Journal of Chemical Health Risks

Journal of Chemical Health Risks (2015) 5(2), 81–98

ORIGINAL ARTICLE

Quantitative Analysis of Mixtures of Monoprotic Acids Applying Modified Model-Based Rank Annihilation Factor Analysis on Variation Matrices of Spectrophotometric Acid-Base Titrations

Ebrahim Ghorbani-Kalhor[*], **Jafar Abolhasani, Mahsa Veladi**

Department of Chemistry, Tabriz Branch, Islamic Azad University, Tabriz, Iran

(Received: 29 December 2014 Accepted: 28 February 2015)

KEYWORDS

Chemical equilibrium model
Monoprotic acid
Rank annihilation factor analysis
Spectrophotometric titration
Variation matrix

ABSTRACT: In the current work, a new version of rank annihilation factor analysis was developed to circumvent the rank deficiency problem in multivariate data measurements. Simultaneous determination of dissociation constant and concentration of monoprotic acids was performed by applying model-based rank annihilation factor analysis on variation matrices of spectrophotometric acid-base titrations data. Variation matrices can be obtained by subtracting first row of data matrix from all rows of the main data matrix. This method uses variation matrices instead of multivariate spectrophotometric acid-base titrations matrices to circumvent the rank deficiency problem in the rank quantitation step. The applicability of this approach was evaluated by simulated data at first stage, then the binary mixtures of ascorbic and sorbic acids as model compounds were investigated by the proposed method. At the end, the proposed method was successfully applied for resolving the ascorbic and sorbic acid in an orange juice real sample. Therefore, unique results were achieved by applying rank annihilation factor analysis on variation matrix and using hard soft model combination advantage without any problem and difficulty in rank determination.

INTRODUCTION

In analytical chemistry, quantitative analysis of multicomponent systems with overlapping spectrum and complex matrices in black or gray systems without any primitive or some incomplete information about

* Corresponding author: ekalhor@iaut.ac.ir (E. Gh-K).

components is one of the goals of the chemometrics methods. Multivariate calibration methods, a broad branch of chemometrics-based methods, are employed for resolving this problem. First order, second order, and higher order methods are the branches of multivariate calibrations which utilize first, second, and higher order data, respectively [1]. In first-order multivariate calibration which uses first order data, the matrix and nature of standard samples must be similar to those of the unknown samples, otherwise, the effect of unknown components in real samples cannot be modeled [2]. Therefore, accurate results cannot be obtained. In addition, the model building in these methods based on calibration set requires a lot of experiments [3]. Hence, second order calibrations such as parallel factor analysis (PARAFAC) [4], multivariate curve resolution (MCR) [5], and rank annihilation factor analysis (RAFA)[6-8] are applied to overcome the aforementioned disadvantages even in the presence of unknown and uncalibrated interferences. These methods frequently utilize only pure analyte standards to quantify unknown samples. This feature is known as second order advantage [9]. Instead of large set of samples required in first-order calibration, second order calibrations can be performed with a few samples.

Using diverse approaches such as MCR, RAFA etc. second order acid–base spectroscopic titration data have been used in the literature [10-12] for quantitative analysis of spectroscopic and acid base behavior of components simultaneously as a result of higher order dimensions. Among them, RAFA has many individual advantages compared with similar methods. In contrast to some other second order calibrations like MCR, quantitative analysis can be performed using RAFA without any augmentation of the matrix of the unknown and standard samples [10]. Moreover, RAFA avoids the need to estimate initial concentration or spectral profiles using different complex factor analysis based methods such as evolving factor analysis (EFA) and simple to use interactive self-modeling mixture analysis (SIMPLISMA). Therefore, the answers can be easily achieved without any ambiguity. In addition, application of RAFA on second order data obviates the need for selection and implementation of several constraints such as non-negativity, unimodality, and closure which is an important factor to achieve convergency in MCR [13]. RAFA has been used in quantitative analyses of mixtures of monoprotic acids[10], dipropic acids[14], polyproticacids[6], conditional acidity constant of organic acids [15] and some other components based on multivariate pH-spectrophotometric titrations[12, 16].

In monoprotic acids, RAFA can be easily applied to second order acid-base spectrophotometric data in order to find the concentration and corresponding dissociation constant, simultaneously [10]. The procedure uses chemical rank, the number of components for quantification of analytes along with calculation of dissociation constant. The chemical rank–which can be estimated by singular value decomposition or other related factor analysis techniques– is the number of significant contributions to the data variance; In some cases, especially in analyzing complex chemical systems such as the mixtures of substances with acid–base behavior, this rank is lower than the real number of chemical components present in the system and the data matrix is the rank-deficient. Then, the number of independent species will be lower than that of real chemical species in the system. Consequently, the application of this method for quantification of monoprotic acid is not straightforward because obtaining the correct rank of the system and accurate results are not possible.

For resolving this problem in parallel reaction systems, the variation matrix is exploited instead of the original data matrix in this study. Simply, the variation matrix including both analyte and unknown sample can be acquired by subtracting the zero-point spectrum from each spectrum at each measurement point.

In a system involving parallel reactions, if the original data matrix is converted into variation data matrix, a full rank matrix will be found based on the reaction rank instead of the chemical rank. In other words, the matrix reaction rank is the number of independent reactions or processes in which they take place. Using this approach, a chemical rank-deficient system can be converted to full-rank reaction system by a simple pretreatment.

The variation matrix can be decomposed in two vectors containing reaction extent vector and reaction spectrum. The reaction extent vector of monoprotic acid can be expressed as a function of total concentration of analyte and dissociation constant. This vector is calculated based on the respective dissociation constant and the concentration of the analyte. The reaction spectrum of the analyte is estimated by least squares method using reconstructed reaction extent vector and standard variation matrix of analyte. Computed reaction spectrum by least squares method and the reconstructed reaction extent vector based on hard chemical models, are multiplied by each other and hence make some variation matrices with similar dimensions as the unknown sample variation matrix. Over a wide range, the concentration and dissociation constant of an analyte are altered simultaneously and the reaction extent vector is calculated at each step. By applying RAFA on the estimated analyte variation matrix and the unknown sample variation matrix, the best values will be achieved for analyte concentration and the related dissociation constant on condition that the rank of unknown sample variation matrix decreases, correctly.

The main aim of this study was to improve a model based on RAFA strategy for quantification of analytes and subsequent calculations of model parameters based on the reaction rank instead of the chemical rank discarding any problem and ambiguity in rank determination. The modifications of the RAFA were investigated with simulated data at the first stage. Then, the binary mixtures of ascorbic and sorbic acids as synthetic samples were prepared and good analytical results were obtained using the proposed procedure. Finally, the method was successfully applied to the analysis of experimental pH-spectral data of ascorbic and sorbic acids in a real sample. To the best of our knowledge, this is the first study, which focuses on analyzing the acidic-spectrophotometric data by combining the variation matrix and the rank annihilation factor analysis.

MATERIALS AND METHODS

Materials

All materials with analytical grade were purchased from Merck (Darmstadt, Germany) and were used without further purification. Stock solutions of sorbic acid were prepared in brown flask and were stored in dark place. These solutions are stable for six months in dark place. Stock solution of ascorbic acid were prepared and used daily.

