients in agreement with the requirements mentioned for a component of interest, in presence of a ballast range in different samples, their calculation could be performed through calibration with a number of standard samples (let their number S) containing a ballast range as close as possible to that of real samples (of unknown composition) under analysis. Thus, two different standard samples may have the same concentration of the component of interest if they have a different ballast.

The concentrations of the component of interest in the S standard samples and the absorbances $A_s(\lambda_i)$; (s = 1 , 2 , . . . , S) of the standard samples satisfy the equation (32). The equation system obtained with the standard sample data can be rendered in matrix form (33).

If S = N, the number of unknowns equals the number of equations, so we dispose of the minimum number of equations necessary to solve the system (33) in relation to the N unknowns. For the reasons discussed above, the creation of a supra-determined system of equations is preferred (S > N), as well as the search for a solution with an optimal global fit with least squares method.

$$\begin{bmatrix} c_1^{st} \\ c_2^{st} \\ \vdots \\ c_s^{st} \\ \vdots \\ c_S^{st} \end{bmatrix} = \begin{bmatrix} A_1(\lambda_1) & A_1(\lambda_2) & \cdots & A_1(\lambda_i) & \cdots & A_1(\lambda_N) \\ A_2(\lambda_1) & A_2(\lambda_2) & \cdots & A_2(\lambda_i) & \cdots & A_2(\lambda_N) \\ \vdots & \vdots & & \vdots & & \vdots \\ A_s(\lambda_1) & A_s(\lambda_2) & \cdots & A_s(\lambda_i) & \cdots & A_s(\lambda_N) \\ \vdots & \vdots & & \vdots & & \vdots \\ A_S(\lambda_1) & A_S(\lambda_2) & \cdots & A_S(\lambda_i) & \cdots & A_S(\lambda_N) \end{bmatrix} \cdot \begin{bmatrix} f(\lambda_1) \\ f(\lambda_2) \\ \vdots \\ f(\lambda_i) \\ \vdots \\ f(\lambda_N) \end{bmatrix} \tag{33}$$

Values $f(\lambda_i)$; (i = 1 , 2 , . . . , N), representing the solution to the equation system (33), can be positive and negative numbers, both type being relevant for the analysis. If the absolute value of one of the coefficients $f(\lambda_i)$; (i = 1 , 2 , . . . , N) is small (negligible in relation to the mean of the absolute values of all coefficients), their contribution to the equation (32) is insignificant, they can be considered null, and the respective wavelengths are not relevant for the intended quantitative analysis. Therefore, to each wavelength in the spectrum a coefficient is associated expressing the relevance of that wavelength for the quantitative analysis of the component of interest in the presence of the matrix included in the calibration stage.

By excluding the irrelevant wavelengths, which do not improve the selectivity of the analytical method, one may reduce the number of wavelengths at which the measurement of absorbances is imposed when executing a real sample analysis.

In possession of the coefficients $f(\lambda_i)$; (i = 1 , 2 , . . . , N), the concentrations in the standard samples can be recalculated by relation (4.65) (the concentrations obtained are denoted c_1 , c_2 , . . . , c_s , . . . , c_S). Ideally, concentrations for all standard samples can be found. In reality, the correspondance between the set of existing (and known) concentrations in the S standard samples and the set of concentrations recalculated with relation (33) is not perfect. The success of the calibration operation can be expressed through the value of the linear correlation coefficient between the set of existing concentrations in the standard samples and the recalculated ones. Since the arithmetic mean of the existing (and known) concentrations in the S standard samples and the arithmetic mean of the concentrations recalculated with relation (33) are equal (according to a known theorem of mathematical statistics), their notation with a common symbol is justified:

$$\bar{C} = \frac{1}{S} \cdot \sum_{s=1}^{S} c_s^{st} = \frac{1}{S} \cdot \sum_{s=1}^{S} c_s$$

The linear correlation coefficient between the set of concentrations c_s^{st} and c_s (s = 1 , 2 , ... , S) is calculated with relation (34).

$$r = \frac{\sum_{s=1}^{S} \left(c_s^{st} - \bar{C}\right) \cdot \left(c_s - \bar{C}\right)}{\sqrt{\left[\sum_{s=1}^{S} \left(c_s^{st} - \bar{C}\right)^2\right] \cdot \left[\sum_{s=1}^{S} \left(c_s - \bar{C}\right)^2\right]}} \tag{34}$$

If the correlation coefficient (34) has an acceptable value from a statistical point of view (for example r > 0,95), it is likely that the set of coefficients $f(\lambda_i)$; (i = 1 , 2 , ... , N), obtained

by solving the equation system (33) will allow to find the correct concentration of the substance of interest in real samples, provided that the real sample ballast is not completely different from the ballast range covered when calibrating the method (when determining the coefficients $f(\lambda_i)$; (i = 1 , 2 , . . . , N)). This requirement is met to a certain extent in the case of serial analyses, where the nature of individual samples does not differ much, meaning that their ballast is similar.

Presenting a spectrum in a spectral field through pairs of wavelength-absorbance values ($A(\lambda)$ vs. λ , "digitized presentation") implies a large amount of data (for a faithful reprensentation of a spectrum the N number of sampling points is large). It results that, in order to genearate a supra-determinant equation system (33), an even larger number of standard samples is necessary (S > N). This is generally inconvenient to realize in practice because it implies the use of a too large number of standard samples.

