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# Second-order data obtained by beta-cyclodextrin complexes: A novel approach for multicomponent analysis with three-way multivariate calibration methods

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ABSTRACT

This research reports the first application of β-cyclodextrin (β-CD) complexes as a new method for generation of three way data, combined with second-order calibration methods for quantification of a binary mixture of caffeic (CA) and vanillic (VA) acids, as model compounds in fruit juices samples. At first, the basic experimental parameters affecting the formation of inclusion complexes between target analytes and β-CD were investigated and optimized. Then under the optimum conditions, parallel factor analysis (PARAFAC) and bilinear least squares/residual bilinearization (BLLS/RBL) were applied for deconvolution of trilinear data to get spectral and concentration profiles of CA and VA as a function of β-CD concentrations. Due to severe concentration profile overlapping between CA and VA in β-CD concentration dimension, PARAFAC could not be successfully applied to the studied samples. So, BLLS/RBL performed better than PARAFAC. The resolution of the model compounds was possible due to differences in the spectral absorbance changes of the β-CD complexes signals of the investigated analytes, opening a new approach for second-order data generation. The proposed method was validated by comparison with a reference method based on high-performance liquid chromatography photodiode array detection (HPLC-PDA), and no significant differences were found between the reference values and the ones obtained with the proposed method. Such a chemometrics-based protocol may be a very promising tool for more analytical applications in real samples monitoring, due to its advantages of simplicity, rapidity, accuracy, sufficient spectral resolution and concentration prediction even in the presence of unknown interferents.



## 1. Introduction

Analytical chemistry can involve samples that are far from being simple, containing numerous components to be analyzed simultaneously, or a few target analytes in the presence of many chemical interferents. In these cases, sophisticated instrumentation and mathematical tools are available to deal with the complexity [1]. Nowadays, multivariate calibration can be considered as one of the most active research areas in analytical chemistry field to solve the above mentioned problems. Depending on the data complexity, different multivariate calibration methods, such as zero, first, second, third order calibrations and so on [2], are available.

Second-order calibrations are particularly attractive for determinations of complex samples, essentially due to their ability to perform determinations even in the presence of interferences unmodeled in the calibration step, a property known as the second-order advantage. Second-order data for a given sample can be easily produced in a variety of ways, either in a single instrument or by resorting to instrument hyphenation. In second-order multivariate calibration, a data matrix is produced per sample and grouping matrices for all calibration samples give three-dimensional data known as three-way array [3,4].

In the present study, the authors demonstrated for the first time an accurate, reliable, inexpensive and simple way for producing second-order data based on formation of inclusion complexes between target analytes and β-CD. Dielectric properties of the environment are known to affect spectral and molecular properties of molecules, such as absorption intensity and position of peaks in the spectra. Hence, properties of a molecule embedded in a cyclodextrin cavity can be expected to differ from those observed

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in bulk water. In this system, the absorbance changes upon variations in the concentration of β-CD, therefore, it gives the absorbance spectra–concentration of β-CD matrix data, and binding these data matrices for a set of samples will produce a three-way array.

Cyclodextrins (cyclic oligosaccharides composed of seven D-glucose units) are known for their ability to bind covalently or noncovalently and to form inclusion complexes with organic compounds [5]. Complex formation is a dimensional fit between a host cavity and a guest molecule [6]. The lipophilic cavity of cyclodextrin molecules provides a microenvironment, into which appropriately sized non-polar moieties can enter to form inclusion complexes [7]. The main driving force of the complex formation is a release of enthalpy-rich water molecules from the cavity [8–10]. Replacement of water molecules by more hydrophobic guest molecules present in the solution results in a formation of an inclusion complex between the host and the guest. CDs have found extensive applications in many fields, including food industry [11], environmental protection analysis [12] and enzyme modeling [13].

Phenolic acids are secondary metabolites that are commonly found in plant-derived food. Structurally, phenolic acids derive from either hydroxycinnamic or hydroxybenzoic acid skeletons. Caffeic and vanillic acids are among the most abundant hydroxybenzoic derivatives in plants [14]. Unlike hydroxycinnamates, hydroxybenzoic acid derivatives are mainly present in food [15]. Among the variety of phenolic compounds, phenolic acids have attracted considerable interest in the past few years, due to their many potential health benefits. Phenolic acids are bioactive compounds, present in the diary diet and influencing health. They are antioxidative, antitumor, antimutagenic and antibacterial [16–18]. Moreover, phenolic acids can inhibit DNA damage [19]. As a result, consumption of fruits is a major source of phenolic acids in the diet. Interestingly, fruit extracts richer in phenolic acids usually present a larger antioxidative activity than the corresponding pure compounds or even vitamins, which is an evident synergistic effect [20]. Therefore, determination of phenolic acids in food samples, such as fruit juice samples, is an important analytical task.

Many different analytical techniques have been used for the determination of phenolic acids, including gas chromatography–mass spectrometry (GC–MS) [21–23], capillary electrophoresis (CE) methods [24–26], micellar electrokinetic chromatography (MEKC) [27,28]. In addition, the most widely used methods are based on reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with UV–vis detection and/or mass spectrometry [29–31]. The reported methods not only require sophisticated analytical instrumentation, they are also time-consuming and difficult to apply to analysis of a complex matrix sample without any pretreatment. Moreover, such facilities are not commonly available at all laboratories. However, several chemometric procedures have been used as the basis for discrimination and classification of phenolic acids [32–35]. But these studies are based on first-order multivariate calibration and report only qualitative results. Second order calibration methods play an important role in solving the problem of closely overlapping spectra, like in this study. These methods utilize a mathematical separation procedure to carry out the determination using standards that contain only the analyte of interest, and so the interferents do not need to be present in the calibration standards. In addition, these approaches not only determine the concentrations, but can also provide spectral profiles of the target components in the mixtures, even in the presence of uncalibrated components, exploiting the so-called second-order advantage [3,36–39].