Apparatus

Shimadzu UV-1800 spectrophotometer equipped with matched 1 cm quartz cells were applied for recording all UV-spectra over the range 200-350 nm with 1nm intervals. All pH measurements were carried out with Metrohm 744 digital pH meter using a combined glass electrode. For subsequent manipulation of data by RAFA, all spectra data were transferred into a personal computer. Homemade m-files written in MATLAB7 software were used for calculations of concentration and dissociation constant of all analytes.

Preparing the real sample

The real sample (orange juice) was first completely homogenized, filtered through a 0.45 mm filter, and degassed by introducing nitrogen into it.

Procedure

Fresh working solutions were prepared daily by appropriate dilution of stock solution. The adjustment of pH and titration of samples were performed with standard solutions of hydrochloric acid and sodium hydroxide ranging between 0.01–12 mol L^{-1}. In order to adjust the ionic strength of all solutions to approximately 0.1 mol L^{-1}, all experiments were fulfilled in 0.1 mol L^{-1} KCl. In the beginning of titration, all working solutions were prepared with 0.1 mol L^{-1} HCl (pH=1). Following this stage, in order to increase the pH value in each step of titration with 0.5 unit intervals, a few microliter of concentrated NaOH were injected into the solution. Since concentration of NaOH was high enough (12 mol L^{-1}) and a very small amount of it was needed for titration, the added volume did not change the total volume of solutions and therefore it did not significantly affect the initial concentration of all samples.

Theoretical background

Variation matrix, reaction extent vector and reaction spectrum vector

Consider a typical monoprotic acid base equilibrium titration reaction:

$$HA \rightleftharpoons A^- + H^+ \qquad \text{(eqn. 1)}$$

The reaction extent can be defined as follows [17, 18]:

$$\varepsilon_{ra} = -\delta c_{HA} = \delta c_{A^-} = \delta c_J \qquad \text{(eqn. 2)}$$

Where $\boldsymbol{\delta}c_J$ is concentration variation vector whose elements show concentration variation between initial point and that point of titration and $\boldsymbol{\varepsilon}_{ra}$ is the reaction extent vector whose elements represent the reaction extent at these points. Subscript a stands for HA monoprotic acid.

The progress of chemical reaction is being shown by the reaction extent. Generally, the previous equation can be re-written as follows:

$$\varepsilon_{ri} = \delta c_J \quad \text{or} \quad \varepsilon_{ri} = c_J - 1c_J^o \quad \text{or} \quad c_J = \varepsilon_{ri} + 1c_J^o \qquad \text{(eqn. 3)}$$

Where c_J^o is the concentration of component J at zero point and **1** is a column vector all element of which is 1.

Then, the concentration of HA and A^- can be calculated using the following vectors:

$$c_{HA} = -\varepsilon_{ra} + 1c_{HA}^o \qquad \text{(eqn. 4)}$$

$$c_{A^-} = \varepsilon_{ra} + 1c_{A^-}^o \qquad \text{(eqn. 5)}$$

Consider $D_{\mathbf{a}}$ matrix obtained by acid-base spectrophotometric titration of monoprotic acid (HA). This matrix can be divided into concentration and spectral matrix as:

$$D_{\mathbf{a}} = CS^T \qquad \text{(eqn.6)}$$

Where C is concentration matrix the columns of which are pure concentration pH dependent acid components and rows of S^T contains pure spectral profile of related components. Each row of absorbance data matrix, $D_{\mathbf{a}}$, is UV-visible spectra recorded at any step of titration. Hence:

$$D_a = CS^T = [c_{HA}\ c_{A^-}]\begin{bmatrix} s_{HA}^T \\ s_{A^-}^T \end{bmatrix} \qquad \text{(eqn.7)}$$

Where $\mathbf{s}_{HA}$ and $\mathbf{s}_{A^-}$ are the pure spectra of HA and A^-, respectively. Here the following equation can be achieved by replacing equation 7 with the equivalent expressions (equations 4 and 5):

$$D_a = [-\varepsilon_{ra} + 1c_{HA}^o \varepsilon_{ra} + 1c_{A^-}^o]\begin{bmatrix} s_{HA}^T \\ s_{A^-}^T \end{bmatrix} = \varepsilon_{ra}(-s_{HA}^T + s_{A^-}^T) + 1(c_{HA}^o s_{HA}^T + c_{A^-}^o s_{A^-}^T) \qquad \text{(eqn.8)}$$

$$\text{Let } s_{ra}^T = -s_{HA}^T + s_{A^-}^T \text{ and } d_a^{oT} = c_{HA}^o s_{HA}^T + c_{A^-}^o s_{A^-}^T$$

Hence, the equation 8 can be summarized as follows:

$$D_a = \boldsymbol{\varepsilon}_{ra}\mathbf{s}_{ra}^{T} + 1d_a^{oT} \quad \text{(eqn.9)}$$

Where $\mathbf{s}_{ra}^{T}$ and d_a^{oT} are the reaction spectrum and zero point spectrum of monoprotic acid (HA), respectively. In this case, d_a^{oT} can be obtained simply by recording the absorbance spectrum at the initial point of reaction.

On the other hand, reaction spectrum, a constant vector, is achieved by linear combination of the pure spectra of the components, which contribute to the reaction. In this vector, the coefficient of pure spectrum will be negative if the component is reactant and positive if it is product. The variation matrix is constructed by multiplying reaction spectrum by their related reaction extent vector, which is related to, the concentration [17, 19]. Hence:

$$D_a = \varepsilon_{ra}s_{ra}^{T} + 1d_a^{oT} = V_a + 1d_a^{oT} \text{ or } V_a = D_a - 1d_a^{oT} \quad \text{(eqn.10)}$$

Where V_a is the variation matrix for HA acid.

When zero point spectrum of HA is subtracted from any row of the data matrix D_a, the variation matrix is concluded. In other words, if one subtracts the first row of data matrix from all rows of the same data matrix the variation matrix will easily be obtained as:

$$V_a = D_a - 1D_a(1,:) \quad \text{(eqn.11)}$$

Variation matrix, reaction extent matrix, and reaction spectra matrix

The variation matrix of mixture of monoprotic acids is calculated in the same way as single monoprotic acid.

Consider a system in which titration reaction for a mixture of two monoprotic acids (HA, HB) takes place. The independent reactions are as follows:

$$HA \rightleftharpoons A^- + H^+ \quad \text{(eqn.12)}$$

$$HB \rightleftharpoons B^- + H^+ \quad \text{(eqn.13)}$$

According to the beer law, considering the independent behavior of the components, the total absorbance data matrix of mixture (D_x) can be expressed as:

$$D_x = D_a + D_b \quad \text{(eqn.14)}$$

Subscripts a and b stand for HA and HB monoprotic acid, respectively.

