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Second-order advantage obtained from standard addition first-order instrumental data and multivariate curve resolution-alternating least squares. Calculation of the feasible bands of results

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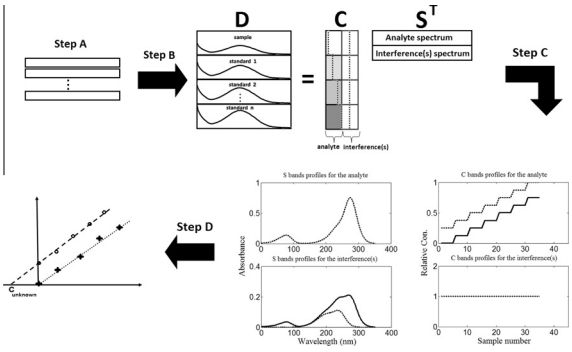
HIGHLIGHTS

- The replicate spectra are used to build a virtual two-way data.
- This data matrix is rank deficient.
- Augmentation of this data with standard addition data will break the rank deficiency.
- The algorithm MCR-BANDS has been employed to evaluate the rotational ambiguity.

GRAPHICAL ABSTRACT



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ABSTRACT

In order to achieve the second-order advantage, second-order data per sample is usually required, e.g., kinetic-spectrophotometric data. In this study, instead of monitoring the time evolution of spectra (and collecting the kinetic-spectrophotometric data) replicate spectra are used to build a virtual second order data. This data matrix (replicate mode × λ) is rank deficient. Augmentation of these data with standard addition data [or standard sample(s)] will break the rank deficiency, making the quantification of the analyte of interest possible. The MCR-ALS algorithm was applied for the resolution and quantitation of the analyte in both simulated and experimental data sets. In order to evaluate the rotational ambiguity in the retrieved solutions, the MCR-BANDS algorithm was employed. It has been shown that the reliability of the quantitative results significantly depends on the amount of spectral overlap in the spectral region of occurrence of the compound of interest and the remaining constituent(s).

Introduction

The problem of the appearance of unknown interference(s) is common in chemical analysis. In most cases, analysts have to deal with natural samples such as biological matrices, pharmaceuticals and environmental specimens, which are far from simplicity. To cope with these issues, many sophisticated instrumentations which provide multidimensional (multi-way) data have been developed. Multi-way data include second-order (matrices) or third-order (three-mode arrays) data for a single sample, which can be organized in a three- or four-way array, respectively, for a group of samples. For these data one mode refers to the compositional variation of the system and the others are related to the

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variation in the collected responses in the instrumental modes. When the number of data modes increase, different data-processing and mathematical algorithms are required for the convenient study of the collected data [1]. A calibration model constructed using multi-way measurements makes the quantitation of the analyte of interest possible in new samples containing unknown component(s) which do not take part in the calibration data set [2–7]. This property is known as the second-order advantage [8]. However univariate calibration, which employs a single response per sample (known as zero order data) or multivariate calibration e.g. collecting a vector data for a sample (known as 1st order data), are not usually able to quantitate the analyte of interest in the presence of unknown and non-calibrated component(s) [8]. This means that the first-order calibration may compensate for interferences only if they are included in the calibration set. This explains why a large number of samples are needed in first-order calibration in comparison with second-order calibration, which can be performed using a few standards (in an extreme case, with only a single calibration sample). Second-order data are provided by advanced hyphenated instrumentations such as two-dimensional NMR, capillary electrophoresis or chromatographic systems coupled to mass spectroscopy or diode-array detectors, whereas first-order instrumental data can be measured using fairly simple equipments employing spectroscopic, chromatographic and voltammetric tools.

Analyte quantitation using first-order multivariate data in the presence of unexpected components (to achieve the second-order advantage) is a very recent subject and to the best of our knowledge only a few reports exist in the literature [9–13]. It has been shown that the correlation-constrained MCR-ALS version facilitates the analyte quantitation in the presence of unexpected interferences using first-order data [9–13]. MCR-ALS with the proposed correlation constraint has been applied to resolution and quantification of mixtures of metal ions with overlapping voltammetric peaks [9]. Also the determination of the major components in complex mixtures using first-order spectrophotometric data [10,11], quantification of industrial mixtures from the vinyl acetate monomer process using near infrared spectroscopic data [12] and urinary quantification of nicotine in the presence of metabolite cotinine and the alkaloid anabasine using surface enhanced Raman spectroscopy [13] have been presented recently. In the latter case [13], standard addition in combination with the MCR-ALS method has been employed to deal with matrix effects and non-calibrated interferences in the quantification of nicotine present in human urine.

In the presence of analyte-background interactions, chemical analysis can be further complicated by matrix effects [14]. When the sensitivity of the calibration depends on the matrix composition, quantitative predictions using pure standards may be expected to be biased. This problem can only be solved by the standard addition method. A proper calibration model should reflect the complexity of the matrix composition, otherwise poor predictions may result when using calibration curves obtained from pure standards [15].

Kinetic-spectroscopic second-order data have been employed recently for analyte quantitation in the presence of non-calibrated interferences, achieving the second-order advantage [16]. In some particular kinetic-spectral experiments, the kinetics of all constituents are identical, so the selectivity in the time direction is zero. In these cases, the second-order advantage can be achieved, however, by augmenting the data matrices in the direction of time, creating selectivity in the augmented direction and using extended MCR-ALS with correspondence restrictions [16].

In the present study, which was inspired by the Ref. [16], we aimed to avoid the time-consuming kinetic experiments and gain the second-order advantage using first-order data by recording a spectrum for each sample and its replicates. Usually, as mentioned, in order to achieve the second-order advantage second-order data, e.g., kinetic-spectrophotometric data are required. In this study, instead of monitoring the spectra *versus* time (collecting kinetic-spectrophotometric data) spectral replicates are used to build a replicated spectrophotometric data matrix (i.e. replicate number $\times$ λ). The constructed data matrices are rank deficient. Augmentation of these data with standard addition samples or a few external standard test samples will break the rank deficiency problem and make the quantification of the analyte of interest possible. These data are the same as if the kinetics of all sample constituents were identical employing the second-order kinetic-spectroscopic measurements.