If S < N, the equation system (33) allows several sets of wavelengths for which the concentrations in standard samples correlate satisfactorily with the absorbance values, and the remaining problem is to identify at least one of these sets. This method is frequently used in practice, and establishing a profitable set of wavelengths involves the following stages:

(1) The matrix of absorbance values **A**[S,N] turns into a new square matrix **B**[S,S] whose columns are a complete orthogonal basis. The orthogonality of columns in the new matrix **B**[S,S] can be realized, for example, by multiplying the matrix **A**[S,N] on the right by a matrix **Q**[N,S] chosen conveniently (35), so that the elements of matrix **B**[S,S] = **A**[S,N]·**Q**[N,S] satisfy the orthogonality relation of columns (36). The construction of such a matrix **Q**[N,S] is not unique; theoretically, there is an infinite number of such matrices capable of generating orthogonal columns satisfying the requirement (36). In spectrophotometric practice a diagonal-superior form of the matrix **Q**[N,S] is sometimes used (where only elements on the main diagonal and those above this diagonal are different from zero).

$$\begin{bmatrix} A_1(\lambda_1) & \cdots & A_1(\lambda_i) & \cdots & A_1(\lambda_N) \\ \vdots & & \vdots & & \vdots \\ A_s(\lambda_1) & \cdots & A_s(\lambda_i) & \cdots & A_s(\lambda_N) \\ \vdots & & \vdots & & \vdots \\ A_S(\lambda_1) & \cdots & A_S(\lambda_i) & \cdots & A_S(\lambda_N) \end{bmatrix} \cdot \begin{bmatrix} Q_{11} & \cdots & Q_{1j} & \cdots & Q_{1S} \\ \vdots & & \vdots & & \vdots \\ Q_{j1} & \cdots & Q_{jj} & \cdots & Q_{jS} \\ \vdots & & \vdots & & \vdots \\ Q_{N1} & \cdots & Q_{Nj} & \cdots & Q_{NS} \end{bmatrix} = \begin{bmatrix} B_{11} & \cdots & B_{1j} & \cdots & B_{1S} \\ \vdots & & \vdots & & \vdots \\ B_{s1} & \cdots & B_{sj} & \cdots & B_{sS} \\ \vdots & & \vdots & & \vdots \\ B_{S1} & \cdots & B_{Sj} & \cdots & B_{SS} \end{bmatrix} \quad (35)$$

$$\sum_{s=1}^{S} B_s(\lambda_j) \cdot B_s(\lambda_{j^*}) = 0 \quad ; \quad for\ any \quad j \neq j^* \quad (36)$$

(2) Calculate the correlation coefficient of the elements of matrix $\mathbf{C}_{et}$[S,1] in relation (33) one by one with the columns of matrix **B**[S,S] (for j = 1 , 2 , . . . , S), thus obtaining N correlation coefficient values, in real cases all being smaller than theoretical value 1. The correlation coefficient of the elements of matrix $\mathbf{C}_{st}$[S,1] with the column "j" of matrix **B**[S,S] is calculated by the relation (37).

$$r(\mathbf{C}_{st}[S,1],\mathbf{B}_j[S,1]) = \frac{\sum_{s=1}^{S}\left(c_s^{s_i}-\overline{C}\right)\cdot\left(B_{sj}-\overline{B}_j\right)}{\sqrt{\left[\sum_{s=1}^{S}\left(c_s^{st}-\overline{C}\right)^2\right]\cdot\left[\sum_{s=1}^{S}\left(B_{sj}-\overline{B}_j\right)^2\right]}} \tag{37}$$

In relation (37) $\mathbf{B}_j[S,1]$ represents the column vector made up of the column of number "j" of matrix $\mathbf{B}[S,S]$, $\overline{C}$ is the mean value of the elements of matrix $\mathbf{C}_{st}[S,1]$ and $\overline{B}_j$ is the mean value of elements in column "j" in matrix $\mathbf{B}[S,S]$.

$$\overline{C} = \frac{1}{S}\cdot\sum_{s=1}^{S} c_s^{st} \quad ; \quad \overline{B}_j = \frac{1}{S}\cdot\sum_{s=1}^{S} B_{sj}$$

The correlation coefficients, calculated with relation (37) for j = 1, 2, . . . , S, in relation to "j" and the value "j" is retained (denoted by 1) for which the correlation coefficient is highest (in case of obtaining equal values of the correlation coefficient for more "j" values, one of these "j" values is retained arbitrarily).

(3) By using the multiple linear regression method, the elements of column matrix $\mathbf{C}_{et}[S,1]$ are correlated with all the pairs of columns of matrix $\mathbf{B}[S,S]$ obtained by combining column 1 with all the other columns of matrix $\mathbf{B}[S,S]$. The values of the multiple correlation coefficient $r(\mathbf{C}_{et}[S,1], (\mathbf{B}_1\&\mathbf{B}_j)[S,2])$ are calculated in relation to the values taken by "j" and is retained (and denoted by 2) the value " j" for which the multiple correlation coefficient is highest. Two "j" values are thus obtained (denoted by 1 and 2) indicating the pair of columns in matrix $\mathbf{B}[S,S]$ which correlate conveniently with the column matrix $\mathbf{C}_{et}[S,1]$.