The aim of the present work is to propose a new and reliable spectrophotometric method based on β-CD complexes, as a new method for generation of three way data, for simultaneous quantification of multicomponents with overlapping spectra, in the presence of uncalibrated interferents. Deconvolution of concentration of β-CD-resolved absorbance spectra was carried out using BLLS/RBL and PARAFAC, while these models used the second-order advantage. The results indicated a better predictive ability of the BLLS/RBL procedure in comparison with standard PARAFAC. Finally, predictive ability, figures of merit and accuracy were estimated for BLLS/RBL to demonstrate its potential as an alternative for quantification of CA and VA as an example in fruit juices samples, even in the presence of unexpected or unmodeled interferents and without sample pretreatments. To the best of our knowledge, this is the first attempt to apply BLLS/RBL and PARAFAC with β-CD complexes for this purpose.

## 2. Theory of BLLS/RBL and PARAFAC

### 2.1. Bilinear least squares/residual bilinearization (BLLS/RBL)

The BLLS/RBL [40–43] model has been discussed in detail in the literature; thus only a brief description of this model is presented here. Bilinear least-squares followed by residual bilinearization step has been developed and applied for two-way data modeling [38]. The BLLS method uses analyte concentration introduced into the calibration step, where only standard matrices are present; in order to obtain approximations of pure-analyte matrices at unit concentration ($\mathbf{S}_n$). The first step is the vectorization of each data matrix of the calibration set $\mathbf{X}$ (each of size $J \times K$) and the $I_c$ calibration samples are grouped in a matrix $\mathbf{V}_x$ of dimension ($JK \times I_C$):

$$\mathbf{V}_x = [\mathrm{vec}(\mathbf{X}_1)|\mathrm{vec}(\mathbf{X}_2)|\cdots|\mathrm{vec}(\mathbf{X}_{Ic})] \quad (1)$$

where "vec" is the operation of unfolding the matrix into the vector.

The next step employs direct least squares [40,41] to obtain information of the pure analyte matrices at unit concentration ($\mathbf{V}_s$), in a procedure analogous to first-order classical least squares:

$$\mathbf{V}_S = \mathbf{V}_x\mathbf{Y}^+ \quad (2)$$

where $\mathbf{Y}$ is an $I \times N_C$ matrix of the reference concentrations, $N_C$ is the number of calibrated analytes. $\mathbf{V}_s$ ($JK \times N_C$) contains the required $\mathbf{S}_n$ matrices in the vectorized form:

$$\mathbf{V}_S = [\mathrm{vec}(\mathbf{S}_1)|\mathrm{vec}(\mathbf{S}_2)|\cdots|\mathrm{vec}(\boldsymbol{S}_{Nc})|] \quad (3)$$

To obtain the spectral profiles from the estimated matrix, singular value decomposition (SVD) is employed at each estimated matrix ($\mathbf{S}_n$), obtained after an appropriate reshaping of the unfolded vec ($\mathbf{S}_n$) [40,41]:

$$(\mathbf{b}_n, g_n, \mathbf{c}_n) = \mathrm{SVD}(\mathbf{S}_n) \quad (4)$$

where $g_n$ is the first singular value, and $\mathbf{b}_n$ and $\mathbf{c}_n$ are $J \times 1$ and $K \times 1$, the first left and right singular vectors of $\mathbf{S}_n$, respectively.

The concentration of an unknown sample (which matrix data are $\mathbf{X}_u$) can be estimated, provided that no interference occurs, by a direct least squares procedure [40,41]:

$$\mathbf{y}_u = \mathbf{S}_{\mathrm{cal}}^{+}\mathrm{vec}(\mathbf{X}_u) \quad (5)$$

where $\mathbf{y}_u$ is an $N_C \times 1$ vector of the estimated concentration of the $N_C$ analytes in the sample and $\mathbf{S}_{\mathrm{cal}}$ is a calibration $JK \times N_C$ matrix given by:

$$S_{\mathrm{cal}} = [g_1(\mathbf{c_1} \otimes \mathbf{b}_1)|g_2(\mathbf{c_2} \otimes \mathbf{b}_2)|\cdots|g_{Nc}(\mathbf{c}_{Nc} \otimes \mathbf{b}_{Nc})| \quad (6)$$

where $\otimes$ indicates the Kronecker product.

### 2.2. Parallel factor analysis (PARAFAC)

A brief summary on spectral deconvolution and calibration using PARAFAC is given here. PARAFAC performs the decomposition of a three-way data array **X** (I × J × K), consisting of I training matrices of dimensions J × K, in three loading matrices: **A** (I × N), **B** (J × N) and **C** (K × N), where *N* is the number of factors or responsive components. This decomposition can be represented by Eq. (7), for a generic element $x_{ijk}$ of the three-dimensional array **X**:

$$\mathbf{x}_{ijk} = \sum_{n=1}^{N} \mathbf{a}_{ni}\mathbf{b}_{nj}\mathbf{c}_{nk} + \mathrm{e}_{ijk} \tag{7}$$

where $e_{ijk}$ is an element of the residual error array **E** of the same dimensions as **X**; $\mathbf{a}_{ni}$, $\mathbf{b}_{nj}$ and $\mathbf{c}_{nk}$ are the elements of the column vectors $\mathbf{a}_n$, $\mathbf{b}_n$ and $\mathbf{c}_n$. The column vectors are collected into the three loading matrices **A**, **B** and **C**. The trilinear model is found by minimizing the sum of squares of the residuals given by Eq. (7) by alternating least squares (ALS). The profile of the each compound in the mixture is stored in one factor of the PARAFAC model. Therefore, the model presents low flexibility utilizing low degrees of freedom and determining a unique solution for the system. The regression model is obtained by least squares regression between the column of **A** related to the analyte and the reference concentration (**y**) of the calibration samples [44]:

$$\mathbf{y} = \boldsymbol{\omega} a \tag{8}$$

where **ω** is the liner regression coefficient. This method is not ideal for handling data, which are not strictly trilinear in the latter sense [45]. The number of components to be modeled can be estimated by different methods, such as evaluation of the core consistency or percentage of the fit [46,47]. In the present case, the optimum number of PARAFAC factors is estimated by core consistency for each factor. A core consistency close to 100% implies an appropriate (trilinear) model. A core consistency close to zero or even negative implies an invalid model [46].