In this work we used the standard addition method, which allows to overcome the matrix effects. This means that when each sample arrives at the laboratory, the experimentalist has to perform several measurements and experimental sample preparation activities. Although with external calibration, calibration only needs to be performed once, the standard addition method is unavoidable when it is necessary to overcome the matrix effects. Conventional standard addition in conjunction with the MCR-ALS approach has been employed to quantitate the analyte of interest in the presence of unexpected interference components. Avoiding tedious procedures of complex sample pretreatments, minimizing analyte loss and therefore increasing precision in the results are the advantages provided by the standard addition method. Finally, in order to evaluate the extent of rotational ambiguity in the retrieved solutions, the MCR-BANDS algorithm was applied. The calibration curves were built, similarly to the traditional standard addition method, using the recovered concentration profiles as a function of standard concentrations. In order to demonstrate the applicability of the proposed method, several simulated examples, a number of synthetic binary mixtures and spiked samples including human urine and blood serum samples were analyzed using the proposed method.

Experimental procedure

Reagents

All experiments were performed using analytical reagent grade chemicals. Malachite green (MG), crystal violet (CV), HCl and methanol were obtained from Merck (Darmstadt, Germany) and were used without any purification. Pure paracetamol (PC) and ibuprofen (IB) were provided from an Iranian pharmaceutical company. To perform binary mixture analysis, individual standard solutions of MG and CV (20 μg mL^{-1}) were prepared by dissolving appropriate amounts in distilled water. Also, standard solutions of 100 μg mL^{-1} each of PC and IB were prepared by dissolving appropriate amount of these compounds in a 0.1 mol L^{-1} HCl–methanol mixture (1:3). Different aliquots of the standard solutions of MG and CV, and also of PC and IB within the linear calibration range were transferred into 10 mL voltammetric flasks and completed to the volume with distilled water and a 0.1 mol L^{-1} HCl–methanol mixture (1:3), respectively. Urine and blood serum samples were prepared by spiking these samples with appropriate amounts of the stock solution of PC.

Apparatus

A model T80^{+} UV–Vis double-beam spectrophotometer with a PG mode (China) with 1-cm quartz cells (volume 5 mL) was employed for spectrophotometric measurements.

Theoretical background and the proposed method

Multivariate curve resolution techniques are powerful approaches promoted to tackle many chemical problems that could not be solved otherwise. The common purpose of all multivariate resolution methods is to transform the raw experimental measurements into useful information. MCR-ALS is a well known second-order algorithm that uses an alternative approach to iteratively find the concentration profiles and instrumental responses [17–21]. Bilinear decomposition of the initial mixture data matrix **D** into the product of concentration profiles (**C**) and pure spectra ($\mathbf{S}^T$) according to Beer's law can be expressed as:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E} = \sum_{i=1}^{N} \mathbf{c}_i \mathbf{s}_i^T + \mathbf{E} = \mathbf{D}^* + \mathbf{E} \quad (1)$$

where **E** is the residual data matrix not explained by the model, which should ideally be close to the experimental error, and $\mathbf{D}^*$ is the noiseless approximation to the data matrix. The iterative ALS optimization procedure to find the matrices of concentration profiles and pure spectra, which optimally fits the experimental data matrix **D**, starts with initial estimates of either **C** or $\mathbf{S}^T$ profiles [22,23]. During the optimization, several constraints may be applied depending on the characteristics of the system under study [17,24–26].

It is well known that the main source of uncertainty associated with the solutions obtained by MCR methods (like for any other factor analysis-based methods) are the ambiguities of the recovered profiles. When ambiguity exists, a band of feasible solutions instead of a unique profile will be obtained for a compound. Ambiguities (intensity and rotational) can be mathematically represented by the following equation:

$$\mathbf{D}^* = \mathbf{C}_{old} \times \mathbf{S}_{old}^T = (\mathbf{C}_{old} \times \mathbf{T}^{-1}) \times (\mathbf{T} \times \mathbf{S}_{old}^T) = \mathbf{C}_{new} \times \mathbf{S}_{new}^T \quad (2)$$

where **T** is any non-singular invertible matrix which is responsible for rotation in Eq. (4). Imposing appropriate constraints can considerably reduce the number of possible solutions or the number of possible **T** matrices.

Since several different degrees of overlap will be applied to the simulated systems in this paper, to calculate the degree of spectral overlap between the compound of interest and interference the following expression was used:

$$S_{12} = \frac{||s_1^T \times s_2||}{||s_1|| \, ||s_2||} \quad (3)$$

where s_1 and s_2 are the spectra related to the analyte and interference, respectively.

In order to evaluate the accuracy of the proposed method, the prediction error of analyte concentrations in the mixtures was calculated as the relative standard error (RSE) of the prediction concentrations:

$$\mathrm{RSE}(\%) = \left(\frac{\sum_{j=1}^{N} (\hat{C}_j - C_j)^2}{\sum_{j=1}^{N} (C_j)^2} \right)^{1/2} \times 100 \quad (4)$$

where N is the number of samples, C_j the real concentration of the component in the jth mixture and $\hat{C}_j$is the estimated concentration.

Relative error of prediction (REP) for quantitative measurements in analyte concentrations was calculated according the following equation:

$$\mathrm{REP}(\%) = \frac{(C_{found} - C_{true})}{C_{true}} \times 100 \quad (5)$$

where C_{true} is considered the known concentration value for analyte and C_{found} is the prediction concentration.

