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Standard Addition Connected to Selective Zone Discovering for Quantification in the Unknown Mixtures

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Univariate calibration method is a simple, cheap and easy to use procedure in analytical chemistry. A univariate analysis will be successful if a selective signal can be found for the analyte(s). In this work, two simple ways were used to find the selective signals, spectral ratio plot (SRP) and loading plot (LP). Both of them were able to discover the selective regions in the recorded data sets. For SRP, the spectral profiles of unknown mixture and standard sample of analyte were necessary. However, in LP, multivariate data of standard addition procedure were necessary to discover the selective zones. After discovering the selective wavelengths, the standard addition method can be used to determine the concentration of given analyte. The standard addition curve was interpolated to reduce any bias error. To demonstrate the ability of LP and SRP, several synthetic and real datasets were analyzed and the results were reported. The SRP and LP were used to determine some additives in food and hygienic real samples using spectrophotometric data.

Keywords: Principal component analysis, Spectral ratio plot, Loading plot, Spectral selective region, Preservatives, Standard addition

INTRODUCTION

New instruments have been developed and are developing to deal with complexity of chemical and biological samples. The complexity of analyzing samples with numerous unknown components presents a major challenge in modern instrumental analysis. Most analytes of interest are accompanied by other compounds being absorbed within the same spectral region leading to inherent lack of resolution in the classical Ultra Violet (UV) spectral measurement. In such cases, resolution of the components is often associated with cumbersome sample clean up and separation procedures. However, separation methods are associated with risks such as loss of analyte, contamination of sample, possibility of incomplete separation, and, above all, the procedure can be expensive and time consuming [2]. Simultaneous multi-component analysis by UV-Vis molecular absorption spectrophotometry is mainly developed for the purpose of minimizing the cumbersome task of separating interferents allowing an increasing number of analytes to be determined and, consequently, analysis time and cost to be reduced.

Quantification of given analyte in different biological and chemical samples is a critical topic in different sciences and industries. Analytical measurement in complex samples such as foods, cosmetic products for quality control and biological samples for the diagnosis of diseases is very important issue [3-8]. In chemistry and chemometrics a key challenge is designing and developing new, accurate and reliable quantification methods for analyzing complex systems.

In some samples, a selective spectral zone can be existed for the given analyte. The presence or absence window of all components can be extracted by using Evolving Factor Analysis [4], Window Factor Analysis [5] and Heuristic Evolving Latent Projection [6]. However it seems very interesting and important to extract presence or absence window of different components in the wavelength direction. This can be very useful in quantification purpose. For example by discovering the selective region of the

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given analyte in the spectral mode it is possible to determine the concentration of the analyte by univariate calibration method which is very simple and low-cost.

Standard addition method is a well-known analytical technique. This is used where sample matrix also contributes to the analytical signal as matrix effect, and makes it impossible to compare the analytical signal between sample and standard matrix using the traditional calibration curve approach [7]. However, standard addition method cannot eliminate the bias error and it is necessary to use in the modified standard addition procedures such as H-point standard addition method [8] and generalized H-point standard addition method [9]. Besides eliminating the matrix effect in quantitation, standard addition method can be extended to the second order standard addition method leading to second order advantages [10-13]. Lately, analyte quantitation using first order dataset in the presence of unknown interferents has been published in the literature [14,15]. In 1993, Meier and Zund claimed that interpolation is more precise than extrapolation. Besides, they prevented us from always extrapolating to a zero signal level, because in the presence of interferents, one should extrapolate to a higher signal value. Interpolation is a way to get predictions on the central part of the regression line, thus minimizing bias in the prediction and associated variance with the analytical result [16].

In this work, we have used standard addition method along with interpolation to quantify the preservatives of food and cosmetic products. As production of processed and convenience products increases, application of chemical food additives is becoming an increasingly important practice in modern food and herbal pharmaceutical industries. Chemical additives play an important role in healthy maintenance of food products. Preservatives are used to lengthen the shelf-life of foods and promote their robust status. They can delay or stop disintegration process undertaken by microorganisms [17]. Preservatives such as benzoates, sorbates, propionates, sulphur dioxides, sulphites, parabones, sodium nitrites, sodium nitrates, natamycine and nicine are widely used, especially in production of cheese, meet products, fruit-based products, and various beverages. Various methods such as thin layer chromatography (TLC), gas chromatography (GC), capillary electrophoresis, spectrophotometry, and high performance liquid chromatography (HPLC) have been applied to determine food preservation levels in food products in the literature.

Lack of a straight forward method to discover selective and adoptive zones within spectral mode of datasets motivated us to work on and introduce some methods already available in literature; though not as a main purpose. The aim of this work is to use PCA loading plot as well as spectral ratio plot to find selective zones and quantify an analyte in the presence of unknown components. Standard addition method was used to get data matrix, implement PCA, and also remove the matrix effect.