### 2.3. Figures of merit

The determination of figures of merit (FOM), such as accuracy, sensitivity and selectivity, is an important necessity for the validation of these kinds of chemometric methods. In chemometrics, the root mean squares error of prediction (RMSEP) generally expresses the accuracy of the model. It reports the closeness of agreement between the reference value and the value found by the model:

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{n=1}^{I}(y_n - \hat{y}_n)^2}{I-1}} \tag{9}$$

where *y* is the reference concentration value for each of the *I* test samples, and $\hat{y}$ is the concentration value estimated by the second-order model. When expressing FOM for multivariate calibration methods, the part of the signal that relates uniquely to the analyte of interest is more important than the total signal. This unique signal is termed net analyte signal (NAS). For second-order data, the estimation of NAS is analogous to those for first-order procedures. In the present work, the NAS is the pure analyte data obtained by PARAFAC and BLLS/RBL and can be calculated according to Eq. (10) [39].

$$\mathrm{NAS}_{ij} = a_{ij}(\mathbf{b}_j \otimes \mathbf{c}_j^T) \tag{10}$$

where $\mathrm{NAS}_{ij}$ is the net analyte signal for the *i*th sample and *j*th analyte, $a_{i,j}$ is the obtained score, $\mathbf{b}_j$ and $\mathbf{c}_j$ are the loading vectors for other dimensions and ⊗ means the kronecker product. When using second-order advantage, each *i*th sample will have a specific value of NAS, and sensitivity (SEN*i*) is estimated as the NAS at unit concentration, as shown in Eq. (11). Selectivity (SEL*i*) is estimated as the ratio between SEN*i* and the total signal, according to Eq. (12) [39].

$$\mathrm{SEN}_i = ||\mathrm{NAS}_i||_{\mathrm{F}} \tag{11}$$

$$\mathrm{SEL}_i = \frac{||\mathrm{NAS}_i||_{\mathrm{F}}}{||\mathrm{N}_i||_{\mathrm{F}}} \tag{12}$$

$\mathbf{N}_i$ is the matrix of the total signal and the symbol $\|\ \|_{\mathrm{F}}$ means the Frobenius norm of a matrix. A more informative FOM is the analytical sensitivity ($\gamma$), which is defined, as the ratio between $\mathrm{SEN}_i$ and the variance of instrumental signal, which may be estimated by replicate blank measurements [39]:

$$\gamma_i = \frac{\mathrm{SEN}_i}{s(0)} \tag{13}$$

The inverse of this parameter ($\gamma^{-1}$) reports the minimum concentration difference between two samples that can be determined by the model. Finally, according to Eq. (14) the limit of detection (LOD) can be estimated as 3.3 times the standard deviation for a sample of low or zero analyte concentration.

$$\mathrm{LOD} = 3.3\, s(0) \tag{14}$$

As the second-order advantage was applied, SEN and SEL determinations are sample specific and cannot be defined for the multi-way method as a whole. In such cases, average values for a set of samples can be estimated and reported.

## 3. Experimental

### 3.1. Reagents and solutions

All chemicals used in this work were of analytical grade. All chemicals were purchased from Merck (Darmstadt, Germany) or Aldrich (Chemical Co., Milwaukee, WI, USA). Beta-cyclodextrin (β-CD) ( > 98%) and phenolic acids, caffeic acid (CA) and vanillic acid (VA), were purchased from Merck. Double distilled water was used throughout the experiments. Stock solutions of 200 mg $L^{-1}$ CA and VA were prepared in double distilled water. Working standard solutions and mixtures of target phenolic acids were freshly prepared by appropriate dilution of stock solutions with double distilled water to the required concentrations. Both stock and working solutions were stored in a refrigerator at 4 °C in darkness. The HCl (37%) and $H_3PO_4$ acids were used to adjust the pH to 3.0.

### 3.2. Instrumentation and software

A UV–vis spectrophotometer (Perkin Elmer, Lambda 25, www.perkinelmer.com) with 10 mm quartz cells was used to measure the absorbance of the phenolic acids. A Universal 320R refrigerated centrifuge equipped with an angle rotor (6-place, 9000 rpm, Cat. No. 1620A) was from Hettich (Kirchlengern, Germany). pH of aqueous solution was measured by a digital pH meter (Metrohm, model 692, Herisau, Switzerland) equipped with a glass combination electrode.

Chromatographic analyses were performed with a HPLC instrument including a Knauer S 1000 HPLC pump (Germany), with a 20 μL sample loop, equipped with a Knauer S 2800 PDA detector. Chromatographic data were recorded and analyzed using EZChrom Elite software.

The UV–vis spectra were recorded between 200 and 700 nm, in steps of 1 nm. All spectra were exported in ASCII format, and the data treatment was carried out using MATLAB version 7.9.0 on a personal computer. Second order multivariate calibration using BLLS/RBL and PARAFAC was carried out using multivariate calibration for second-order (MVC2) program, which is available on the internet and performs under Matlab environment [48].

### 3.3. Formation of inclusion complexes of β-CD with vanillic and caffeic acids procedure

For formation of inclusion complexes of β-CD under optimum conditions, an appropriate volume of CA and VA working solutions or of the sample solution was placed in a 10.0 mL volumetric flask. Then, desired amount of 0.02 mol $L^{-1}$ β-CD solution (in the range of 0.0001–0.0013 mol $L^{-1}$) was added and the pH of solution was adjusted to pH=3.0. The mixed solution was diluted to the final volume with distilled water and sonicated for 30 min at room temperature (25 °C). The absorbance data of samples were taken in the range of 200 and 700 nm against the reagent blank by spectrophotometer with a good reproducibility. Then the data were transferred to a personal computer and processed by applying chemometric analysis based on the second order algorithms, using BLLS/RBL and PARAFAC, which was carried out using MVC2 program.

### 3.4. Calibration and validation sample sets

The first step in the simultaneous determination of different analytes by second-order multivariate calibration methodologies involved construction of the calibration matrix for a mixture of analytes. Eleven standard solutions with a random design for concentrations of both analytes were prepared and used for the calibration set. The concentrations were between 0–6.0 and 0–9.0 μg $mL^{-1}$ for CA and VA, respectively. The analyte concentrations are shown in Table 1.