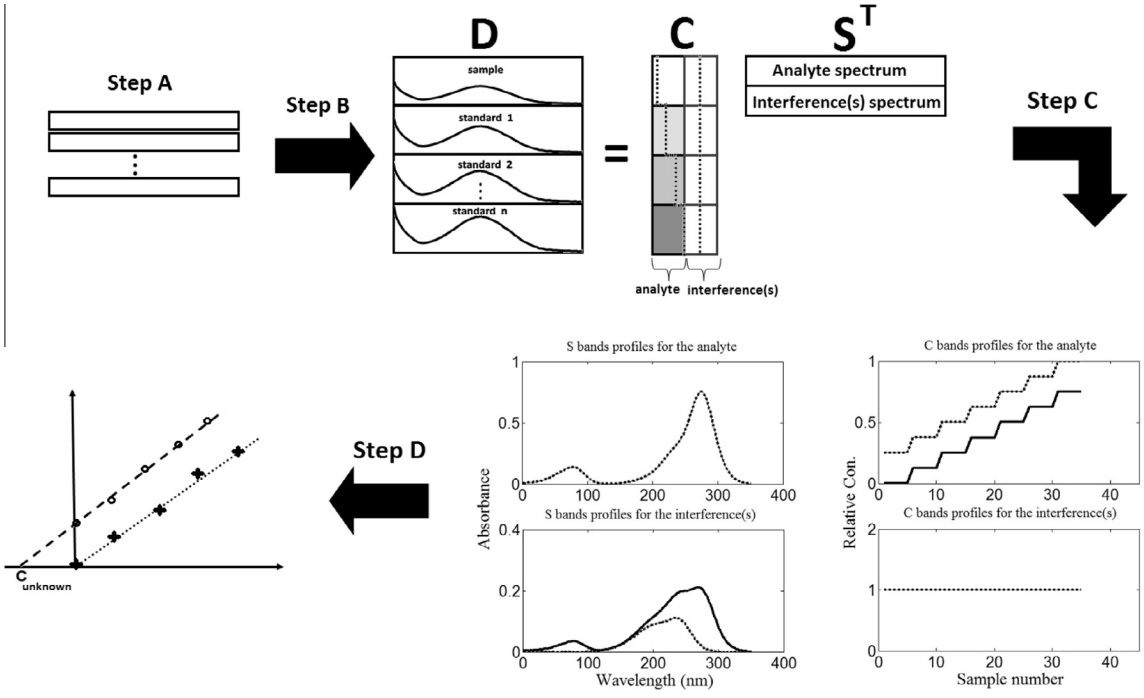

Fig. 1. MCR-ALS in conjunction with standard addition method for quantitative analysis of the analyte in the presence of interferences, considering the kinetics of all sample constituents is identical. Step A: Setting repeatedly the spectrophotometric data vector for a sample under each other. Step B: Column-wise augmentation of standard added samples followed by MCR-ALS decomposition. Step C: MCR-BANDS analysis and calculating the maximum and minimum band boundaries of the retrieved solutions. Step D: Quantitative analysis by plotting standard addition calibration curve.

The data arranging strategy

A graphical description of the proposed method is presented in Fig. 1, and further expanded below.

Construction of a data matrix

Absorbance for a series of samples prepared according to the standard addition method was measured within a given wavelength range and a data vector (spectrum) was obtained (first-order data) for each sample. Each of these vectors provides the spectrum of a mixed sample. Then, the row data vector for every standard added sample was arranged repeatedly below each other (arbitrarily, 5 replications per sample) and a virtual second-order data matrix was created. This kind of data arrangement may be considered as a second-order kinetic-spectroscopic data matrix where the kinetic mode (row direction) represents an invariant reaction rate during the time. A particular case occurs when the kinetics of all sample constituents are identical and as a consequence there is no selectivity in the time mode.

Column-wise augmentation of the standard addition data matrices

By successive standard addition of an analyte, the concentrations of the remaining components (interferences) remain constant and introduce linear dependency between interference concentrations in the samples mode (replicate mode). Therefore the individual virtual data matrix and its standard addition data (replicate $\times$ λ) are rank deficient. It is possible to break the linear dependency by augmenting the data matrices in the rank deficient direction. This was carried out by organizing the individual data matrices corresponding to each standard added sample under the data matrix of unknown sample (column-wise augmentation). Then, the number of components was simply estimated by singular value decomposition of augmented matrices, which implies the presence of two components including the analyte of interest and the interference(s).

MCR-ALS analysis

As mentioned the iterative ALS optimization starts with the initial estimates of either $\mathbf{C}$ or $\mathbf{S}^T$. In general, the use of chemically meaningful estimates is an essential factor that can help not only to rapid convergence of the results but also to decrease the ambiguity of the solutions. Different methods can be used to find suitable initial estimates to start the MCR-ALS. Based on the strategy used for data construction in this work, after augmentation there will be two components, one for analyte of interest and the other for linear combination of the interferents. Pure spectrum of the analyte along with the purest cumulative spectrum of the interferents were used as initial estimate. The purest cumulative spectrum can be obtained by pure variable selection methods such as orthogonalization or subtraction of the analyte spectrum from that of mixture (taking into account the non-negativity constraint). Other pure variable selection methods such as SIMPLISMA can be used alternatively [25,27].

MCR-ALS was implemented on the augmented data matrix comprising an unknown sample and those of the standard addition which is simply $\boldsymbol{D}_{aug} = \boldsymbol{C}_{aug}\boldsymbol{S}^T + \boldsymbol{E}_{aug}$, where the augmented data matrix ($\boldsymbol{D}_{aug}$) is of size $I \times J$ (I equals X-times repeated spectrum below each other $\times$ the number of standard added samples and J is the number of wavelengths), the columns indicate the concentration variations in the standard added samples and the rows involve the absorption spectrum for each sample. Bilinear decomposition of the data matrix $\boldsymbol{D}_{aug}$ into the matrix of concentration profiles $\boldsymbol{C}_{aug}$ (size $I \times N$) and pure spectra $\boldsymbol{S}^T$ (size $N \times J$), where N represents the number of components, achieved according to the MCR-ALS approach. For our case N equals 2. According to the nature and structure of the data, non-negativity for both concentration and spectral profiles and equality for the