EXPERIMENTAL

Apparatus

Absorbance measurements were carried out on a UV-Vis double beam spectrophotometer model Cary-100 using 1 cm quartz cells. All spectral measurements were performed using the blank solution as a reference. The pH measurements were made with a Metrohm 713 pH-meter using a combined glass electrode. The measured data were recorded and processed on a Pentium IV computer with codes written in MATLAB 11 (Mathworks).

Sample Collection

All samples, including tooth paste, shampoo, sterile eye drops, moisturizing cream, juice (Rani and Sunich), yoghurt and cola were collected from the industries in Tabriz, Iran.

Chemicals and Reagents

Benzoic acid, sorbic acid, methylparaben and saccharin were purchased from Merck Co. (Darmstadt, Germany). Furthermore, phosphoric acid, boric acid and acetic acid were also purchased from Merck Co. in order to make Britton-Robinson (B-R) buffer solution.

Solutions

All solutions were prepared in and used throughout double distilled water. Stock solutions of benzoic acid (BA), sorbic acid (SA) methylparaben (MP), and saccharin (SC) were prepared by dissolving appropriate amount of the crystalline compounds in doubly distilled water. In order to prepare of working solutions with proper concentration,

suitable amounts of stock solutions of BA, SA, MP and SC were transferred into 10 ml volumetric flasks followed by the addition of 3.33 ml of the B-R buffer (pH = 7). The resultant solution was diluted with doubly distilled water. The absorbance spectrum of each solution was measured with respect to reagent blank within 200 and 300 nm wavelengths. Different commercial samples were digested and diluted to obtain a solution, namely unknown solution. Different amounts of standard solutions of preservatives were separately added to unknown solutions before their spectrophotometric spectra were recorded within UV-Vis ranges.

Britton-Robinson aqueous, universal buffer solutions of pH values of 2-12 were prepared by mixing appropriate volumes of acidic and basic buffer components. The prepared buffers contained 0.04 M phosphoric acid, 0.04 M boric acid and 0.04 M acetic acid.

Real Sample Solution Preparation

Real sample solutions were prepared for determination of methyl paraben, saccharin, sorbic acid and benzoic acid. 3.34 g of toothpaste sample was mixed with 50 ml of methanol and stirred for 20 min. Then, 3 ml of the supernatant solution was poured into a 9 ml centrifuge tube and diluted with deionized water to the mark. The solution was centrifuged at 10000 rpm for 15 min before being filtered by nylon membrane. Then, 800 µl of an unknown solution was transferred to a 10 ml volumetric flask followed by addition of 3.33 ml of the buffer solution and different amounts of standard solutions of saccharin (20, 30, 40, 60, 80, 100 and 120 µl). The resultant solution was diluted to the mark with double distilled water and mixed completely. This was repeated for all unknown solutions and different amounts standard solutions of saccharin.

The eye drop solution was prepared by dissolving the sample into deionized water in a 25 ml volumetric flask. Then, 100 µl of an unknown solution was transferred to a 10 ml volumetric flask followed by addition of 3.33 ml of the buffer solution and different amounts of standard solutions of methyl paraben (40, 60, 80, 100, 120, 140 and 160 µl). This was repeated for all unknown solutions and different amounts of standard solutions of methyl paraben. The preparation of shampoo solution was started by dissolving 2.8 g of the sample in 50 ml of deionized water and stirring for 10 min. Then, 3 ml of the supernatant solution were poured into a 9 ml centrifuge tube and diluted with deionized water. The solution was centrifuged at 10000 rpm for 15 min before being filtered by nylon membrane. Afterward, 1 ml of unknown solutions was transferred to a 10 ml volumetric flask followed by the addition of 3.33 ml of the buffer solution and different amounts of standard solutions of benzoic acid (10, 20, 30, 40, 50, 60 and 70 µl). This was repeated for all unknown solutions and different amounts of standard solutions of benzoic acid. The cola (Fanta) solution was prepared by dissolving the sample into deionized water in 25 ml volumetric flask. Then, a suitable amount of unknown solution, 400 µl, was transferred to 10 ml volumetric flasks followed by the addition of 3.33 ml of the buffer solution and different amounts of standard solutions of sorbic acid (14, 20, 30, 40, 50, 60 and 80 µl). This was repeated for all unknown solutions and different amounts of standard solutions of sorbic acid.

The preparation of moisturizing cream solution was started by dissolving 3.1 g of the sample in 50 ml of deionized water and stirring for 30 min. Then, 3 ml of the supernatant solution were poured into a 9 ml centrifuge tube and diluted with deionized water. The solution was centrifuged for 15 at 10000 rpm min before being filtered with nylon membrane. Then, an adequate amount of the unknown solution was transferred to a 10 ml volumetric flask followed by addition of 3.33 ml of the buffer solution and different amounts of standard solution of methylparaben (10, 20, 30, 40, 50, 60 and 70 µl). This was repeated for all unknown solutions and different amounts of standard solutions of methylparaben.