**Table 1**
Composition of the phenolic acids mixtures used for calibration set.

| Calibration samples | VA (μg $mL^{-1}$) | CA (μg $mL^{-1}$) |
|---|---|---|
| C1 | 7.0 | 0 |
| C2 | 9.0 | 0 |
| C3 | 0 | 4.0 |
| C4 | 0 | 6.0 |
| C5 | 6.0 | 2.0 |
| C6 | 8.0 | 1.0 |
| C7 | 2.0 | 3.0 |
| C8 | 3.0 | 3.0 |
| C9 | 1.0 | 5.0 |
| C10 | 5.0 | 2.0 |
| C11 | 4.0 | 0 |

A seven sample set (validation set) was built to validate the employed multivariate model. The analyte concentrations arose from a random design (see Table 2). Calibration and validation samples were prepared by measuring appropriate aliquots of standard solutions of each phenolic acid, placing them into 10.0 mL volumetric flasks to obtain the desired concentrations, and completing to the mark with double distilled water.

Each calibration, validation and real samples mixtures were measured according to the procedure described in Section 3.3. The absorption spectra were recorded between 200 and 700 nm with the scan rate of 450 nm $min^{-1}$ against the blank. The spectral region between 255 and 315 nm was selected for the analysis, because this is the zone with the maximum spectral information about the component mixture of interest.

### 3.5. Pretreatment and arrangement of spectral data for multi-way modeling

No pre-processing of the data was performed. In present study, the three-way data (concentration of β-CD-resolved absorbance data) for calibration, validation and real samples (fruit juices samples) as data sets (samples × absorbance × concentration of β-CD) were arranged as: calibration data: $11 \times 61 \times 7$ ($I \times J \times K$), validation set: $7 \times 61 \times 7$ ($I \times J \times K$), real samples: $8 \times 61 \times 7$ ($I \times J \times K$). Second order multivariate calibration using BLLS/RBL and PARAFAC were carried out using MVC2 program. In fact, the current study exhibited the absorbance of CA and VA changes upon variations in the concentration of β-CD.

### 3.6. Preparation of fruit juices samples

Four commercial fruit juices containing 100% natural juices (barberry and pomegranate juices), 45% juice (sour cherry juice) and 50% juice (orange juice) were obtained from a local supermarket in Tehran. The samples were stored at 4 °C before use. 20 mL of each fruit juice samples were centrifuged at 5000 rpm for 10 min. The supernatant was filtered through a 0.45 μm pore size membrane filter and diluted to 200 mL with double distilled water.

Generally, all of these real samples were clear and no sediments or suspensions were observed. Finally, an accurate volume of 10 mL of each of the prepared real samples were analyzed under the recommended procedure (see Section 3.3) and the exact CA

**Table 2**
Validation set samples and prediction results of CA and VA (μg $mL^{-1}$) using BLLS/RBL and PARAFAC methods.

| Validation samples | | | Predicted concentration ($n=3$) | | | |
|---|---|---|---|---|---|---|
| | | | BLLS/RBL[a] | | PARAFAC[b] | |
| Mixture | VA | CA | VA | CA | VA | CA |
| V1 | 4.5 | 3.5 | 4.4 (97.77)[c] | 3.6 (102.85) | 4.38 (97.33) | 4.00 (114.28) |
| V2 | 0 | 4.0 | 0.01 (–) | 4.12 (103.00) | 0 (–) | 4.3 (107.5) |
| V3 | 5.0 | 1.5 | 4.95 (99.00) | 1.63 (108.66) | 4.90 (98.00) | 1.8 (120.00) |
| V4 | 7.5 | 2.5 | 7.42 (98.93) | 2.39 (95.6) | 7.30 (97.33) | 2.88 (115.2) |
| V5 | 1.0 | 5.0 | 0.94 (94.00) | 5.16 (103.2) | 0.90 (90.00) | 5.40 (108.00) |
| V6 | 6.5 | 0 | 6.53 (100.46) | 0 (–) | 6.40 (98.46) | 0.47 (–) |
| V7 | 3.0 | 3.0 | 2.90 (96.66) | 3.14 (104.66) | 2.91 (97.00) | 3.4 (113.33) |
| Mean recovery | | | 97.80 | 102.99 | 96.35 | 113.05 |
| RMSD[d] | | | 0.112 | 0.117 | 0.116 | 0.28 |
| REP%[e] | | | 3.08 | 4.53 | 3.18 | 10.79 |

[a] BLLS/RBL was carried out maintaining number of solutes and number of interferences at 2 and 0, respectively.
[b] PARAFAC modeling was carried out using two factors as selected by core consistency criterion.
[c] Recovery in parenthesis.
[d] Root-mean-square difference.
[e] Relative error of prediction, $REP = \frac{100}{\bar{c}}\left[\frac{1}{I}\sum_1^I (C_{act} - C_{pred})^2\right]^{1/2}$, where $I$ is the number of samples, cact and cpred are the actual and predicted concentrations, and $\bar{c}$ is the mean concentration.

and VA concentrations in the original samples were then determined with the aid of second-order calibration method.

### 3.7. Chromatographic separation and quantification of compounds of interest in real samples

The concentrations of CA and VA in fruit juices samples were verified by the HPLC-PDA method. The separations were carried out on a C18 column (250 cm × 4.0 mm, with the particle size of 5 μm). A mixture of acetonitrile and phosphoric acid (25 mmol $L^{-1}$) (15:85 v/v) for 10 min at a flow rate of 1.0 mL $min^{-1}$ were used as a mobile phase at room temperature. The linear calibration range was from 1 to 15 μg $mL^{-1}$ for both analytes. The detections were performed at 214 nm for VA and at 252 nm for CA. Retention times were used for identification of compounds of interest and peak areas obtained at different concentrations were used to plot calibration lines for each analyte. Prior to injection into a column, all solutions were filtered using 0.45 μm filters to remove insoluble materials.