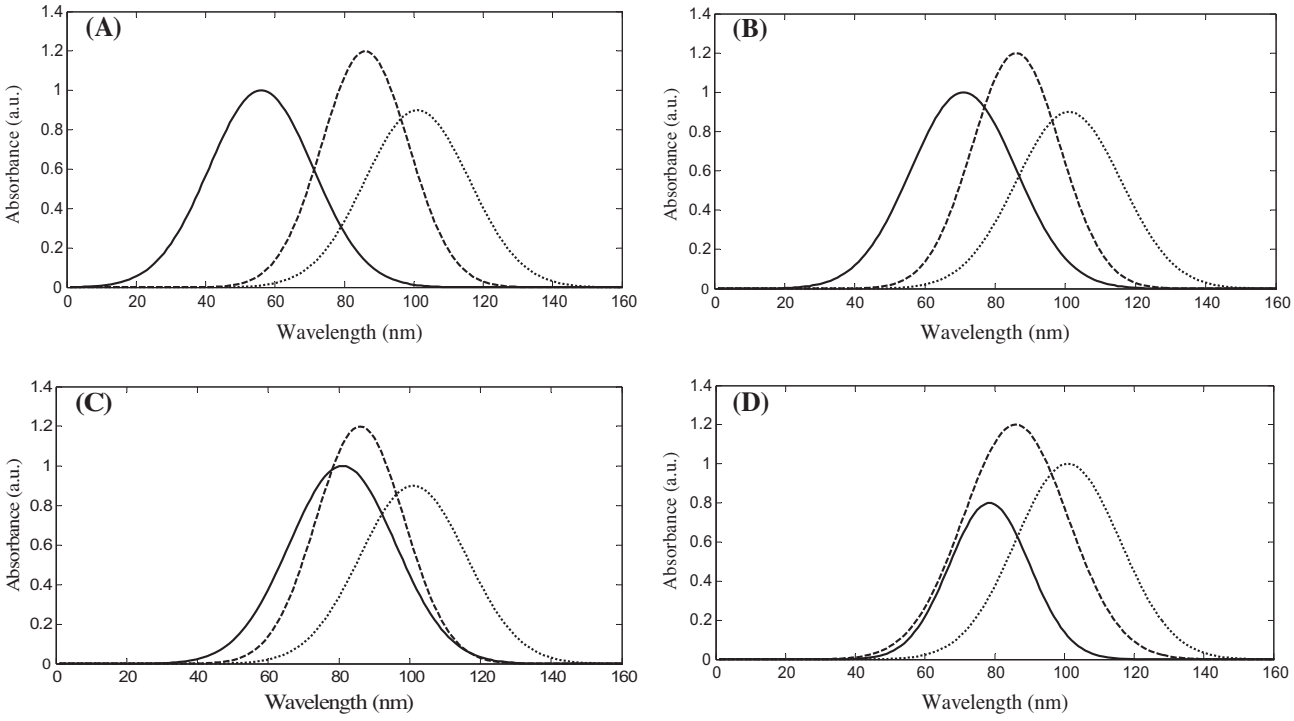

Fig. 2. Simulated spectra for three component systems with different degrees of overlap: (A) data set 1, (B) data set 2, (C) data set 3 and (D) data set 4. The solid line is the analyte of interest, the dashed line and the dotted line indicate the interferences.

analyte spectrum were imposed as suitable constraints. The number of iterative cycles was set in a way that convergence was fulfilled in each case.

Evaluation of rotational ambiguity

After the MCR-ALS decomposition, the extent of rotational ambiguity remaining in the retrieved profiles was investigated. Concentration and spectral profiles as the initial input values were submitted to the MCR-BANDS program. During the optimization, the constraints implemented in the previous MCR-ALS procedure were used herein. Optimization was carried out and maximum and minimum band boundaries of concentration and spectral profiles were obtained. The differences between the maximum and minimum component relative contribution optimization function ($f_n^{\max} - f_n^{\min}$) were calculated as a criterion of the rotational ambiguity for the analyte concentration profiles [28,29].

Quantitative analysis

The calibration curves were built, similarly to the conventional standard addition method. The relative concentration values in matrix **C** to each addition were plotted *versus* the standard concentration. Extrapolation of the calibration curve, i.e., the intercept of the calibration line with the abscissa, gave the concentration of analyte in the sample.

Data and modeling

Simulated data

In order to evaluate the performance of the proposed method, it was employed to analyze several simulated data. Four data sets with different degrees of spectral overlap were prepared. The spectrum for the analyte was intentionally constructed so that the degrees of spectral overlap gradually increased from data set 1 to data set 4, as presented in Fig. 2(A–D). Spectral overlap for the simulated data sets 1, 2, 3 and 4 were calculated 0.23, 0.61, 0.87 and 0.96, respectively, using Eq. (3). For every sample, several successive additions of the analyte were done, while concentrations of the other two components (interferences) were kept constant in all the samples according to the standard addition model. The data sets were generated from noiseless UV–Vis spectral and concentration profiles. To built up a data matrix, the spectrum (row vector) corresponding to each standard added sample was repeated five times (this number is arbitrary) below each other. Simulated spectral profiles, concentration profiles and the constructed data matrix are shown in Fig. 3(A–C), respectively. Each simulated sample contained three chemical components two interferents and one as the analyte of interest. The constructed data matrix was used for subsequent calculations.

Binary synthetic mixture analysis

To demonstrate the analytical applicability of the proposed method, binary mixtures of malachite green (MG) and crystal violet (CV), which were assumed alternatively as the analyte and the unknown interference, and also of paracetamol (PC) in the presence of ibubrophen (IB) as an interference were created. The absorption spectra of the mixture samples were recorded within the wavelength range of 350–700 nm for MG and CV and 200–310 nm for PC and IB with the increment of 1 nm against the appropriate solvent blank. The data were processed as the simulated data sets, with the spectrum corresponding to each standard added sample repeated five times below each other.

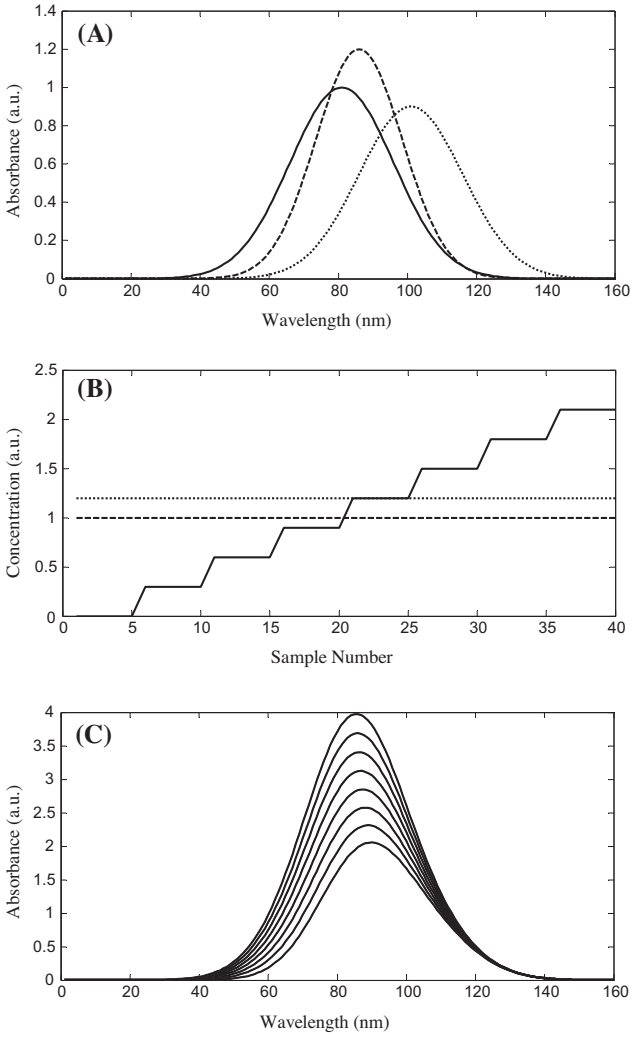

Fig. 3. (A) Spectrum, (B) concentration profiles (solid line is the analyte, dashed and dotted lines indicate the interferences) and (C) simulated standard added data matrix.