(4) By using the multiple linear regression method, the elements of column matrix $\mathbf{C}_{et}[S,1]$ are correlated with all sets of three columns of matrix $\mathbf{B}[S,S]$, obtained by combining columns 1 and 2 with all the other columns of matrix $\mathbf{B}[S,S]$. The values $r(\mathbf{C}_{et}[S,1], (\mathbf{B}_1\&\mathbf{B}_2\&\mathbf{B}_j)[S,3])$ are calculated in relation to the values taken by "j" and is retained (and denoted by 3) the value " j" for which the multiple correlation coefficient is highest. Three "j' values result this way (denoted by 1 , 2 , and 3), indicating the set of three columns of matrix $\mathbf{B}[S,S]$ which correlates conveniently with the column matrix $\mathbf{C}_{et}[S,1]$.

(5) The procedure described above continues by increasing progressively the number of columns of matrix $\mathbf{B}[S,S]$ with which is correlated, by multiple linear regression, the column matrix $\mathbf{C}_{et}[S,1]$. The columns of matrix $\mathbf{B}[S,S]$, involved at this phase, include those retained in the previous phase and a column which hasn not been yet retained. It is obvious that, by increasing the number of columns in $\mathbf{B}[S,S]$, involved in the multiple correlation, the optimal correlation coefficient approaches progressively the ideal value r = 1. Because the columns in matrix $\mathbf{B}[S,S]$ are orthogonal, there is no danger that, at a certain phase, the maximum correlation coefficient will be exceeded by a correlation coefficient corresponding to a combination of columns including a column (therefore a "j" value) which has not been retained in a previous phase. If the columns in matrix $\mathbf{B}[S,S]$ were not orthogonal, the above-mentioned danger would have appeared. This justifies the transformation of matrix $\mathbf{A}[S,N]$ (whose columns are not generally orthogonal) into a matrix $\mathbf{B}[S,S]$ with orthogonal columns. In practice, the procedure continues until obtaining a compromise situation, namely a satisfactory multiple correlation coefficient at a minimum number of involved columns if matrix $\mathbf{B}[S,S]$.

(6) Following the correlations described above, a set of columns of matrix **B**[S,S] results. These have been retained and denoted by $\underline{1}$, $\underline{2}$, . . . , $\underline{J}$. A convenient set is then established, made up of wavelength values or, in other words, a set of $\underline{J}$ columns of the **A**[S,N] matrix. The set of $\underline{J}$ columns of matrix **A**[S,N] (conceived as $\underline{J}$ vectors in an imaginary S-dimensional space) is chosen so that each column of matrix **A**[S,N] presents a maximum covariance with a column of matrix **B**[S,S] retained during the above-mentioned operations. More concretely, if one suppose that the column of order "j" of matrix **B**[S,S] is associated to the column of order "i" of matrix **A**[S,N], it means that for the value "j" the column of order "i" of matrix **A**[S,N] ensures a maximum value of the covariance (of the correlation coefficient) calculated with relation (38).

$$r(\mathbf{A}_i[S,1],\mathbf{B}_j[S,1]) = \frac{\sum_{s=1}^{S}\left(A_s(\lambda_i)-\overline{A}(\lambda_i)\right)\cdot\left(B_{sj}-\overline{B}_j\right)}{\sqrt{\left[\sum_{s=1}^{S}\left(A_s(\lambda_i)-\overline{C}\right)^2\right]\cdot\left[\sum_{s=1}^{S}\left(B_{sj}-\overline{B}_j\right)^2\right]}} \tag{38}$$

In relation (38) $\overline{A}(\lambda_i)$ and $\overline{B}_j$ represent the arithmetic means of the corresponding matrix elements in columns of order "i", and "j" respectively.

$$\overline{A}(\lambda_i) = \frac{1}{S}\cdot\sum_{s=1}^{S} A_s(\lambda_i) \quad ; \quad \overline{B}_j = \frac{1}{S}\cdot\sum_{s=1}^{S} B_{sj}$$

In what follows, the wavelengths selected during phase (6) will be denoted by λ_1^* , λ_2^* , . . . , λ_J^*. By applying relation (38) for i = 1 , 2 , . . . , N and j = 1 , 2 , . . . , $\underline{J}$, the matrix **R**[N,$\underline{J}$] of the correlation coefficients is obtained (39).

$$\begin{bmatrix} r_{11} & \cdots & r_{1j} & \cdots & r_{1J} \\ \vdots & & \vdots & & \vdots \\ r_{i1} & \cdots & r_{ij} & \cdots & r_{iJ} \\ \vdots & & \vdots & & \vdots \\ r_{N1} & \cdots & r_{Nj} & \cdots & r_{NJ} \end{bmatrix} \tag{39}$$

In each column "j" of the matrix (39) an element r_{ij} with maximum absolute value is sought. The set of order numbers "i", which associates an "i" for each column"j", corresponds to the researched set of wavelengths.