## 4. Results and discussion

In order to achieve maximum formation of inclusion complexes of β-CD with target phenolic acids, at first various experimental parameters were investigated and optimized. Then under the optimum conditions, BLLS/RBL and PARAFAC algorithms were applied for the simultaneous spectrophotometric quantification of CA and VA.

### 4.1. Optimum conditions for formation of inclusion complexes of CA and VA-β-CD

#### 4.1.1. Effect of pH on formation of inclusion complexes of CA and VA with β-CD

Caffeic and vanillic molecules contain a benzene ring group, which size and geometry are suitable for the hydrophobic cavity of β-CD; therefore, they are easily included by β-CD from aqueous solution to form a supramolecular complex. The species of CA and VA molecules in aqueous solutions are changeable with pH of the solution, due to ionization. In acidic conditions, CA and VA are very stable and exist with neutral molecules. On the contrary, stability of the target phenolic acids decreases in basic medium, because hydroxyl group takes place of ionization to form the related phenyl salt.

As β-CD cavity is hydrophobic and the major inclusion interactions are hydrophobic interactions between the guest and β-CD cavity, the neutral molecules of CA and VA in acidic medium are more easily included by β-CD than the related salt of CA and VA in basic medium. The results of β-CD including CA and VA in different pH values are shown in Fig. 1. The results showed that maximum absorbance intensities appeared at pH 3.0 and decreased with the increasing pH value. Based on Fig. 1, pH 3.0 was selected for further experiments.

#### 4.1.2. Effects of temperature and time on β-CD including CA and VA

Effect of temperature on β-CD including CA and VA is shown in Fig. 2. It can be seen that in the inclusion process of CA and VA, the absorbance intensities gradually decrease, as the temperature increases. The maximum absorbance intensities were observed at room temperature (25 ± 1 °C). In addition, with higher temperature it was easier to reach the inclusion equilibrium and also the inclusive time was shorter, which is in accordance with general absorption reaction rules.

For inclusion of CA and VA on β-CD, sonication was applied in the present work. The sonication time required for attaining the inclusion equilibrium depended on the system. The results showed that for the time of 10 min, the inclusion equilibrium was reached at 60 °C, for 15 min at 50 °C, for 25 min at 30–40 °C, and for 30 min at 25 °C, respectively. Based on these results, the room temperature (25 °C) and the time of 30 min were chosen for further experiments.

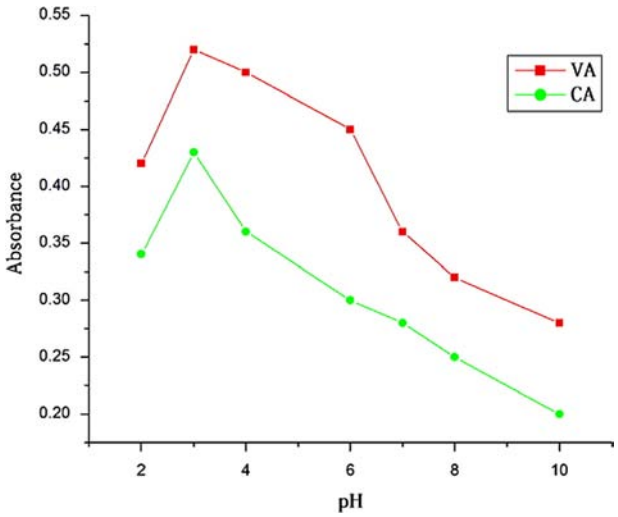


**Fig. 1.** Effect of pH on β-CD including CA and VA. Conditions: concentration of β-CD: 0.7 mmol $L^{-1}$, Concentration of each phenolic acid: 5 μg $mL^{-1}$, sonication time: 30 min, room temperature, total volume: 10 mL.

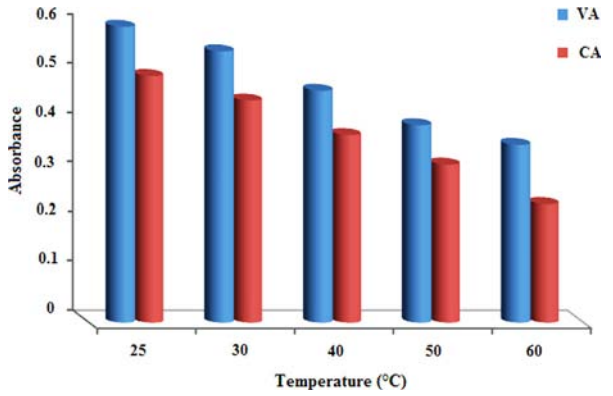


**Fig. 2.** Effect of temperature on β-CD including CA and VA. Conditions: concentration of β-CD: 0.7 mmol $L^{-1}$, Concentration of each phenolic acid: 5 μg $mL^{-1}$, pH = 3.0, sonication time: 30 min, total volume: 10 mL.

#### 4.1.3. Effect of ionic strength on β-CD including CA and VA

Effect of ionic strength on the formation of β-CD including CA and VA complexes was investigated. The concentration of sodium nitrate ($NaNO_3$) varied from 0.0 to 0.5 mol $L^{-1}$. The results showed a little increase in absorbance when the concentration of $NaNO_3$ was changed from 0.01 to 0.1 mol $L^{-1}$. Furthermore, it was approximately constant when the concentration of $NaNO_3$ was between 0.2 and 0.5 mol $L^{-1}$. In conclusion, the ionic strength has little effect on the inclusion of target phenolic acids in β-CD. Therefore, the ionic strength was controlled at 0.1 mol $L^{-1}$ in further research.