Real samples

The wide applicability of the proposed method was investigated. Human urine and blood serum samples spiked with PC were analyzed for this purpose. The absorption spectra of the spiked samples were recorded within the wavelength range of 200–310 nm with the increment of 1 nm. The spectrum corresponding to each standard added sample repeated five times below each other and the data were processed as the simulated data sets.

Software

All simulations and initial estimates prior to MCR-ALS algorithm were carried out using MATLAB (version 7.10.0 R2010a) computer environment. Data processing was done in Microsoft Excel for Windows. MCR-ALS was performed with the graphical user-friendly interface provided by Tauler [21]. Calculations related to rotational ambiguities were implemented using MCR-BANDS graphical user interface [28].

Table 1
Results obtained by applying MCR-ALS and MCR-BANDS analysis to the simulated data sets 1 and 2.

Simulated concentration[a]	Predicted concentration	Recovery (%)	REP (%)[b]	Feasible band[c]
Simulated data set 1				
0	0	–	–	0
0.3	0.3	100	0	0.0462
0.6	0.6	100	0	0.0937
0.9	0.9	100	0	0.1419
1.2	1.2	100	0	0.1904
1.5	1.5	100	0	0.2389
1.8	1.8	100	0	0.2870
2.1	2.1	100	0	0.3346
2.4	2.4	100	0	0.3815
Mean recovery		100		
RSE (%)[d]		0		
Simulated data set 2				
0	0	–	–	0
0.2	0.1989	99.44	−0.55	0.0544
0.4	0.3992	99.79	−0.20	0.1097
0.6	0.5994	99.91	−0.10	0.1651
0.8	0.7997	99.96	−0.04	0.2201
1	1	100.0	0	0.2740
1.2	1.2003	100.0	+0.02	0.3269
Mean recovery		99.85		
RSE (%)		0.081		

[a] The concentration of interference components were fixed at 1 (in arbitrary unit) during all simulation as standard addition modeling.
[b] Relative error of prediction (Eq. (5)).
[c] Corresponds to the difference between $f_n^{max} - f_n^{min}$.
[d] Relative standard error of prediction (Eq. (4)).

Results and discussion

Simulated data

As illustrated in the previous section, four data sets with different degrees of spectral overlap were simulated and analyzed. For data set 1, nine successive additions of the analyte were made and a data matrix of size 50 (5 replications per sample × 10 standard addition mode) × 201 (number of wavelengths) was obtained. MCR-ALS decomposition of the data matrix was done using the initial estimate explained in the third step of the proposed method. A set of solutions $\mathbf{C}$ (50×2) and $\mathbf{S}^T$ (2×201) were obtained and used as initial inputs for the MCR-BANDS program. In both procedures, non-negativity constraints for concentration and spectral profiles and equality constraint for the analyte spectrum were imposed. In each case, one of the standard added data matrices was removed (five out of fifty) and the new data matrix was analyzed. Quantitative analysis was performed for every sample as illustrated in the fifth step of the proposed method. In Table 1 (upper part), the obtained results for data set 1 are given.

MCR-BANDS results for three samples with the simulated concentrations of 0, 0.3 and 0.6 (in arbitrary units) for the analyte and constant concentration of 1 for both interferences are shown in Fig. 4. Maximum and minimum band boundaries for the analyte concentration profiles imply the range of feasible solutions (f_n^{max} and f_n^{min}) where the maximum band boundaries (continuous blue line) coincide with the red dotted line of the initial profiles. As can be seen from Fig. 4, with increasing the analyte concentration, the range of feasible concentration profiles also increases, while the lower concentration level (minimum band boundary) remains invariant and equals to zero concentration. Therefore, the upper level (maximum band boundary) defines the analyte concentration. Extrapolation of the standard addition calibration curve for the upper boundary determines the analyte concentration in each sample. Acceptable recoveries were obtained which indicate that the results are accurate.

Likewise, other three data sets were built up and analyzed with MCR-ALS and MCR-BANDS programs. Tables 1 (lower part) and 2 collect the results for all data sets 2, 3 and 4, respectively. In each case, relative standard error (RSE), quantitation error and also the differences between the maximum and minimum optimization function values were calculated. The same as data set 1, for these data sets the lower concentration level was invariant and equal to zero concentration. The extrapolation of the standard addition calibration curve for the upper level ascertained the analyte concentrations in samples.

From the obtained results for the analyte quantitation in four simulated data systems it can be concluded that with increasing the degrees of spectral overlap between the analyte and interferences, the value of relative error in the predicted concentrations for the upper boundary increases, whereas for the lower one it always equals −100%. For data set 1, the proposed method yields excellent recoveries. This may be due to the fact that the degree of spectral overlap between the analyte and interferences is small (0.23 as calculated from the Eq. (3)). Also for the case of data sets 2 and 3, with degrees of overlap 0.61 and 0.87, respectively, satisfactory quantitation results were obtained. However, analysis of data set 4 led to apparently worse recoveries. In fact, the latter data set provides the opportunity to test an extreme spectral overlap effect, where the spectrum for the compound of interest is completely embedded in the sample background and there is no selective region for it. This may be ascribed to the fact that the analyte spectrum becomes mixed up with those of the interferences and the analyte contribution is not totally removed from the rest of the mixture. As a consequence, the proposed method overestimates the concentration of the analyte.