(7) The equation system (15) is reconstructed, using only the set of wavelengths λ_1^* , λ_2^* , . . . , λ_J^* selected in previous phases.

$$\begin{bmatrix} c_1^{et} \\ c_2^{et} \\ \vdots \\ c_s^{et} \\ \vdots \\ c_S^{et} \end{bmatrix} = \begin{bmatrix} A_1(\lambda_1^*) & A_1(\lambda_2^*) & \cdots & A_1(\lambda_J^*) \\ A_2(\lambda_1^*) & A_2(\lambda_2^*) & \cdots & A_2(\lambda_J^*) \\ \vdots & \vdots & & \vdots \\ A_s(\lambda_1^*) & A_s(\lambda_2^*) & \cdots & A_s(\lambda_J^*) \\ \vdots & \vdots & & \vdots \\ A_S(\lambda_1^*) & A_S(\lambda_2^*) & \cdots & A_S(\lambda_J^*) \end{bmatrix} \cdot \begin{bmatrix} f(\lambda_1^*) \\ f(\lambda_2^*) \\ \vdots \\ f(\lambda_J^*) \end{bmatrix} \tag{40}$$

In order for the equation system (40) to be solvable in relation to the unknowns $f(\lambda_1^*)$, $f(\lambda_2^*)$, . . . , $f(\lambda_J^*)$, it is necessary that the number of selected wavelengths (J) be smaller than (or equal) to the number of standard samples (S). It is also essential that the determinant of matrix $\mathbf{D}[J,J]$, resulting after multiplying the transpose of system matrix $(\mathbf{A}^*)^{\mathbf{T}}[J,S]$ by the system matrix $(\mathbf{A}^*)[S,J]$ be significantly different from zero.

$$(\mathbf{A}^{*\mathbf{T}}[J,S]\cdot(\mathbf{A}^*)[S,J] = \mathbf{D}[J,J];\ \det(\mathbf{D}[J,J])\neq 0$$

At the simultaneous determination of several chemical components which do not interact chemically, the equation system (1) and (2) has been constituted, with the help of N standard solutions, measured at Λ distinct wavelength values. In order to correctly solve the analytical problem, it is recommendable that the spectra of the N standard solutions be "as distinct as possible", because in the extreme (and imaginary) case where two standard solutions had identical spectra, the equation system would be undetermined, so impossible to solve. It is necessary to rigorously express the requirement that the spectra be as "different as possible". A method of characterizing the difference between spectra consists in considering the absorbances of a standard solution, measured at the selected set of wavelengths, as components of a vector in the Λ-dimensional space. The N spectra of standard solutions will thus form a set of N vectors.

$$D_{Gramm} = \begin{vmatrix} \sum_{\lambda=1}^{\Lambda} A^1(\lambda)\cdot A^1(\lambda) & \cdots & \sum_{\lambda=1}^{\Lambda} A^1(\lambda)\cdot A^n(\lambda) & \cdots & \sum_{\lambda=1}^{\Lambda} A^1(\lambda)\cdot A^N(\lambda) \\ \vdots & & \vdots & & \vdots \\ \sum_{\lambda=1}^{\Lambda} A^n(\lambda)\cdot A^1(\lambda) & \cdots & \sum_{\lambda=1}^{\Lambda} A^n(\lambda)\cdot A^n(\lambda) & \cdots & \sum_{\lambda=1}^{\Lambda} A^n(\lambda)\cdot A^N(\lambda) \\ \vdots & & \vdots & & \vdots \\ \sum_{\lambda=1}^{\Lambda} A^N(\lambda)\cdot A^1(\lambda) & \cdots & \sum_{\lambda=1}^{\Lambda} A^N(\lambda)\cdot A^n(\lambda) & \cdots & \sum_{\lambda=1}^{\Lambda} A^N(\lambda)\cdot A^N(\lambda) \end{vmatrix} \quad (41)$$

The value of the *Gramm* determinant (41) of the vector set expresses quantitatively the difference between vectors. The higher the value of the determinant (41), the more satisfied the requirement that the standard spectra be "as different as possible". At a higher value of the *Gramm* determinant the absorbance measurement error affects to a smaller extent the precision of the final results.

4. Generalization the standard addition method for several components of interest

In a real sample, subjected to be analyzed, one must take into consideration that the sample contains, besides the substance of interest, various other ingredients. Although it is possible to choose a wavelength at which the absorbance of the substance of interest should be significant and the absorbance of the ingredients negligible, it may happen that the ingredients, through their presence, modify the molar absorptivity of the component of interest, and thus modify the sensitivity of the spectrophotometric response to the component of interest. This possibility is more plausible in real pharmaceutical products,

where the ingredients are found, as a rule, in a larger quantity than the active components. In this case, comparing the absorbance of the sample with that of a standard solution (which does not contain any ingredients) could provide erroneous analytical results. In order to realize even in these cases the quantitative determination of the active substance (the component of interest), one may resort to the "standard addition method" [Bosch-Reigh et al., 1991; Lozano et al., 2009 ; Szabadai, 2005; Valderrama & Poppi, 2009].