The data matrices (D_a and D_b) are replaced with the related equation (equation 10):

$$D_x = D_a + D_b = \varepsilon_{ra}s_{ra}^{T} + 1d_a^{oT} + \varepsilon_{rb}s_{rb}^{T} + 1d_b^{oT}$$
$$= [\varepsilon_{ra}\varepsilon_{rb}]\begin{bmatrix} s_{ra}^{T} \\ s_{rb}^{T} \end{bmatrix} + 1\left[d_a^{oT} + d_b^{oT}\right]$$

$$D_x = E_{rx}S_{rx}^{T} + 1d_x^{oT} = V_x + 1d_x^{oT} \quad \text{(eqn.15)}$$

Where E_{rx}, S_{rx}^{T}, V_x and $\mathbf{d}_x^{oT}$ are the reaction extent matrix, reaction spectra matrix, variation matrix and zero point spectrum of the mixture, respectively. The variation matrix can be constructed by subtracting the first row of data matrix D_x from all rows of this data matrix:

$$V_x = D_x - 1d_x^{oT} \text{ or } V_x = D_x - 1D_x(1,:) \quad \text{(eqn.16)}$$

Finally, the abstract form of variation data matrix can be re-written as follows:

$$V_x = \varepsilon_{ra}s_{ra}^{T} + \varepsilon_{rb}s_{rb}^{T} \text{ or } V_x = V_a + V_b \quad \text{(eqn.17)}$$

The rank of a matrix is called reaction rank on condition that it is based on a number of independent reactions in which they take place. Then the variation matrix will be a full rank matrix based on the number of monoprotic acids as analytes involved in one step reaction. In other words, the rank of the variation matrix containing K independent reaction or processes is equal to the number of reactions (K) for a closed system [17, 18].

Hence, RAFA can be applied on the variation matrix easily to quantify the dissociation constant and concentration of monoprotic acids without any ambiguity, as it is explained in the next section.

Performing modified model-based RAFA on variation matrices

Consider the acid-base behavior of a monoprotic acid (HA) in unknown samples containing some inert interference and other some pH-dependent interference. The equilibrium, which explains the dissociation of monoprotic acid (HA), is as follows:

$$HA \rightleftharpoons A^- + H^+ \quad \text{(eqn.18)}$$

With equilibrium constant equal to:

$$k = \frac{[H^+]c_{A^-}}{c_{HA}} \quad \text{(eqn.19)}$$

Here, the total concentration of acid (c_{HAt}) can be shown as:

$$c_{HAt} = c_{HA} + c_{A^-} \quad \text{(eqn.20)}$$

By combining this equation with the equilibrium constant of an analyte, one can calculate the equilibrium concentration values of each form of the monoprotic acid:

$$c_{HA} = c_{HAt} \times \frac{[H^+]}{[H^+]+k} \quad \text{(eqn.21)}$$

$$c_{A^-} = c_{HAt} \times \frac{k}{[H^+]+k} \quad \text{(eqn.22)}$$

As mentioned before in equation 5, the reaction extent vector can be calculated as follows:

$$\varepsilon_{ra} = c_{A^-} - 1c^{o}_{A^-} \quad \text{(eqn.23)}$$

The concentration of A^- at initial point ($c^{o}_{A^-}$) will be zero on condition that the start point of titration is placed at low pH values. Given this:

$$\varepsilon_{ra} = c_{A^-} \quad \text{(eqn.24)}$$

$$\varepsilon_{ra} = c_{HAt} \times \frac{k}{[H^+]+k}$$

It is obvious that the reaction extent vector of monoprotic acid (HA) is explained as a function of two kinds of parameters: the total acid-base system concentration (c_{HAt}) related to the analytical information sought and acidic dissociation constant (k) related to the physicochemical behavior of the monoprotic acid.

As stated above, the variation matrix of monoprotic acid (V_a) is constructed by multiplying reaction spectrum($\mathbf{s}^T_{ra}$) by their related reaction extent vector ($\boldsymbol{\varepsilon}_{ra}$) which is related to the concentration. This bilinear combination of ($\boldsymbol{\varepsilon}_{ra}$) and($\mathbf{s}^T_{ra}$) can be shown as follows:

$$V_a = \varepsilon_{ra} s^T_{ra} \quad \text{(eqn. 25)}$$

In addition, the variation matrix of unknown sample (V_x) which is containing some inert interference or other some pH-dependent interference can be obtained easily by subtracting the first row of data matrix$\mathbf{D_x}$ from all rows of this data matrix.

$$V_x = D_x - 1D_x(1,:) \quad \text{(eqn. 26)}$$

$$V_x = \varepsilon_{ra} s^T_{ra} + \varepsilon_{rb} s^T_{rb} + \varepsilon_{rc} s^T_{rc} + \cdots = V_a + V_R \quad \text{(eqn. 27)}$$

A portion of V_x matrix which expresses contribution of analyte (HA monoprotic acid) in the model is the variation matrix of monoprotic acid V_a and the other portion is residual variation matrix V_R, containing any other independent reaction or process in the sample except analyte.

In principal component analysis (PCA) the contribution of reaction rank in the variance of the data matrix is much greater than the noise effects or instrumental contributions. In addition, the variation matrix is a full-rank matrix in terms of the number of the independent reaction or process. Hence, the reaction rank of variation

matrix V_x can be easily obtained by applying singular value decomposition (SVD). In this way, the reaction rank of V_x matrix is the number of singular values whose variance is larger than that of noise. Therefore, correct reaction rank quantification of variation matrix even in the presence of inert interfering agents or with any number of closed equilibrium systems besides analyte is very simple and straightforward. It is evident that there is not any rank deficiency problem and difficulty in the matrix quantitation. For this reason, we do not require any further approach for resolving rank deficiency problem such as matrix augmentation or entering other constraints in the model.

Given these points, consider the unknown sample variation matrix V_x and standard (monoprotic acid) variation matrix V_a possesses rank of n and of 1, respectively. Since these matrices aren't rank deficient, if one subtract matrix V_a from V_x, the obtained matrix will comprise rank of n-1.

As mentioned before, in equation 24, the reaction extent vector of monoprotic acid ($\boldsymbol{\varepsilon}_{ra}$) in the sample can be expressed as a function of two kinds of known parameters, the total concentration of analyte (c_{HAt}) and dissociation constant (k). If reaction spectrum ($\mathbf{s}_{ra}^{T}$) for analyte is known, RAFA procedure can be performed as follows:

$$V_R = V_x - V_a = V_x - \varepsilon_{ra} s_{ra}^{T} \qquad \text{(eqn. 28)}$$

The aim of RAFA application in monoprotic acid quantitation is to find the best parameters at reaction extent vector of monoprotic acid that can reduce the rank of V_x matrix to n-1 unit. For this purpose, the analyte concentration (c_{HAt}) and the dissociation constant (k) of HA are changed over a wide range and then rank of V_x matrix is investigated. By using the reconstructed reaction extent vector and standard variation matrix of HA, the reaction spectrum of the analyte was computed by the least squares method. It should be noted that the calculated reaction extent vector is multiplied by α scalar, defined as:

$$\alpha = \frac{C_{est}}{C_s} \qquad \text{(eqn. 29)}$$

Where C_s is the concentration of analyte in standard pH-spectral data and C_{est} is the estimated concentration of analyte in the reconstructed reaction extent vector.

Reaction spectrum derived from the least squares method and the reconstructed reaction extent vector based on hard chemical models, is multiplied by each other and hence makes some variation matrix similar dimension as V_x matrix. Concentration (c_{HAt}) and the dissociation constant (k) of analyte are changed simultaneously in a wide range and at each value; the reaction extent vector is calculated. The best answers for c_{HAt} and the related k are obtained whenever the rank of V_x matrix decreases correctly.