Random noise was added to the simulated data sets in order to test the method more rigorously. A random homosadistic noise of ±0.005 absorbance unit was added to each matrix and then the procedure was followed as explained above where the concentration of analyte of interest in the noisy data was predicted. For data sets 1, 2 and 3, there was a good agreement between simulated and

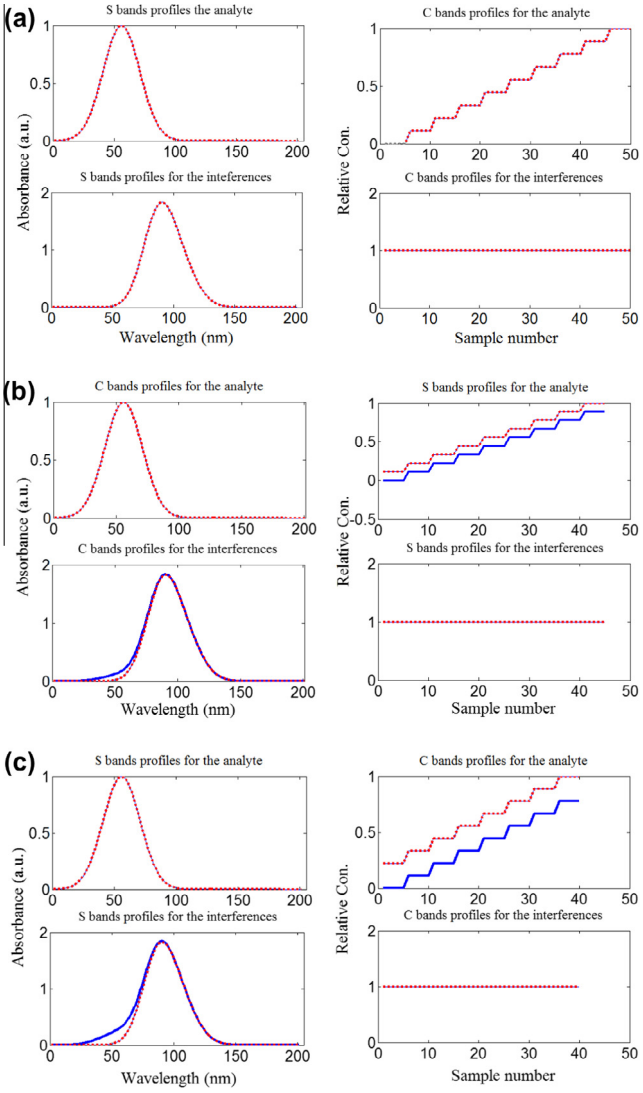

Fig. 4. MCR-BANDS results for the data set 1 with simulated concentrations of (a) 0 (b) 0.3 and (c) 0.6 (in arbitrary units) for the analyte and constant concentration of 1 (in arbitrary unit) for both interferences. Red dotted lines indicate the initial profiles and the solid blue lines are the calculated band boundaries. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

predicted concentrations which shows the applicability of the method for noisy systems. The results are given in Tables 3 and 4.

Binary synthetic mixture analysis

In order to illustrate the proposed method with experimental examples, quantitation of binary mixtures of MG and CV, which were assumed alternately as an analyte and unknown interference, and also PC in the presence of IB as interference were performed.

Malachite green and crystal violet determination

Beer's law was obeyed in the concentration range 0.2–1.8 μg mL^{-1} for MG and CV using standard solutions. As Fig. 5 shows, the absorption spectra of MG and CV overlapped in the wavelength region of 450–650 nm. The degree of spectral overlap was calculated 0.53. Quantitation analysis of this binary system was carried out through eight successive additions of the analyte, while the concentration of CV and MG, assumed as interference components, respectively, were fixed at 1 μg mL^{-1} in all samples. A two-way data matrix of size 45 × 351 (5 replications per sample × 9 standard addition mode and 351 wavelengths) was constructed. The number of components, estimated using singular value decomposition, was two, as expected. Initial estimation obtained from subtraction of the pure analyte spectrum from the first spectrum of the standard added data matrix was used. Under

Table 2
Results obtained by applying MCR-ALS and MCR-BANDS analysis to the simulated data sets 3 and 4.

Simulated concentration[a]	Predicted concentration	Recovery (%)	REP (%)[b]	Feasible band[c]
Simulated data set 3				
0	0	–	–	0.0002
0.3	0.3004	100.1	+0.13	0.0476
0.6	0.6008	100.1	+0.13	0.0958
0.9	0.9013	100.1	+0.14	0.1438
1.2	1.1996	99.97	−0.03	0.1914
1.5	1.5000	100.0	0	0.2381
1.8	1.8004	100.0	+0.02	0.2838
Mean recovery		100.06		
RSE (%)[d]		0.058		
Simulated data set 4				
0	1.3347	–	–	0.0915
0.4	1.7427	435.7	+335.6	0.1201
0.8	2.1323	266.5	+166.5	0.1483
1.2	2.5330	211.1	+111.1	0.1769
1.6	2.9339	183.4	+83.37	0.2053
2	3.3347	166.7	+66.74	0.2337
2.4	3.7355	155.6	+55.64	0.2620
Mean recovery		236.5		
RSE (%)		92.58		

[a] The concentration of interference components were fixed at 1 and 1.5 (in arbitrary units) during all simulation as standard addition modeling.
[b] Relative error of prediction (Eq. (5)).
[c] Corresponds to the difference between $f_n^{max} - f_n^{min}$.
[d] Relative standard error of prediction (Eq. (4)).

Table 3
Results obtained by applying MCR-ALS and MCR-BANDS analysis to the simulated noised data sets 1 and 2.

Simulated concentration[a]	Predicted concentration	Recovery (%)	REP (%)[b]	Feasible band[c]
Simulated data set 1				
0	0	–	–	0
0.3	0.3	100	0	0.0462
0.6	0.6	100	0	0.0935
0.9	0.9	100	0	0.1418
1.2	1.2	100	0	0.1901
1.5	1.5	100	0	0.2386
1.8	1.8	100	0	0.2866
2.1	2.1	100	0	0.3339
2.4	2.4	100	0	0.3815
Mean recovery		100		
RSE (%)[d]		0		
Simulated data set 2				
0	0	–	–	0
0.2	0.1989	99.44	−0.55	0.0541
0.4	0.3997	99.93	−0.08	0.1096
0.6	0.5980	99.67	−0.33	0.1664
0.8	0.8096	101.2	+0.01	0.2218
1	1.0070	100.7	+0.70	0.2747
1.2	1.1828	98.57	+1.43	0.3240
Mean recovery		99.92		
RSE (%)		1.10		

[a] The concentration of interference components were fixed at 1 (in arbitrary unit) during all simulation as standard addition modeling.
[b] Relative error of prediction (Eq. (5)).
[c] Corresponds to the difference between.
[d] Relative standard error of prediction (Eq. (4)).