The reasoning of the addition method in the general case, when aiming to determine several components quantitatively, can be described with the help of the matrix calculation formalism [Szabadai, 2005]. The primary sample, in which the concentrations c_1 , c_2 , . . . , c_j , . . . , c_M of the M chemical components are analysed, is dissolved with an adequate solvent, bringing it to the final known volume V_a . A number of S + 1 equal portions (each having the volume "v") will be drawn from this solution. The portion number "0" is diluted to the final known volume V_b , thus obtaining the final solution of number "0" in which the concentrations of the components of interest are c_{10} , c_{20} , . . . , c_{M0}, and the concentration of ingredients is $c_{b(ing)}$. The portions number 1 , . . . , M are supplemented with known quantities of the M components of interest, so that, after completing to the final volume V_b, "S" solutions with modifications of known concentrations are obtained. In the final solution number "i", which was prepared by adding the masses m_{1i} , m_{2i} , . . . , m_{Mi} of individual components, the concentration modifications of components are Δc_{1i} , Δc_{2i} , . . . , Δc_{Mi} , whereas the concentration of ingredients remains the same in all S solutions, independent of "i". For each final solution the absorbance is measured at the same set of wavelengths λ_1 , λ_2 , . . . , λ_Λ. For the final solution number "0" the values $A_0(\lambda_1)$, $A_0(\lambda_2)$, . . . , $A_0(\lambda_L)$ are obtained. When measuring the absorbances of the final solutions of number 1 , 2 , . . . , S, at the same set of wavelengths and using the same optical path "d", the values $A_i(\lambda_1)$, $A_i(\lambda_2)$, . . . , $A_i(\lambda_L)$, i = 1 , 2 , . . . , S are obtained. The measured absorbances and the concentration modifications, generated by additions, can be arranged in matrix form. If $\varepsilon_j(\lambda)$ denotes the molar absorptivity of the component of order "j" at the wavelength "λ", the absorbances satisfy relations (42) and (43).

$$\frac{1}{d}\cdot\begin{bmatrix} A(\lambda_1) \\ \vdots \\ A(\lambda_\Lambda) \end{bmatrix} = \begin{bmatrix} \varepsilon_1(\lambda_1) & \cdots & \varepsilon_M(\lambda_1) \\ \vdots & & \vdots \\ \varepsilon_1(\lambda_\Lambda) & \cdots & \varepsilon_M(\lambda_\Lambda) \end{bmatrix}\cdot\begin{bmatrix} c_1 \\ \vdots \\ c_M \end{bmatrix} \tag{42}$$

$$\begin{aligned} \frac{1}{d}\cdot\begin{bmatrix} A^1(\lambda_1) & \cdots & A^S(\lambda_1) \\ \vdots & & \vdots \\ A^1(\lambda_\Lambda) & \cdots & A^S(\lambda_\Lambda) \end{bmatrix} &= \begin{bmatrix} \varepsilon_1(\lambda_1) & \cdots & \varepsilon_M(\lambda_1) \\ \vdots & & \vdots \\ \varepsilon_1(\lambda_\Lambda) & \cdots & \varepsilon_M(\lambda_\Lambda) \end{bmatrix}\cdot\begin{bmatrix} c_1+\Delta c_1^1 & \cdots & c_1+\Delta c_1^S \\ \vdots & & \vdots \\ c_M+\Delta c_M^1 & \cdots & c_M+\Delta c_M^S \end{bmatrix} = \\ &= \begin{bmatrix} \varepsilon_1(\lambda_1) & \cdots & \varepsilon_M(\lambda_1) \\ \vdots & & \vdots \\ \varepsilon_1(\lambda_\Lambda) & \cdots & \varepsilon_M(\lambda_\Lambda) \end{bmatrix}\cdot\left(\begin{bmatrix} c_1 & \cdots & c_1 \\ \vdots & & \vdots \\ c_M & \cdots & c_M \end{bmatrix}+\begin{bmatrix} \Delta c_1^1 & \cdots & \Delta c_1^S \\ \vdots & & \vdots \\ \Delta c_M^1 & \cdots & \Delta c_M^S \end{bmatrix}\right) = \\ &= \frac{1}{d}\cdot\begin{bmatrix} A(\lambda_1) & \cdots & A(\lambda_1) \\ \vdots & & \vdots \\ A(\lambda_\Lambda) & \cdots & A(\lambda_\Lambda) \end{bmatrix}+\begin{bmatrix} \varepsilon_1(\lambda_1) & \cdots & \varepsilon_M(\lambda_1) \\ \vdots & & \vdots \\ \varepsilon_1(\lambda_\Lambda) & \cdots & \varepsilon_M(\lambda_\Lambda) \end{bmatrix}\cdot\begin{bmatrix} \Delta c_1^1 & \cdots & \Delta c_1^S \\ \vdots & & \vdots \\ \Delta c_M^1 & \cdots & \Delta c_M^S \end{bmatrix} \end{aligned} \tag{43}$$

If the column matrix on the left member of the quation (42) is denoted by **A**[Λ,1], the matrix of molar absorptivities on the right member of the equation (42) by **E**[Λ,M] and the column matrix of the concentrations on the right member of the same equation by **C**[M,1], the equation (42) takes the form (44).

$$(1/d)\cdot\mathbf{A}[\Lambda,1] = \mathbf{E}[\Lambda,M]\cdot\mathbf{C}[M,1] \tag{44}$$

If equation (42) is subtracted, member by member, from equation (43) the result is equation (45).