As mentioned earlier, the titration at the start point is carried out in extreme low pH value while the concentration of A^- at the initial point ($c_{A^-}^{o}$) was equal to zero. Therefore, the reaction extent vector of monoprotic acid at this study will be computed using equation 24.

Algorithm of the proposed method

Modified RAFA algorithm can be explained in the following steps:

1. The dissociation constant (k) and the concentration (c_{HAt}) of analyte were changed simultaneously in a given range and at each value; the reaction extent vector of analyte was calculated using equation 24. The resulted reaction extent vector was multiplied by corresponded α scalar, ε_{ra}^{re}.

2. The variation matrixes of standard V_a and the unknown sample V_x were obtained using equations 11 and 16, respectively.

3. The reaction spectrum($\mathbf{s}_{ra}^{T(re)}$) of the analyte was computed using $\boldsymbol{\varepsilon}_{ra}^{re}$ by applying the least squares method on the standard variation matrix (V_a).

4. The calculated analyte reaction spectrum($\mathbf{s}_{ra}^{T(re)}$) and the reaction extent vector ($\boldsymbol{\varepsilon}_{ra}^{re}$)were multiplied by each other and hence made the reconstructed standard variation matrix (V_a^{re}).

5. The residual variation matrix, V_R was calculated by applying equation 28.

6. The rank analysis was done on V_R and then RSD value was calculated using equation 30 for all values of parameters. The best values were obtained for the dissociation constant (k) and analyte concentration (c_{HAt}), when the RSD was minimum and the process came to its end. Hence, using the proposed algorithm, the dissociation constant and analyte concentration can be obtained simultaneously.

RESULTS AND DISCUSSION

Simulation

To evaluate the performance of the proposed method, simulated data were investigated at first. For this purpose, the mixture of two monoprotic acids and an inert agent were considered as unknown matrices. This means that in the unknown matrix, there are some unknown components, some of which have acid-base behavior and some are inert.

Simulated pH-dependent concentration profiles of all components in acid-base titration system were constructed by means of equations 21 and 22. Absorbance spectra were created by the normal Gaussian distribution function. The absorbance data matrix were then constructed by multiplying concentration profiles of each species by their related spectral profiles, and then random errors with mean zero and standard deviation equal to the 0.2% of the absorbance values were added to the absorbance data matrix in order to test the method with enough rigidity. The variation matrices were obtained by subtracting the first row of the data matrix from all rows of the data matrix.

Considering known values of c_{HAt} and $k_{(HA)}$, in the presence of an interfering acid with c_{HBt} and $k_{(HB)}$ or in the presence of an inert agent with certain C_I concentration, profiles of all components can be calculated. The simulated data is not presented for space constraints. All of the simulated data were created in MATLAB7 software. In addition, different pk values and different spectral overlapping were simulated to evaluate the ability of the method for resolving different kinds of data.

Simulated data

The proposed method was utilized for analyzing the simulated chemical systems. RAFA procedure was applied to the simulated variation matrix V_x and the relationship between the residual standard deviation (RSD)[10] of the residual variation matrix (V_R) and values of model fitted parameters (c_{HAt}, k) were examined. When the RSD of the residual variation matrix (V_R) reached to its minimum, the best solution was obtained. RSD as the criterion of the lack of fit of a principal component modeled to a data set can be defined as follows:

$$\mathrm{RSD(pc)} = \left(\sum_{i=pc+1}^{s} \frac{E_i}{[pc(s-1)]}\right)^{1/2} \qquad \text{(eqn. 30)}$$

Where E_i is the eigenvalue and pc is the number of considered principal components (independent reactions or process in the case of variation matrix) and s is the number of samples. RSD value of matrix V_R can be plotted versus values of pk_{HA} and the total concentration of acid c_{HAt} and hence the 3D surface plot can be obtained. For each value of c_{HAt}, the reaction extent vector ($\boldsymbol{\varepsilon}_{ra}$) for all values of pk_{HA} were calculated and the corresponding V_R matrices were determined. The proposed method was applied in the simulated data and its ability as a function of concentration of analyte, the pk value and the magnitude of noise level were tested.

Several types of simulated systems were analyzed. The spectral profile, the concentration profile, and the data matrix of one of these simulated data system are shown in Figure 1.