### 4.2. Absorption spectra—Concentration of β-CD matrix data

With three-way data, a matrix of data is collected from each analyzed sample. Such data are generally collected from excitation–emission matrix (EEM) fluorescence spectroscopy and

'hyphenated' analytical methods, such as HPLC with diode-array detection (HPLC-DAD), GC–MS, and capillary electrophoresis with DAD (CE-DAD) [3]. The absorption spectra-pH matrix data can also present three-way data [49]. The main purpose of this study is to introduce a new method for generation of three-way data by changing the β-CD concentration for each set of binary mixtures of both analytes. β-CD is one of the most important host molecules in supramolecular chemistry. β-CD has the peculiar 'interior hydrophobic, exterior hydrophilic' structure forming a 1:1 or 1:2 inclusion complex with guest molecules, thus the physical, chemical and biochemical characters of guest molecules are modified [50,51]. Properties of β-CD affect spectroscopic properties, absorption and fluorescence spectra and especially molar absorptivities of CA and VA. The addition of β-CD to the solution mixture of CA and VA and the subsequent increase of the absorbance in the wavelength range help to have a series of spectra, which can make a bilinear data matrix. By changing the initial concentration of binary mixtures of target phenolic acids and addition of β-CD, a three dimensional data matrix can be generated. To avoid non-linearity due to high concentrations of analytes, the range of the applied concentration of two analytes were kept low enough to limit the absorbances to be lower than 1.0.

### 4.3. Spectral behavior of the compounds of interest

Fig. 3a shows the normalized absorbance spectra of the pure mixture of CA and VA, recorded under the optimum conditions. As it can be seen, the spectra for the two analytes are considerably overlapped. Considering this spectral overlapping, it can be concluded that the simultaneous determination of binary mixtures of CA and VA in their synthetic mixtures and in fruit juices samples cannot be carried out by applying conventional methods. In order to overcome this problem, a mathematical separation procedure based on chemometrics methods can be applied. For this purpose, second-order data, such as concentration of β-CD-resolved absorbance data matrices, were obtained and processed with second-order algorithms based on BLLS/RBL and PARAFAC, achieving the second-order advantage.

### 4.4. Prediction of CA and VA in validation samples

The performance of second-order calibration methods based on BLLS/RBL and PARAFAC were initially tested for determination of CA and VA in validation samples. For BLLS/RBL model, the number of calibrated solutes and the number of expected interferents were set as 2 and 0, respectively. Number of interferents was set as 0, because validation samples were free from any interferents. For PARAFAC analysis, core consistency criterion, outlined earlier, was adopted to find the optimum number of factors leading to optimum data deconvolution. For the first three factors, the corresponding core consistency values were 100, 98 and −33, and as mentioned in Section 2.2, the optimum number of factors was 2. Under the calibration conditions designed earlier, prediction of CA and VA in validation samples by BLLS/RBL and PARAFAC are presented in Table 2. Root-mean-square difference (RMSD) [52] and relative error of prediction (REP) criteria were applied as indicators for the quality of model's prediction, the final results are shown in Table 2. Due to an intense overlap concentration profile between CA and VA in β-CD concentration dimension (Fig. 3b), BLLS provides better results than PARAFAC. The idea behind the BLLS algorithm can explain these results, since BLLS employs concentration information in the calibration step, a direct least squares procedure to obtain the pure-analyte information and no initialization and constraining procedures, yielding analyte profiles and concentrations in samples where strong overlapping occurs. Finally, BLLS/RBL algorithm was chosen for processing of

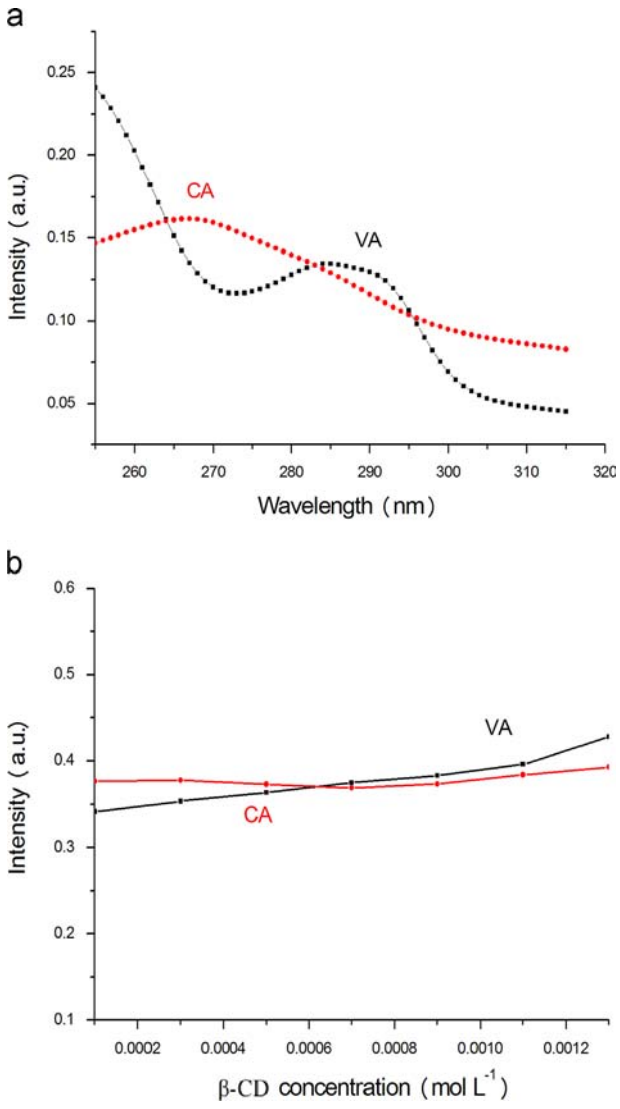


**Fig. 3.** (a) Individual normalized spectra of target analytes under the optimum conditions. (b) Normalized β-CD concentration profile.

data to resolve each of the analyte profiles from any uncalibrated interferences in subsequent studies.

Validation samples show 3-D plots similar to those of calibration samples, therefore satisfactory results in the prediction step by applying BLLS/RBL were reasonably expected (Fig. 4a and b). The prediction results for the validation set were reasonably good, leading to a mean recovery of 97.80% and 102.99%, a REP of 3.08% and 4.53%, and a RMSD of 0.112 and 0.117 for VA and CA, respectively (see Table 2). These parameters indicate that the proposed method is a feasible methodology for achieving the second-order advantage in cases of sample components with similar spectra. Regarding the pseudounivariate calibration graphs using BLLS/RBL models, they are displayed in Fig. 4c and d. As it can be seen, a very good fitting was obtained for both components. This fact reflects a high calibration power of the model.