Table 4
Results obtained by applying MCR-ALS and MCR-BANDS analysis to the simulated noised data sets 3 and 4.

Simulated concentration[a]	Predicted concentration	Recovery (%)	REP (%)[b]	Feasible band[c]
Simulated data set 3				
0	0	–	–	0
0.3	0.3032	101.0	+1.07	0.0480
0.6	0.6000	100.0	0	0.0955
0.9	0.8970	99.67	−0.33	0.1420
1.2	1.2021	100.2	+0.18	0.1910
1.5	1.4979	99.86	−0.14	0.2376
1.8	1.7924	99.58	−0.42	0.2824
Mean recovery		100.1		
RSE (%)[d]		0.32		
Simulated data set 4				
0	1.2008	–	–	0.0826
0.4	1.6064	401.6	+301.6	0.1112
0.8	2.0040	250.5	+150.5	0.1398
1.2	2.4453	203.8	+103.8	0.1713
1.6	2.8577	178.6	+78.61	0.2001
2	3.1960	159.8	+59.80	0.2255
2.4	3.5960	149.8	+49.83	0.2570
Mean recovery		224.0		
RSE (%)		84.27		

[a] [a]The concentration of interference components were fixed at 1 and 1.5 (in arbitrary units) during all simulation as standard addition modeling.
[b] Relative error of prediction (Eq. (5)).
[c] Corresponds to the difference between.
[d] Relative standard error of prediction (Eq. (4)).

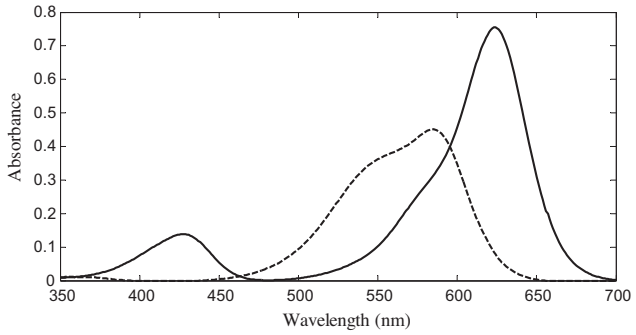

Fig. 5. Absorption spectra for malachite green (solid line) and crystal violet (dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the enforcement of non-negativity constraints for concentration and spectral profiles and equality constraint for analyte spectrum, MCR-ALS decomposition was implemented. MCR-BANDS retrieved profiles for the determination of MG which are shown in Fig. 6. As for the simulated data, one of the standard added data matrices was left out in each case, and the new data matrix was analyzed. It should be noted that the lower concentration level was zero for all cases and the upper level determined the analyte concentration in samples. Extrapolation of the standard addition calibration curve for the upper level specified the analyte concentration in each sample. Table 5 gives the recovery and relative standard error of prediction for the determination of MG and CV. Comparing the prediction performance of the proposed method for both examples indicates that good recoveries are obtained for MG, which is in excellent agreement with the actual content. This could have been expected, because the extent of the selective spectral region for MG is wider compared to that of the CV.

Paracetamol determination

Beer's law was obeyed in the concentration range of 0.6–11 μg mL^{-1} for PC in 0.1 mol L^{-1} HCl–methanol (1:3) mixture. As

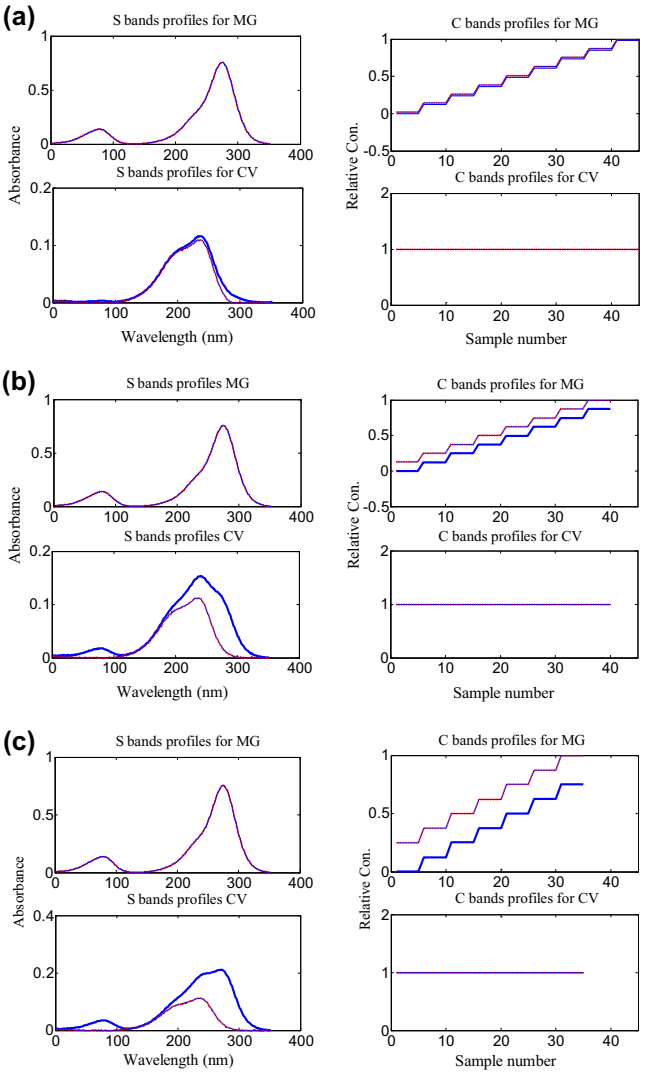

Fig. 6. MCR-BANDS results for determination of MG and CV with concentrations of (a) 0 (b) 0.2 and (c) 0.4 μg mL^{-1} for MG and constant concentration of 1 μg mL^{-1} for CV. Red dotted lines indicate the initial profiles and the solid blue lines are the calculated band boundaries. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 7 shows, the absorption spectra of PC and IB overlapped in the wavelength region of 200–240 nm. In this case, the degree of spectral overlap is 0.61. Quantitation analysis of PC was done by five successive addition of the analyte, while the concentration of IB, as interference, was fixed at 5 μg mL^{-1} in all samples. A two-way data matrix of size 30 × 111 (5 replications per sample × 6 standard addition mode and 111 wavelengths) was constructed. The data matrix was analyzed as before, and good quantification results were obtained, which are presented in Table 6.