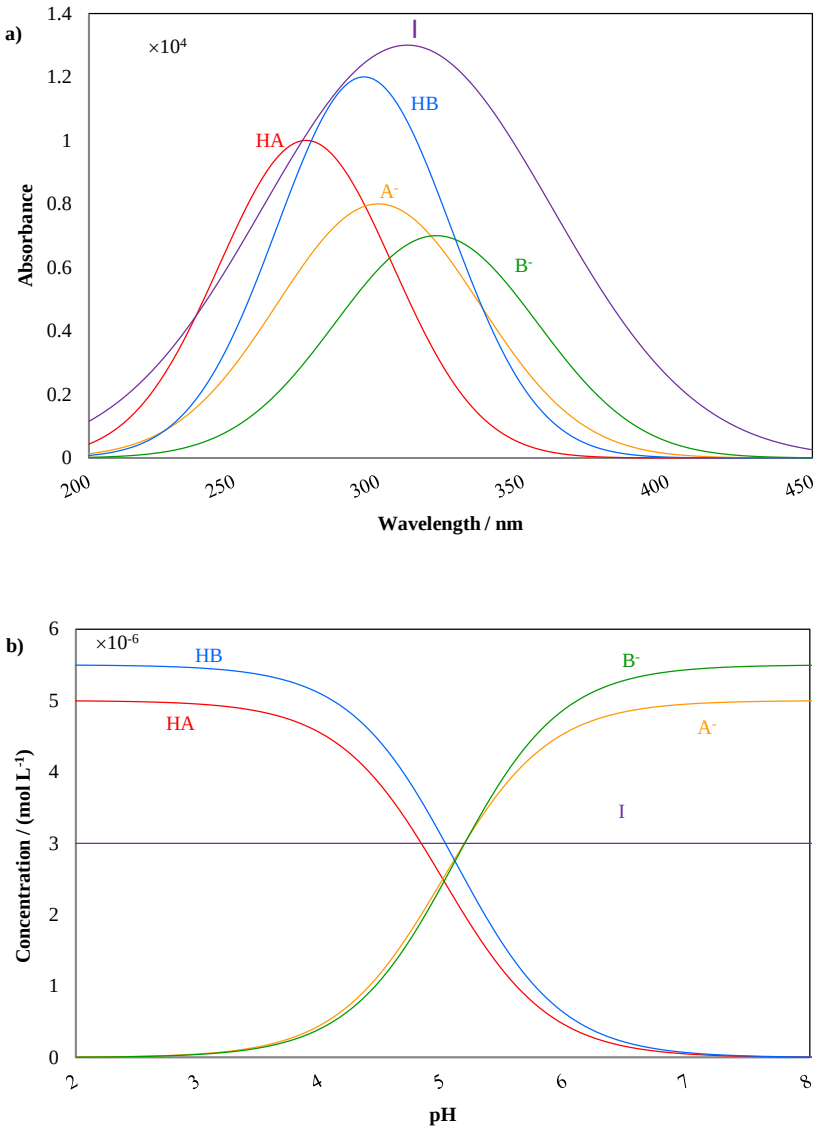


Figure 1. The Simulated (a) spectral profile (b) concentration profile

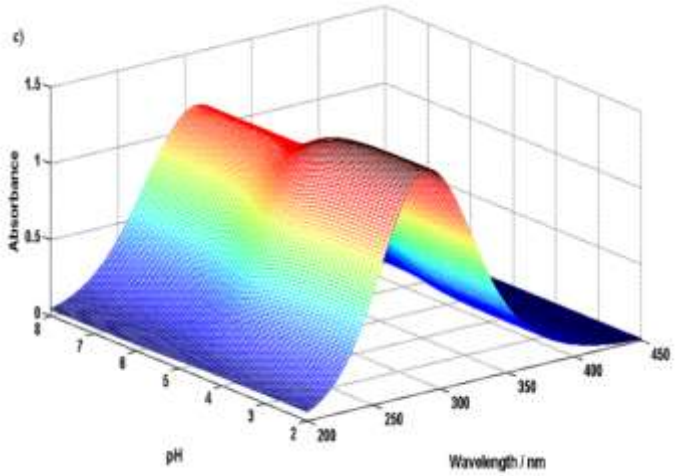


Figure 1Continued. (c) the data matrix of monoprotic acid (HA) in the presence of an interfering acid (HB) and an inert component (I)

The procedure was utilized to resolve the simulated data in order to find the total concentration and the dissociation constant of the monoprotic acid. As can be seen in Figure2, the optimum values for parameters can be obtained when the RSD values of the residual variation matrix reaches to its minimum.

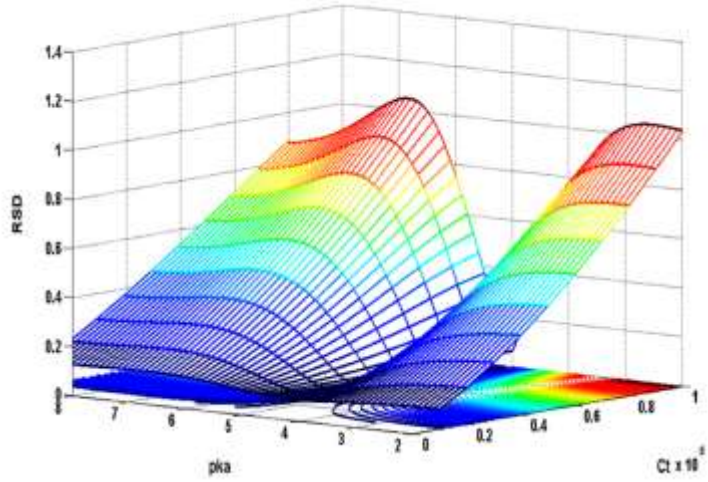


Figure 2. RSD surface and counter plot obtained by using modified RAFA for the simulated data which has been presented in Figure 1 (pk_{HA}= 5; pk_{HB}= 5.1)

RAFA was applied to different data set including analyte in the presence of another monoprotic acid (HB) with or without inert reagent under different Δpk conditions. Δpk value was the difference between pk_a of the analyte (HA) and that of the interference with acid-base behavior (HB).The real values are in good agreement with those obtained from the computer simulations shown in Table 1.

Table 1. The results obtained by using modified procedure on the mixtures of two monoprotic acids and an inert interference as a function of Δpk and concentration, in simulated data set

Δpk	0.1	0.1	0.1	0.5	0.5	0.5	1	1	1
pk_{HA} (Real)	5	5	5	5	5	5	5	5	5
pk_{HA} (Cal.[b])	4.9	5	5	5	5	5	5	5	5
pk_{HB} (Real)	5.1	5.1	5.1	5.5	5.5	5.5	6	6	6
pk_{HB} (Cal.[b])	5.2	5.1	5.1	5.5	5.5	5.5	6	6	6
C^{a}_{HA} (Real)	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}
C^{a}_{HA} (Cal.[b])	3.8×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}	4×10^{-6}
C^{a}_{HB} (Real)	4.1×10^{-6}	4.5×10^{-6}	5×10^{-6}	4.1×10^{-6}	4.5×10^{-6}	5×10^{-6}	4.1×10^{-6}	4.5×10^{-6}	5×10^{-6}
C^{a}_{HB} (Cal.[b])	3.9×10^{-6}	4.6×10^{-6}	4.9×10^{-6}	4.1×10^{-6}	4.5×10^{-6}	5×10^{-6}	4.1×10^{-6}	4.5×10^{-6}	5×10^{-6}

[a]Concentration / (mol L^{-1}); [b]Calculated.

The obtained optimum values for pk and C were employed for the concentration profile calculation of analyte or pH-dependent interference. Figure 3 shows the reconstructed concentration profiles for the analyte and interference.

As can be seen, in any case, in spite of severe overlapping spectral and concentration profiles, the calculated results are in good agreement with the simulated profiles at a reasonable noise level (Figure 3).

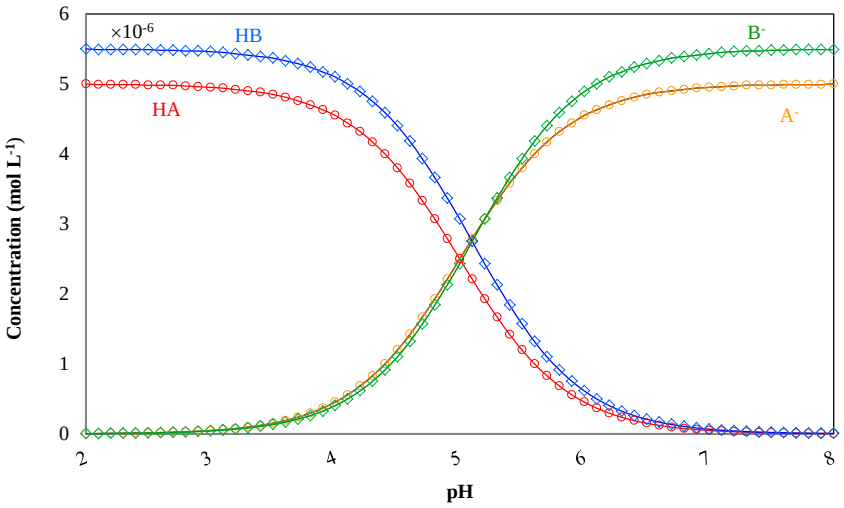


Figure 3. The Simulated (circle and diamond markers) and calculated (solid line) Concentration profiles obtained by modified model based RAFA

As mentioned earlier, the correct determination of the chemical rank of a black or gray sample with unknown constituents is really one of the most important steps in performing the rank annihilation factor analysis. In some cases, the system is ranked deficient and the real chemical rank especially in complex matrices cannot be acquired simply. For this reason, correct resolution and quantitation of the analytes is not possible. In order to circumvent this problem, this approach uses the rank annihilation factor analysis based on the reaction rank instead of chemical rank. To evaluate the performance of the proposed method for the rank quantitation, several sets of the simulated original and the variation data matrix containing monoprotic acids in the presence of inert reagents were created at different noise levels, according to the defined model in the previous section.

Random homoscedastic noise having equal variance with zero mean and different relative standard deviations was added to the set of the simulated data. Added noise levels were 0.1%, 0.2%, and 0.3% of the maximum absorbance of the mixtures. The applied extent of spectral overlap of components was at high level. The ratio of consecutive eigenvalues method was used to determine principal components in any sets.