Figures of merit for the proposed BLLS/RBL method were estimated using MVC2 program where net-analyte signal concept (see Section 2.3) was applied [39]. Figures of merit including sensitivity (SEN), analytical sensitivity (γ), selectivity (SEL), limit of detection (LOD) and RMSEP are summarized in Table 3. From Table 3, it can be seen that the BLLS/RBL model offers a very sensitive and selective method for simultaneous determination of CA and VA.

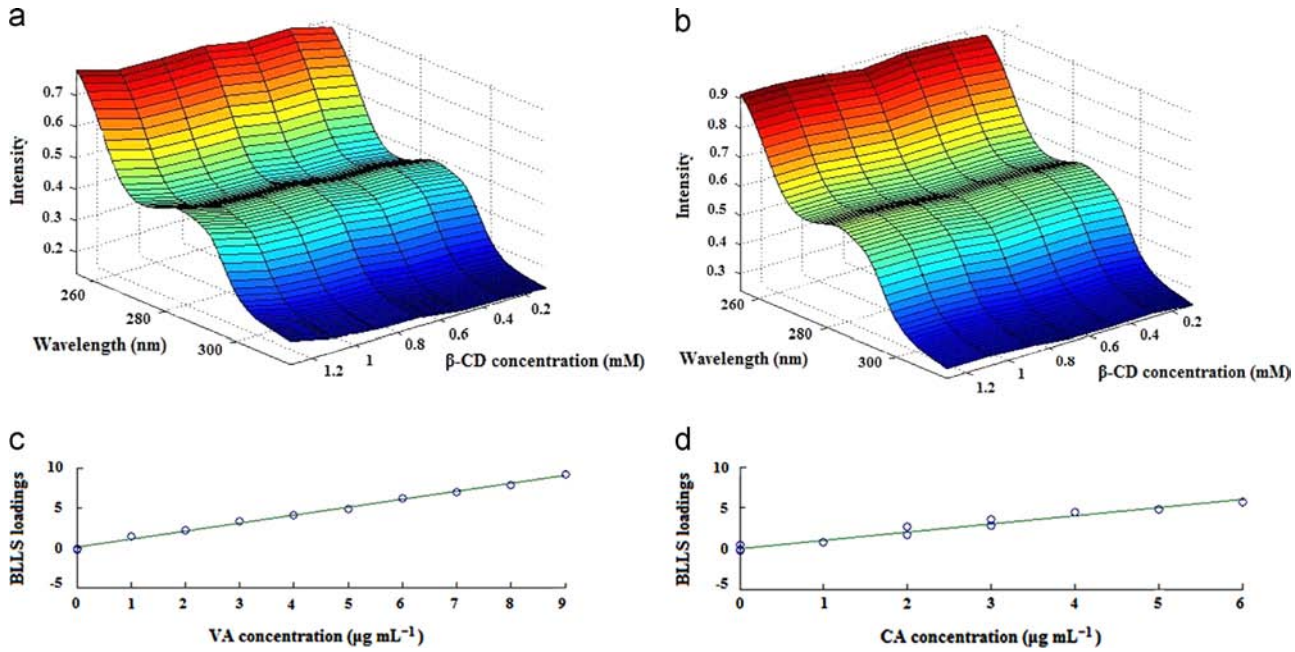


**Fig. 4.** 3-D plots of: (a) calibration sample and (b) validation sample. Pseudounivariate calibration graphs corresponding to the BLLS/RBL models: (c) VA model and (d) CA model.

### 4.5. Simultaneous quantification of CA and VA in real samples

Determination of CA and VA in fruit juices samples was carried out using BLLS/RBL. Numbers of solutes and interferents were set as 2 and 1, respectively. The results are given in Table 4. Prediction of CA and VA in sour cherry juice (for unspiked sample) by BLLS/RBL provides the spectral and concentration of β-CD profiles for each phenolic acid along with the interferents profile, as shown in Fig. 5. Even in the presence of interferents, BLLS/RBL was satisfactory for delivering the spectral profiles of phenolic acids, and this would explain its high prediction power. The results shown in Table 4 clearly demonstrated the ability of the proposed method to successfully determine CA and VA in the fruit juices samples. In addition, according to the delivered spectra depicted in Fig. 5a, a high similarity between these spectral profiles and those of the pure solutions (Fig. 3a) confirmed the accuracy and reliability of the proposed strategy, which fully exploited second-order advantage, regardless of the complexity of the studied matrix. The applied β-CD concentration range in this study was based on the preliminary experiments and previous reports about the spectroscopic properties of inclusion complexes of CA and VA with β-CD [53,54].

In order to validate the performance of the proposed method, the samples were also analyzed by the HPLC-PDA detection method and the predicted CA and VA concentrations are displayed in Table 4. In the proposed method, determination of CA and VA in the real samples (fruit juices samples) was carried out in the presence of β-CD under the optimum conditions, but in the HPLC method, the real samples were directly analyzed, so then some observed partial differences in the results are quite reasonable. The results of the *t*-test at appropriate confidence level (95%) revealed no significant differences between the reference method (HPLC) and the strategy described in the present report. Therefore, a comparison of BLLS/RBL results with those obtained by the chromatographic method demonstrates an acceptable performance of the proposed methodology. It is worth to mention that the proposed method is more environmentally friendly than the HPLC method. In the gradient elution, use of at least two organic solvents is inevitable and the post column derivatization is also a mandatory part of most HPLC analyses, whereas the present method uses safer and cheaper chemicals in the analysis process.

**Table 3**
Figures of merit for quantification of CA and VA using BLLS/RBL method.

| Phenolic acid | SEN[a] (AAU mL μg$^{-1}$) | SEL | LOD (μg mL$^{-1}$) | RMSEP (μg mL$^{-1}$) | Analytical sensitivity$\gamma$) mL μg$^{-1}$) |
|---|---|---|---|---|---|
| VA | 0.26 | 0.27 | 0.20 | 0.121 | 21.4 |
| CA | 0.39 | 0.27 | 0.13 | 0.126 | 32.8 |

[a] Arbitrary absorbance unit.