$$\begin{aligned}\frac{1}{d}\cdot\left(\begin{bmatrix} A^1(\lambda_1) & \cdots & A^S(\lambda_1) \\ \vdots & & \vdots \\ A^1(\lambda_\Lambda) & \cdots & A^S(\lambda_\Lambda)\end{bmatrix} - \begin{bmatrix} A(\lambda_1) & \cdots & A(\lambda_1) \\ \vdots & & \vdots \\ A(\lambda_\Lambda) & \cdots & A(\lambda_\Lambda)\end{bmatrix}\right) = \\ = \begin{bmatrix} \varepsilon_1(\lambda_1) & \cdots & \varepsilon_M(\lambda_1) \\ \vdots & & \vdots \\ \varepsilon_1(\lambda_\Lambda) & \cdots & \varepsilon_M(\lambda_\Lambda)\end{bmatrix}\cdot\begin{bmatrix} \Delta c_1^1 & \cdots & \Delta c_1^S \\ \vdots & & \vdots \\ \Delta c_M^1 & \cdots & \Delta c_M^S\end{bmatrix}\end{aligned} \tag{45}$$

$$\begin{aligned}\frac{1}{d}\cdot\begin{bmatrix} A^1(\lambda_1)-A(\lambda_1) & \cdots & A^S(\lambda_1)-A(\lambda_1) \\ \vdots & & \vdots \\ A^1(\lambda_\Lambda)-A(\lambda_\Lambda) & \cdots & A^S(\lambda_\Lambda)-A(\lambda_\Lambda)\end{bmatrix} = \\ = \begin{bmatrix} \varepsilon_1(\lambda_1) & \cdots & \varepsilon_M(\lambda_1) \\ \vdots & & \vdots \\ \varepsilon_1(\lambda_\Lambda) & \cdots & \varepsilon_M(\lambda_\Lambda)\end{bmatrix}\cdot\begin{bmatrix} \Delta c_1^1 & \cdots & \Delta c_1^S \\ \vdots & & \vdots \\ \Delta c_M^1 & \cdots & \Delta c_M^S\end{bmatrix}\end{aligned}$$

Denoting by **ΔA**[Λ,S] the matrix of differences of absorbances in equation (45) and the matrix of concentration differences in (45), by **ΔC**[M,S], the resulting relation has the form (46).

$$(1/d)\cdot\mathbf{\Delta A}[\Lambda,S] = \mathbf{E}[\Lambda,M]\cdot\mathbf{\Delta C}[M,S] \tag{46}$$

The matrix **E**[Λ,S] is expressed from equation (46), and in its possession the equation (44) may be solved in relation to the column matrix **C**[M,1]. The necessary (but not sufficient) condition for solvency the equations in relation to matrix **C**[M,1] is that Λ should be higher than (or equal) to M or S should be higher than (or equal to) M.

$$\Lambda \geq M \quad sau \quad S \geq M \tag{47}$$

In order to express the matrix **E**[Λ,M], both sides of the relation (46) will be multiplied on the right by the transpose of matrix **ΔC**[M,S].

$$(1/d)\cdot\mathbf{\Delta A}[\Lambda,S]\cdot\mathbf{\Delta C}^{\mathbf{T}}[S,M] = \mathbf{E}[\Lambda,M]\cdot\mathbf{\Delta C}[M,S]\cdot\mathbf{\Delta C}^{\mathbf{T}}[S,M] \tag{48}$$

Both sides of (48) are then multiplied by the inverse of matrix **ΔC**[M,S]·**ΔC** $^{\mathbf{T}}$[M,S]. Relation (49) is obtained, representing the explicit form of matrix **E**[Λ,M].

$$(1/d)\cdot\mathbf{\Delta A}[\Lambda,S]\cdot\mathbf{\Delta C}^{T}[S,M]\cdot(\mathbf{\Delta C}[M,S]\cdot\mathbf{\Delta C}^{T}[S,M])^{-1}=\mathbf{E}[\Lambda,M] \quad (49)$$

The concentration matrix **C**[M,S] is expressed from relation (44). To this purpose, equation (44) is multiplied on the left by the transpose of matrix **E**[Λ,M].

$$(1/d)\cdot\mathbf{E}^{T}[M,\Lambda]\cdot\mathbf{A}[\Lambda,1]=\mathbf{E}^{T}[M,\Lambda]\cdot\mathbf{E}[\Lambda,M]\cdot\mathbf{C}[M,1] \quad (50)$$

When the above relation is multipled on the left by $(\mathbf{E}^{T}[M,\Lambda]\cdot\mathbf{E}[\Lambda,M])^{-1}$, the explicit form of the concentration matrix results (51).

$$(1/d)\cdot(\mathbf{E}^{T}[M,\Lambda]\cdot\mathbf{E}[\Lambda,M])^{-1}\cdot\mathbf{E}^{T}[M,\Lambda]\cdot\mathbf{A}[\Lambda,1]=\mathbf{C}[M,1] \quad (51)$$

The particular case of standard addition method applied to a system with two components to be determined, is illustrated graphically in Figure 2. In this case, the procedure is reduced to determining the plane π passing through a number of figurative points and to reading the intersection points of this plane with the negative semi-axes of the concentrations.