Table 2 presents the eigenvalues and ratios of consecutive eigenvalues of the simulated original and variation data matrix. The eigenvalues obtained from the singular value decomposition of original and variation data matrix as well as the ratio of consecutive eigenvalues illustrate that in contrast to the original data matrix, the variation matrix is a full-rank matrix in terms of the number of independent reaction or process. Hence, determination of the matrix real rank in systems containing a high level of noise is possible.

Table 2. Results of Eigen analysis for data matrices from simulated original and variation data matrix

Simulated data (noise level)	Original data matrix				Variation data matrix		
	n[a]	EV[b]	ROEV[c]	pc[d]	EV[b]	ROEV[c]	pc[d]
HA[e]⇌A⁻ HB[e]⇌B⁻ Inert[f] (0.1%)	1	123.9112	20573.06		2.614679	137.1352	
	2	0.863895	99.04147		0.223277	7.646060×10^{6}	
	3	0.086807	7.069221×10^{6}	3	8.07×10^{-5}	6.170574	2
	4	3.26×10^{-5}	1.051555		3.25×10^{-5}	1.052427	
	5	3.18×10^{-5}	1.108634		3.17×10^{-5}	1.088092	
HA[e]⇌A⁻ HB[e]⇌B⁻ Inert[f] (0.2%)	1	123.8878	20610.19		2.612399	136.4908	
	2	0.862954	98.84077		0.223608	3.700877×10^{5}	
	3	0.0868	4.421274×10^{5}	3	0.000368	7.954485	2
	4	0.000131	1.06103		0.00013	1.258642	
	5	0.000127	1.334469		0.000116	1.09673	
HA[e]⇌A⁻ HB[e]⇌B⁻ Inert[f] (0.3%)	1	123.8936	20657.7		2.609697	131.1626	
	2	0.862001	95.05065		0.227869	9.524851×10^{4}	
	3	0.088416	8.36127×10^{4}	3	0.0 00738	6.228389	2
	4	0.000306	1.109023		0.000296	1.113439	
	5	0.00029	1.224477		0.00028	1.183434	

[a]Principal components; [b]Eigen values; [c]Ratio of consecutive eigen values; [d]Significant principal components; [e]Monoprotic acid; [f]Inert reagent.

Experimental Data

Sorbic acid and its potassium salt as food additive have been used to prevent the growth of bacteria, yeast and molds in a wide variety of food industry and drinks [20]. Nowadays, this chemical preservative has been recognized as 'generally recognized as safe' (GRAS)[21] and the maximum permitted concentration of it in any kind of food products is set by legislation[22]. However, the excess amount of this preservative can cause the adverse effects such as metabolic acidosis, hyperpnoea and convulsions in humans[23] and hence analytical determination of this additive is of great importance. Ascorbic acid also known as vitamin C, an essential nutrient, plays a major role in health by reducing the oxidative damage which can be caused by free radicals [24]. Ascorbic acid, which benefits health and increase vitality, is found mainly in fruit juices, vegetables, and some pharmaceuticals. In spectrophotometric determinations, ascorbic acid has strong spectral overlap

with sorbic acid and hence developing a spectrophotometry-based method for simultaneous determination of these compounds in food products such as fruit juices is of great interest. Due to the importance attached to these compounds in food industry, in this work ascorbic acid and sorbic acid have been selected as model compounds for evaluation of proposed method in orange juice as a real sample.

Binary mixtures of sorbic and ascorbic acids in different proportions were prepared and analyzed by the proposed method. The measured spectrophotometric titration spectra of one binary mixture as well as the standard of ascorbic and sorbic acid systems are shown in Figure 4, 5, and 6, respectively.

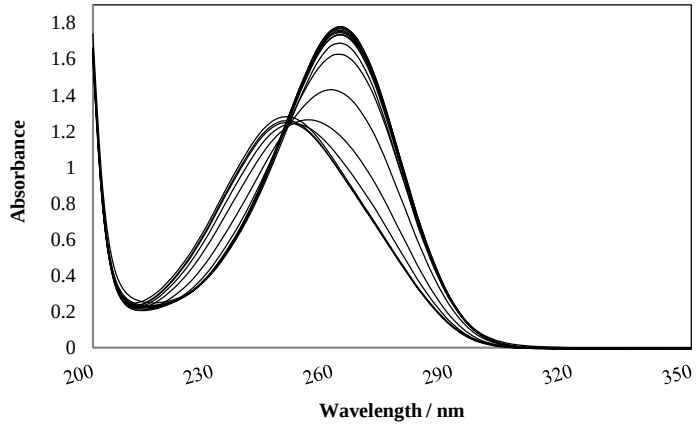


Figure 4. Plots of the measured spectrophotometric titration spectra of mixture of 16 mg L^{-1} ascorbic and 3 mg L^{-1}sorbic acids (pH range was between 1 and 9 with 0.5 unit intervals)

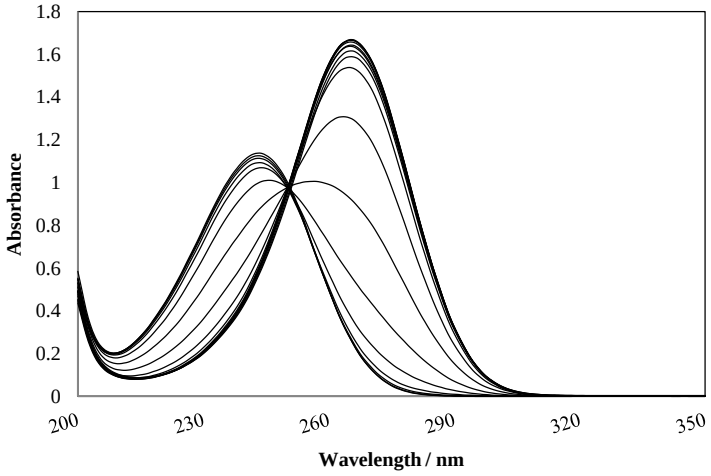


Figure 5. Plots of the measured spectrophotometric titration spectra of 20 mg L^{-1} ascorbic acid (pH range was between 1 and 9 with 0.5 unit intervals).

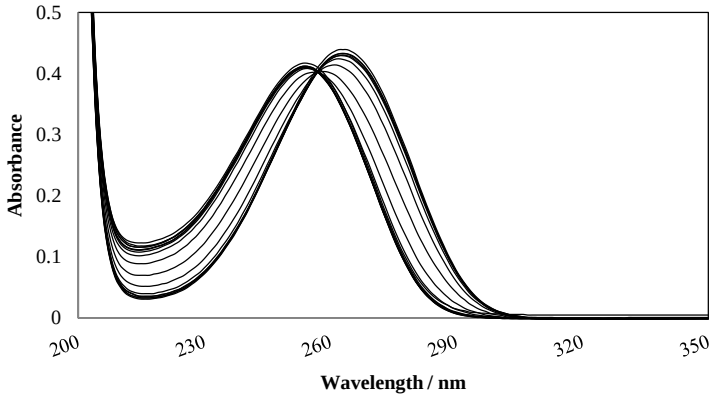
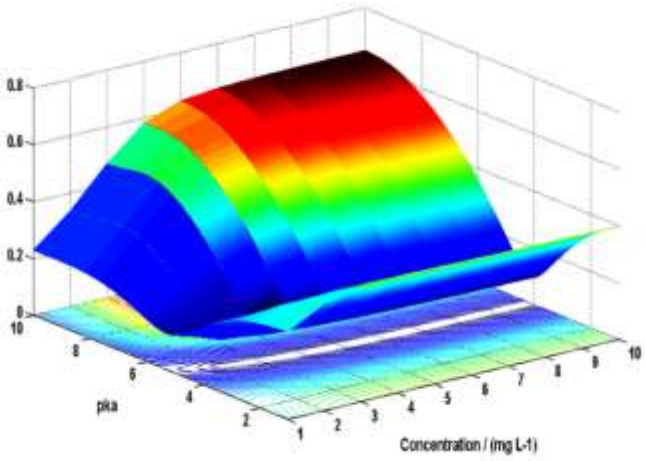


Figure 6. Plots of the measured spectrophotometric titration spectra of 2 mg L-1sorbic acid (pH range was between 1 and 9 with 0.5 unit intervals).