## 5. Conclusion

Various ways have been applied for generation of second-order data until now. This research reports the first application of beta-cyclodextrin (β-CD) complexes as a new method for generation of three way data, combined with second-order calibration based on the BLLS/RBL and PARAFAC algorithms, exploiting the second-order advantage. This combination can be successfully implemented for quantification of multicomponents in the presence of unexpected sample matrix components. Second-order calibration methods could predict accurate concentrations, together with a reasonable resolution of spectral profiles for the analytes of interest from any uncalibrated interferents on account of "second-order advantage".

Both the three-way PARAFAC and BLLS/RBL models predicted concentration of target phenolic acids in validation samples. However, due to an intense overlap present in β-CD concentration dimension, BLLS/RBL provided better results than PARAFAC. BLLS/RBL clearly manifested the utilization of "second order advantage" when quantifying CA and VA as an example in fruit juices samples, even in the presence of uncalibrated/unexpected interferents. When this model is applied to the analysis of real samples, the results are satisfactory and comparable with those delivered by HPLC-PDA detection method. The proposed method could be

**Table 4**
Results for determination of CA and VA in fruit juices samples using BLLS/RBL and HPLC-PDA methods.

| Sample | BLLS/RBL[a] (μg mL$^{-1}$) (RSD %)[b] | | | | | | HPLC-PDA (μg mL$^{-1}$) (RSD %) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | VA | | | CA | | | VA | | CA | |
| | Added | Found | Recovery (%) | Added | Found | Recovery (%) | Added | Found | Added | Found |
| Barberry juice | 0.0 | 6.0 (6.4) | – | 0.0 | 2.3 (5.0) | – | 0.0 | 6.3 (4.3) | 0.0 | 2.27 (6.0) |
| | 0.5 | 6.43 (6.5) | 86.00 | 1.0 | 3.2 (4.8) | 90.00 | 0.5 | 7.00 (4.5) | 1.0 | 3.2 (6.3) |
| Sour cherry juice | 0.0 | 4.5 (6.8) | – | 0.0 | 3.3 (5.5) | – | 0.0 | 4.63 (5.0) | 0.0 | 3.5 (5.5) |
| | 1.0 | 5.53 (6.6) | 103.00 | 1.0 | 4.15 (5.8) | 85.00 | 1.0 | 5.58 (5.3) | 1.0 | 4.6 (5.7) |
| Pomegranate juice | 0.0 | 3.68 (6.3) | – | 0.0 | 4.2 (6.4) | – | 0.0 | 3.76 (6.4) | 0.0 | 4.08 (7.0) |
| | 0.5 | 4.2 (6.7) | 104.00 | 0.5 | 4.73 (6.2) | 106.00 | 0.5 | 4.3 (6.6) | 0.5 | 4.5 (7.4) |
| Orange juice | 0.0 | 2.15 (7.4) | – | 0.0 | 3.77 (6.5) | – | 0.0 | 2.3 (8.0) | 0.0 | 3.85 (8.2) |
| | 2.0 | 4.05 (7.2) | 95.00 | 1.5 | 5.2 (6.3) | 95.33 | 2.0 | 4.1 (8.3) | 1.5 | 5.4 (8.5) |

[a] In BLLS/RBL, number of components and interferents were set at 2 and 1, respectively.
[b] Data were calculated based on three-replicate experiments.

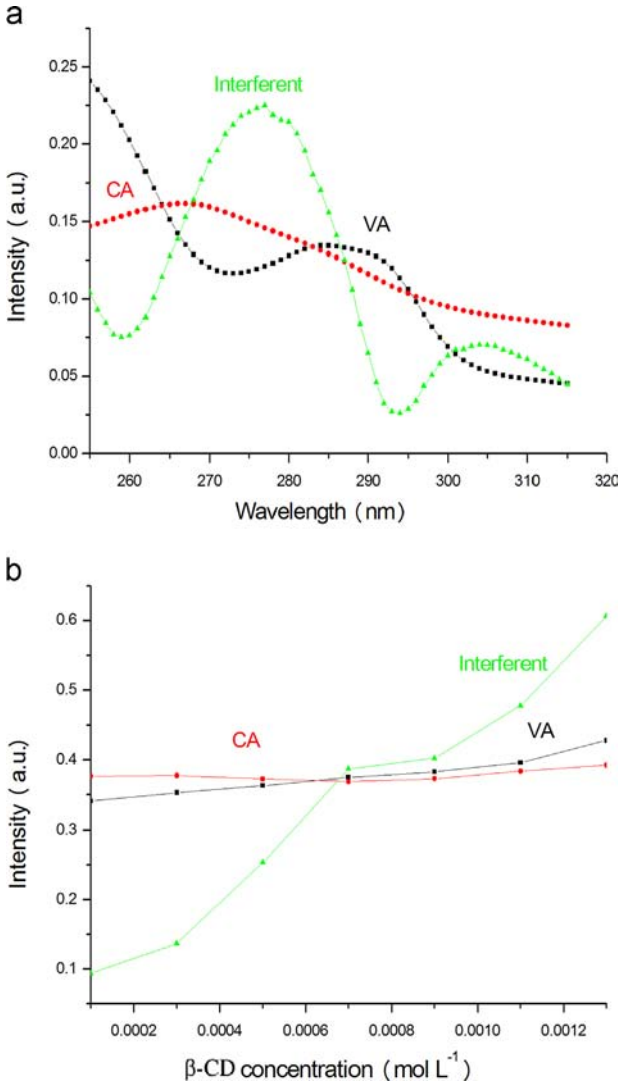


**Fig. 5.** BLLS/RBL outputs for spectral deconvolution of CA and VA in sour cherry juice (for unspiked sample). (a) Normalized spectra predicted by 2-components and 1-interferent with BLLS/RBL model and (b) Normalized β-CD concentration profile.

applied to simultaneous monitoring and quantification of multi-components in real samples with complex matrices and represents an interesting, simple, fast, accurate, and economical alternative to separation methods.

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