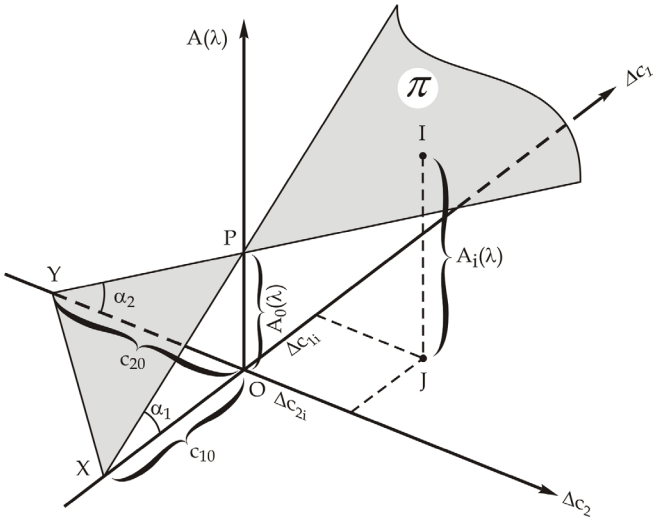

Fig. 2. Graphic representation of absorbances $A_i(\lambda)$ in relation to the modifications of concentrations Δc_{1i} and Δc_{2i} (i = 1 , 2 , . . . , n)

At the graphic representation of absorbances $A_i(\lambda)$ vs. the increase of concentrations Δc_{1i} and Δc_{2i} (i = 1 , 2 , . . . , n), the figurative points are situated theoretically on a plane (denoted by π in Figure 4-20). The axis of absorbances is intersected by plane π in point P, corresponding to the absorbance $A_0(\lambda)$, measured in the case of the solution with i = 0. If at the selected wavelength (λ) the absorbance of the ingredients can be left out, the points X and Y, situated at the intersection of plane π with the negative parts of axes Δc_1 and Δc_2, have the coordinates $-c_{10}$ respectively $-c_{20}$ (in other words, the lengths of the segments OX and OY are proportional to the concentrations c_{10} and c_{20}). From the values c_{10} and c_{20}, and knowing the volumes V_a, v and V_b, one may calculate the concentrations c_1 and c_2 of the components of interest in the first solution, and finally their content in the primary sample.

4.1 Example

In order to illustrate the application of the standard addition method and of the subsequent data processing procedure, let consider the mixture of salicylic acid, caffeine and acetaminophen, discussed in a previous example. The aim is to determine the concentrations of the three chemical components. Table 2 includes the modifications of the component concentrations (5 modifications are performed) and the absorbances both for the original solution (where concentrations have not been modified) and for the five solutions in which the three chemical components have been modified. All absorbance values are read at the same set of 18 wavelengths ($\Lambda = 18$).

The elements of matrix **E** are calculated with relation (49) and are expressed in the tolerated unit of measure l/(mol·cm), employed in spectrophotometric practice, and the elements of matrix **C**, calculated with relation (51) are expressed in μmol/l.

Table 2

	Modified solution 1	Modified solution 2	Modified solution 3	Modified solution 4	Modified solution 5	Original solution
Absorbance values at 18 wavelengths (d = 1 cm)	0.555	0.553	0.579	0.660	0.677	0.453
	0.519	0.525	0.552	0.627	0.637	0.424
	0.454	0.469	0.496	0.564	0.560	0.370
	0.382	0.405	0.430	0.498	0.471	0.311
	0.354	0.374	0.397	0.467	0.431	0.287
	0.368	0.378	0.398	0.476	0.439	0.297
	0.394	0.390	0.408	0.495	0.460	0.317
	0.427	0.410	0.426	0.523	0.490	0.343
	0.466	0.440	0.455	0.560	0.531	0.374
	0.443	0.416	0.430	0.526	0.506	0.356
	0.350	0.329	0.340	0.409	0.405	0.283
	0.207	0.195	0.201	0.229	0.247	0.168
	0.119	0.111	0.115	0.120	0.149	0.098
	0.080	0.074	0.076	0.076	0.101	0.066
	0.045	0.041	0.043	0.042	0.057	0.037
	0.023	0.022	0.022	0.023	0.029	0.019
	0.014	0.014	0.014	0.016	0.018	0.012
	0.012	0.011	0.012	0.013	0.014	0.009
Δc_i μmol/l) Salicylic acid	3.40	1.50	2.20	0.60	10.00	
Caffeine	2.60	1.50	1.80	5.00	4.00	
Acetaminophen	1.20	2.20	2.80	4.00	3.00	

$$\mathbf{E} = \begin{pmatrix} 6721.72 & 17534.60 & 28741.06 \\ 5978.40 & 14156.50 & 32098.95 \\ 4390.01 & 10405.40 & 34854.40 \\ 1832.22 & 8685.62 & 35571.34 \\ 675.67 & 10070.25 & 32288.01 \\ 426.77 & 14575.14 & 26461.11 \\ 463.41 & 20233.58 & 19124.73 \\ 575.88 & 25871.08 & 12545.68 \\ 1045.49 & 29980.13 & 8855.66 \\ 1617.15 & 28153.68 & 7048.81 \\ 2330.58 & 20505.79 & 5443.55 \\ 3248.96 & 8960.11 & 3602.66 \\ 3540.99 & 2713.00 & 1580.26 \\ 2852.64 & 1204.43 & 613.63 \\ 1694.67 & 550.32 & 343.30 \\ 719.08 & 434.12 & 355.28 \\ 292.02 & 443.65 & 350.29 \\ 209.16 & 417.53 & 502.18 \end{pmatrix} \quad ; \quad \mathbf{C} = \begin{pmatrix} 17.819 \\ 10.290 \\ 5.307 \end{pmatrix} \mu mol / l$$

5. Conclusions

The application of matrix algebra to the quantitative spectrophotometry provides a unified formalism for treatment the mathematical issues. Unlike the usual mathematical approaches, the matrix description of the phenomena behind the analytical spectrophotometry promise new dimensions for the automatic processing of results.