The pH range for all spectrophotometric titrations was between 1 and 9 with 0.5 unit intervals. Sorbic acid as monoprotic acid has the dissociation constant of pk_a = 4.77. Ascorbic acid as a diprotic acid has two dissociation constants: pk_{a1} = 4.17 and pk_{a2} = 11.57. However, in this range of pH, ascorbic acid acts like a monoprotic acid[25]. Hence, in this study all considered acids were considered as monoprotic acids.

For evaluating the ability of the proposed method to resolve the chemical systems with rank deficiency problem, the analysis was carried out on binary mixtures of various solutions containing ascorbic and sorbic acids with different concentrations. At first, one binary mixture of analytes (ascorbic=6 mg L^{-1} and sorbic=4 mg L^{-1}) were studied. The related RSD surface of sorbic acid (as one of the monoprotic acids) is shown in Figure7.

Using reaction rank instead of chemical rank, the minimum point of RSD surface can be used for acquiring the concentration and dissociation constant of sorbic acid in the presence of other components without rank deficiency problem.



.

Figure 7. RSD surface and counter plot of sorbic acid obtained by proposed procedure in the binary mixture.

Again, the proposed procedure was repeated for the same binary mixture and the initial concentration and dissociation constant of ascorbic acid in the presence of other components were calculated from minimum point of the related RSD. The results for just three analyses and all analytes are shown in Table 3.

Table 3. pk_a and concentrations of ascorbic and sorbic acids obtained by RAFA in the binary mixture of analytes

	Binary mixture No. 1		**Binary mixture No. 2**		**Binary mixture No. 3**	
Analyte	**Ascorbic**	**Sorbic**	**Ascorbic**	**Sorbic**	**Ascorbic**	**Sorbic**
$\mathbf{C_{Real}(mg\ L^{-1})}$	16	3	10	2	6	4
$\mathbf{C_{Calculated}(mg\ L^{-1})}$	16	2.95	10.04	1.98	5.97	4
$\mathbf{pk_{a(Real)[25]}}$	4.17	4.77	4.17	4.77	4.17	4.77
$\mathbf{pk_{a(Obtained)}}$	4.17	4.75	4.18	4.80	4.19	4.76

As can be seen in Table 3, the analysis of each analyte in the presence of other analytes is possible by using the proposed method even in the presence of the rank deficiency problem in the original data matrix.

It is worth noting that different methods have been used to estimate the principal components such as cross validation, the scree plot and the ratio of consecutive eigenvalues [1, 26]. However, in this study, the latest method was used to determine it in the real sample system.

Lastly, the wider applicability of the proposed method was investigated by analyzing the real sample with the complex matrix. For this purpose, the orange juice produced by Sunich Company (Iran) was used as the real sample. The juice's basic ingredients were natural orange concentrate, water, sugar, and vitamin C. This sample was spiked with 1 mg L^{-1}sorbic acid after the preliminary treatment and dilution. This sample was analyzed for its ascorbic and sorbic content. To eliminate the matrix effect in determination of analytes in the real sample the standard addition strategy was exploited. In this method, the standard matrix of analyte was obtained by standard addition of a certain amount of each component to the unknown samples and then by subtraction the standard addition unknown matrix from the unknown matrix. In this way, not only using an extra titration of the standard and augmentation with sample matrix were avoided, but also the spectra of each standard were obtained in the exact real matrix of samples[10].

Determination of sorbic and ascorbic acids in the orange juice was conducted by the modified RAFA. Titration of the orange juice has been performed in pH ranges between 1 and 9 with 0.5 unit intervals and the results are shown in Figure 8.

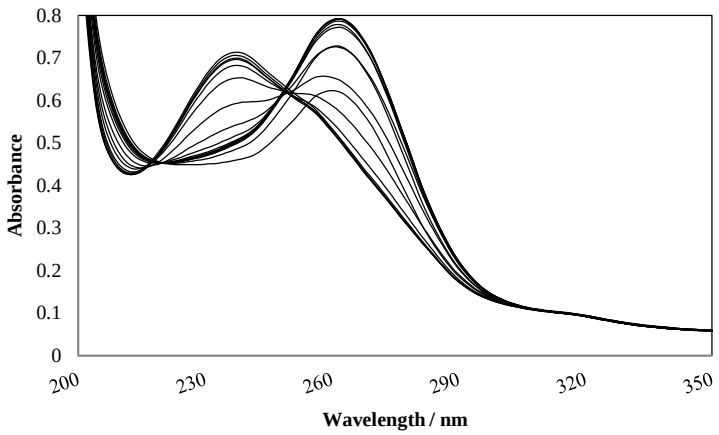


Figure 8. Plots of the measured spectrophotometric titration spectra of orange juice as real sample (pH range was between 1 and 9 with 0.5 unit intervals).

In order to examine the accuracy of the results in the real sample quantification, recoveries of analytes in the standard added samples were investigated. Various amounts of ascorbic and sorbic acids were added to the juice and the concentration of analytes in the real sample as well as recoveries of them in standard added samples were determined with the proposed method. The results obtained by applying the modified RAFA on real sample are shown in Table 4.

Table 4. The results obtained by RAFA on orange juice sample spiked by different concentrations of ascorbic and sorbic acids

Sample	**OJ[b]**		**Spiked OJ[b] No.1**		**Spiked OJ[b] No.2**		**Spiked OJ[b] No.3**	
Analyte	**Ascorbic**	**Sorbic**	**Ascorbic**	**Sorbic**	**Ascorbic**	**Sorbic**	**Ascorbic**	**Sorbic**
Added (C[a])	-	-	3	4	5	2	7	2.5
Found (C[a])	7	1.02	9.76	4.82	12.2	3.18	13.86	3.59
Recovery (%)	-	-	92	95	104	108	98	102.8

[a]Concentration /(mg L^{-1});[b]Orange juice.

As shown in Table 4, satisfactory results were obtained. Recoveries of analytes were between 92-108% and these values fell within acceptable range for such a real sample.