Real samples

Absorption spectra for paracetamol, blood serum and urine samples are also presented in Fig. 7. The degree of spectral overlap for PC in serum and urine samples are 0.65 and 0.85, respectively. Quantitation analysis was carried out by five successive addition of PC as the analyte of interest. A two-way data matrix of size 30 × 111 (5 replications per sample × 6 standard addition mode and 111 wavelengths) was constructed. The data matrix was

Table 5
Obtained results for determination of MG and CV considered alternately as an analyte and unknown interference by applying the proposed method.

Taken ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	Recovery (%)	REP (%)[a]	Feasible band[b]
Malachite green				
0	0	–	–	0
0.2	0.1987	99.40	−0.65	0.1501
0.4	0.3968	99.20	−0.80	0.2854
0.6	0.6045	100.7	+0.75	0.4172
0.8	0.7939	99.24	−0.76	0.5292
1	0.9904	99.04	−0.96	0.6329
1.2	1.1911	99.26	−0.74	0.7273
1.4	1.4022	100.2	+0.16	0.8203
Mean recovery		99.58		
RSE (%)[c]		0.66		
Crystal violet				
0	0	–	–	0
0.2	0.2006	100.3	+0.30	0.1019
0.4	0.3990	99.76	−0.25	0.1934
0.6	0.6026	100.4	+0.43	0.2830
0.8	0.7927	99.08	−0.91	0.3691
1	0.9810	98.10	−1.90	0.4498
1.2	1.1785	98.20	−1.79	0.5402
1.4	1.4151	101.1	+1.08	0.6108
Mean recovery		99.56		
RSE (%)		1.41		

[a] Relative error of prediction (Eq. (5)).
[b] Corresponds to the difference between $f_n^{max} - f_n^{min}$.
[c] Relative standard error of prediction (Eq. (4)).

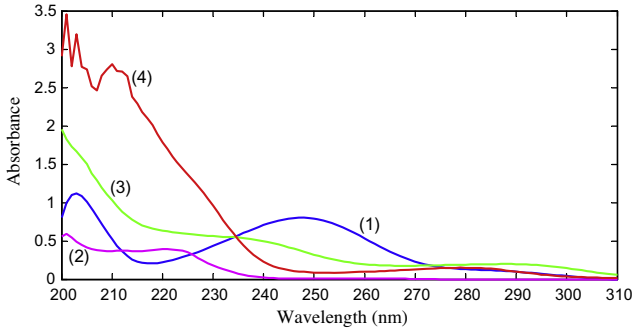

Fig. 7. Absorption spectra for paracetamol (1), ibuprofen (2), blood serum (3) and urine samples (4).

Table 6
Obtained results for determination of PC in the presence of IB by applying the proposed method.

Taken ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	Recovery (%)	REP (%)[a]	Feasible band[b]
0	0.0101	–	–	0
2	1.9494	97.47	−2.53	0.1051
4	4.3021	97.00	−7.55	0.2118
6	6.0606	101.0	+1.01	0.2339
8	7.6990	96.24	−3.76	0.2578
Mean recovery		97.93		
RSE (%)[c]		3.96		

[a] Relative error of prediction (Eq. (5)).
[b] Corresponds to the difference between $f_n^{max} - f_n^{min}$.
[c] Relative standard error of prediction (Eq. (4)).

analyzed as before, and the results are given in Table 7. Comparing the prediction performance of the proposed method for both serum and urine samples indicates that good recoveries are obtained for quantitation of PC in serum samples. This could have been expected, because the extent of the selective spectral region for PC in serum samples is wider compared to that of the urine samples.

Table 7
Results obtained by applying the proposed method to human blood serum and urine samples spiked with PC.

Taken ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	Recovery (%)	REP (%)[a]	Feasible band[b]
Serum				
0	0.1224	–	–	0.0038
2	2.1428	107.1	+7.14	0.0648
4	4.1633	104.1	+4.08	0.1274
6	6.1837	103.1	+3.06	0.1902
8	8.2041	102.6	+2.55	0.2525
Mean recovery	104.2			
RSE (%)[c]	3.38			
Urine				
0	0.3438	–	–	0.0197
2	2.3542	117.7	+17.7	0.1327
4	4.3750	109.4	+9.38	0. 2425
6	6.3854	106.4	+6.42	0.3469
8	8.3958	105.0	+4.95	0.4450
Mean recovery	109.6			
RSE (%)	7.58			

[a] Relative error of prediction (Eq. (5)).
[b] Corresponds to the difference between $f_n^{max} - f_n^{min}$.
[c] Relative standard error of prediction (Eq. (4)).

Conclusion

The main objective of this study was to investigate the possibility of achieving the second-order advantage from first-order spectrophotometric data when the kinetics of all sample constituents are identical. Instead of collecting two-way kinetic-spectrophotometric data, virtual replicate × spectrophotometric data were used in the present work. Standard addition in combination with the MCR-ALS method was applied as an alternative to, circumvent the matrix effect firstly and quantitation of the analyte in the presence of unknown interference components secondly. Despite a band boundary of feasible solutions for analyte concentration profiles recovered from MCR-ALS, the maximum band boundary can be used for determination of analyte concentrations while the minimum one is always invariant and equals to zero concentration. It may be noted that successful analyte quantitation in the presence of interference components (second-order advantage) based on the proposed method, depends significantly on the degree of selectivity in the rows of the standard added data matrix. The degree of selectivity, in turn, depends on the amount of overlap in the region of occurrence for the compound of interest with the rest of constituents (the spectra mode). With increasing degrees of spectral overlap between the analyte and interferences, the uncertainty for the maximum band boundary also increases. This study showed that the proposed method succeeded in the analyte quantitation in interfering systems, where there is a minimum selective spectral region for the analyte of interest.

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