6. References

Bosch-Reigh, F., Campins-Falco, P., Sevillano-Cabeza, A., Herraes-Hernandez, R., & Molins-Legua C. (1991). Development of the H-Point Standard Addition Method for Ultraviolet-Visible Spectroscopic Kinetic Analysis of Two-component Systems, *Analytical Chemistry*, Vol.63, No.21, pp. 2424-2429

Burnius, E. (1959). Assay of Vitamin A Oils, *Journal of the American Oil Chemistry's Society*, Vol.35, No.4, pp. 13-14

Ewing, D.T., Sharpe L.H., & Bird O.D. (1953). Determination of Vitamin A in Presence of Tocopherols, *Analytical Chemistry,* Vol.25, No.4, pp. 599-604, ISSN 0003-2700

Fox, S.H. & Mueller, A. (1950). The Influence of Tocopherols on U.S.P. XIV Vitamin Assay, *Journal of the American Pharmaceutical Association*, Vol.39, No.11, pp.621-623

Garrido, M., Lázaro, I., Larrechi, M.S., & Rius F.X. (2004). Multivariate Resolution of Rank-deficient Near-infrared Spectroscopy Data fron the Reaction of Curing Epoxi Resins Using the Rank Augmentation Strategy and Multivariate Curve Resolution Alternating Least Squares Approach, *Analytica Chimica Acta*, Vol.515. No.1, pp.65-73

Garrido, M., Rius, F.X., & Larrechi, M.S. (2008). Multivariate Curve Resolution-alternating Least Squares (MCR-ALS) Applied to Spectroscopic Data from Monitoring Chemical Reaction Processes, *Anal. Bioanal. Chem.*, Vol.390, No.8, pp. 2059-2066

Li, N., Li, X.Y., Zou, Z.X., Lin, L.R., & Li, Y.Q. (2011). A Novel Baseline-correction Method for Standard Addition Based Derivative Spectra and its Applications to Quantitative Analysis of benzo(a)pyrene in Vegetable Oil Samples, *Analyst*, Vol.136, pp. 2802-2810

Lozano, V.A., Ibañez, G.A., & Olivieri, A.C. (2009). A Novel Second-order Standard Addition Analytical Method Based on Data Processing with Multidimensional Partial Least-squares and Residual Bilinearization, *Analytica Chimica Acta*, Vol.651, No.2, pp. 165-172

Lykkesfeld, J. (2001). Determination of Malonaldehyde as Dithiobarbituric Acid Adduct in Biological Samples by HPLC with Fluorescence Detection: Comparison with Ultraviolet-Visible Spectrophotometry, *Clinical Chemistry*, Vol.47, pp.1725-1727

Melnick, D., Luckmann, F.H., & Vahlteich, H.W. (1952). Estimation of Vitamin A in Margarine. I. Collaborative Study of Assay Methods for Estimating the Potencu of the Vitamin A Concentrates, *Journal of the American Oil Chemistry's Society*, Vol.29, No.3, pp. 104-108

Morton, R.A., & Stubbs A.L., (1946). *Photoelectric Spectrophotometry Applied to the Analysis of Mixtures and Vitamin A Oils*, Vol.71, pp. 348-350

Morton, R.A., & Stubbs, A.L. (1947). A Re-examination of Halibut-liver Oil. Relation Between Biological Potency and Ultraviolet Absorption Due to Vitamin A, *Biochem. J.*, Vol.41, pp. 525-529

Morton, R.A., & Stubbs, A.L. (1948). Studies in Vitamin A. 4. Spectrophotometric Determination of Vitamin A in Liver Oils. Correction for Irrelevant Absorption, *Biochem. J.*, Vol.42, No.2, pp. 195-203

Oka, K., Oshima, K., Inamoto, N., & Pishva D. (1991). Chemometrics and Spectroscopy, *Analytical Sciences*, Vol.7, pp.757-760, ISSN 0910-6340

Owen, A.J. (1995). Quantitative UV-Visible Analysis in the Presence of Scattering" Application Notes – Agilent Technologies, *Pharmaceutical Analysis*, (publication number: 5963-3937E)

Page, R.C., & Berkovitz, Z. (1943). The Absorption of Vitamin A in Chronic Ulcerative Colitis, *American Journal of Digestive Diseases*, Vol.10, No.5, pp. 174-177

Ruckenbusch, C., De Juan, A., Duponchel, L., & Huvenne, J.P. (2006). Matrix Augmentation for Breaking Rank-deficiency: A Case Study, *Chemometrics and Intelligent Laboratory Systems*, Vol.80, No.2, pp. 209-214

Sánchez, E.; & Kowalski, B.R. (1986). Generalized Rank Annihilation Factor Analysis, *Analytical Chemistry,* Vol.58, pp. 496-499, ISSN 0003-2700

Szabadai Z. (2005). *Bazele fizico-chimice ale metodelor de control analitic al medicamentelor*, Editura Mirton, Vol. II., pp. 4.38-4.96, ISBN: 973-661-677-0, Timişoara, Romania

Valderrama, P., & Poppi R.J., (2009). Second Order Standard Addition Method and Fluorescence Spectroscopy in the Quantification of Ibuprofen Enantiomers in Biological Fluids, *Chemometrics and Intelligent Laboratory Systems*, Vol.106, No.2, pp. 160-165.