As can be seen from results, the procedure is successful in the simultaneous determination of concentration and the dissociation constant of an analyte in the presence of interferent(s) reaction and inert components in the rank deficient data. The modified RAFA was used to analyze the rank deficient data, in which inert components or other reactions in addition to the present analyte. It should be noted that the original rank annihilation factor analysis (RAFA) of such cases may be difficult and fails to give the correct concentration of the analyte of interest. Hence, to circumvent this issue, the rank deficiency problem is solved using the variation matrix prior to analyzing. In other words, this preprocessing strategy has been applied prior to the rank annihilation factor analysis to analyze rank-deficient spectrophotometric acid-base titrations data and decrease ambiguity in the rank quantitation step.

CONCLUSIONS

The proposed procedure combines the advantages of the variation matrix and hard soft modeling through applying the rank annihilation factor analysis on full rank variation pH-UV data matrix. Accordingly, by

performing a simple mathematical pretreatment on pH-absorbance data matrix, the rank deficient data was converted to variation data with full rank in terms of the number of independent reaction or process. The ambiguity in the matrix rank determination step is negligible because the studied matrix is full rank and the rank of which is equal to the reaction numbers. It was also shown that the possible rank-deficiency problem was circumvented using the variation matrix concept. The obtained results (theoretical and experimental) confirm that applying RAFA on the variation matrix produces unique results in concentration and the dissociation constant of monoprotic acids without any ambiguity in the rank quantitation step.

ACKNOWLEDGEMENTS

The authors acknowledge support from Islamic Azad University, Tabriz Branch. The authors declare that there is no conflict of interests.

NOTES

In equations, lowercase normal letters, lowercase bold letters, and capital letters are used as scalars, vectors and matrices, respectively.

REFERENCES

1. Malinowski E.R., 2002. Factor Analysis in Chemistry. Wiley. New York.

2. Haaland D.M., Thomas E.V., 1988. Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. Anal Chem. 60 (11), 1193-1202.

3. Thomas E.V., 1994. A primer on multivariate calibration. Anal Chem. 66 (15), 795A-804A.

4. Abdollahi H., Sajjadi S. M., 2009. Evaluation of variation matrix arrays by parallel factor analysis. J Chemom. 23 (3), 139-148.

5. Pasamontes A., Callao M.P., 2004. Determination of amoxicillin in pharmaceuticals using sequential injection analysis and multivariate curve resolution. Anal Chim Acta. 515 (1), 159-165.

6. Ghorbani-kalhor E., Sajjadi S.M., Naseri A., Abolhasani J., Vardini M.T., 2014. Modified model-based rank annihilation factor analysis to quantitative analysis of pH-modulated mixture samples.Curr Anal Chem.10 (4), 552-564.

7. Abdollahi H., Golshan A., 2011. Rank annihilation factor analysis method for spectrophotometric study of second-order reaction kinetics. Anal Chim Acta. 693 (1–2), 26-34.

8. Kompany-Zareh M., Vasighi M., 2010. Analysis of solvents mixtures employing rank annihilation factor analysis on near infrared spectral data from sequential addition of analyte. Fuel Process Technol. 91 (1), 62-67.

9. Smilde A.K., Tauler R., Saurina J., Bro R., 1999. Calibration methods for complex second-order data. Anal Chim Acta. 398(2–3), 237-251.

10. Abdollahi H., Safavi A., Zeinali S., 2008. Model-based rank annihilation factor analysis for quantitative analysis of mixtures of monoprotic acids using multivariate spectrophotometric acid-base titrations. Chemom Intell Lab Syst. 94 (2), 112-117.

11. Izquierdo-Ridorsa A., Saurina J., Hernández-Cassou S., Tauler R., 1997. Second-order multivariate curve resolution applied to rank-deficient data obtained from acid-base spectrophotometric titrations of mixtures of nucleic bases. Chemom Intell Lab Syst. 38 (2), 183-196.

12. Khalafi L., Rohani M., Afkhami A., 2008. Acidity constants of some organic acids in the presence of β-cyclodextrin in binary ethanol−water mixtures by rank annihilation factor analysis. J Chem Eng Data. 53(10), 2389-2392.

13. De Juan A., Tauler R., 2006. Multivariate curve resolution (MCR) from 2000: progress in concepts and applications. Crit Rev Anal Chem. 36 (3-4), 163-176.

14. Hemmateenejad B., Abbaspour A., Maghami H., Foroumadi A., 2008. Spectrophotometric determination of acidity constants by two-rank annihilation factor analysis. Anal Chim Acta. 607(2), 142-152.

15. Afkhami A., Khalafi L., 2006. Spectrophotometric determination of conditional acidity constant as a function of β-cyclodextrin concentration for some organic acids using rank annihilation factor analysis. Anal Chim Acta. 569 (1–2), 267-274.

16. Niazi A., Zolgharnein J., Davoodabadi M. R., 2008. Spectrophotometric determination of acidity constant of some indicators in various micellar media solutions by rank annihilation factor analysis. Spectrochim Acta. Part A. 70 (2), 343-349.

17. Jiang J.H., Ozaki Y., Kleimann M., Siesler H.W., 2004. Resolution of two-way data from on-line Fourier-transform Raman spectroscopic monitoring of the anionic dispersion polymerization of styrene and 1,3-butadiene by parallel vector analysis (PVA) and window factor analysis (WFA). Chemom Intell Lab Syst. 70 (1), 83-92.

18. Xu C.J., Gourvénec S., Liang Y.Z., Massart D.L., 2006. Using orthogonal projection approach (OPA) for rank-deficient reaction processes. Chemom Intell Lab Syst. 81 (1), 3-12.

19. Jiang J.H., Sasic S., Yu R.Q., Ozaki Y., 2003. Resolution of two-way data from spectroscopic monitoring of reaction or process systems by parallel vector analysis (PVA) and window factor analysis (WFA): inspection of the effect of mass balance, methods and simulations. J Chemom. 17 (3), 186-197.

20. Garcia I., Ortiz M.C., Sarabia L., Vilches C., Gredilla E., 2003. Advances in methodology for the validation of methods according to the International Organization for Standardization: Application to the determination of benzoic and sorbic acids in soft drinks by high-performance liquid chromatography. J Chromatogr A. 992 (1–2), 11-27.

21. Renner T., Baer-Koetzle M., Scherer G., 1999. Determination of sorbic acid in urine by gas chromatography–mass spectrometry. J Chromatogr A. 847 (1–2), 127-133.

22. Mota F.J.M., Ferreira I.M.P.L.V.O., Cunha S.C., Beatriz M., Oliveira P.P., 2003. Optimisation of extraction procedures for analysis of benzoic and sorbic acids in foodstuffs. Food Chem. 82(3), 469-473.

23. Naseri A., Ghorbani-Kalhor E., Vallipour J., Jafari S., Shahverdizadeh G.H., Asadpour-Zeynali K., 2009. Simultaneous spectrophotometric determination of benzoic acid, sorbic acid, and ascorbic acid using a net analyte signal-based method. J AOAC Int. 92(6), 1807-1814.

24. Du J., Cullen J.J., Buettner G.R., 2012. Ascorbic acid: Chemistry, biology and the treatment of cancer. Biochim Biophys Acta. 1826(2), 443-457.

25. Dean J.A. 1999. Lange's Handbook of Chemistry. McGraw-Hill. New York.

26. Abdollahi H., Nazari F., 2003. Rank annihilation factor analysis for spectrophotometric study of complex formation equilibria. Anal Chim Acta. 486(1), 109-123.