The preparation of juice (Sunich and Rani) and yoghurt drink samples were started by pouring appropriate amounts of samples into separate 9 ml centrifuge tubes before being diluted with deionized water. The solutions were centrifuged at 10000 rpm for 15 min before being filtered with nylon membranes. Then, adequate amounts of the resultant solution were transferred to 10 ml volumetric flasks followed by addition of 3.33 ml of the buffer solution and different amounts of standard solutions of benzoic acid (14, 20, 30, 40, 50, 60 and 80 µl). The resultant solution was then diluted with double distilled water. This was repeated for all unknown solutions and different amounts of standard

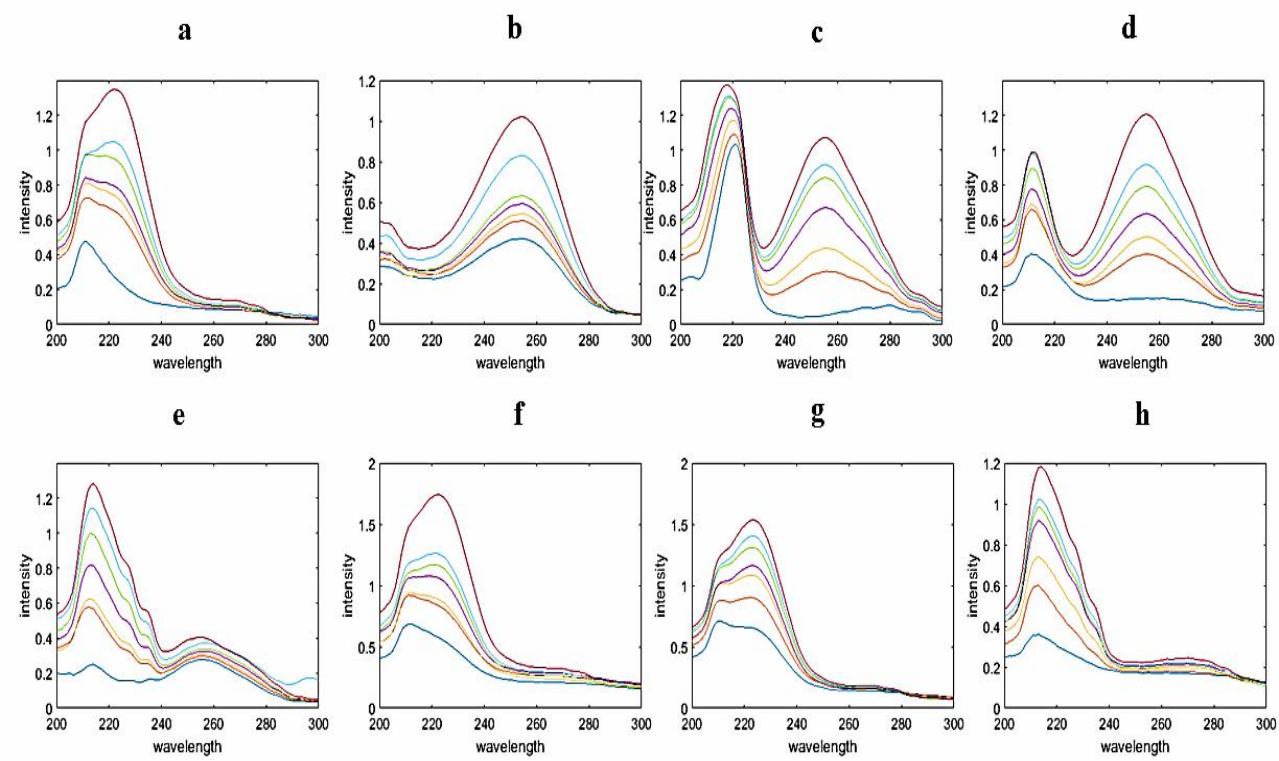

Fig. 1. The recorded data sets of different samples are visualized. (a) Rani, (b) yoghurt, (c) moisturizing cream, (d) tooth paste, (e) sunich, (f) shampoo, (g) sterile eye drops and (h) cola.

solutions of benzoic acid. For all cases, the absorbance spectrum of the prepared solution was measured with respect to the reagent blank within the wavelength range of 200-300 nm. The recorded data sets are visualized in Fig. 1.

Simulations

Performing a simulation rather than analyzing real experimental data will come up with some advantages. Most of the time, simulation testing is cheaper and faster than real data analysis. The second major advantage is the level of detail you can get from a simulation process. Simulation can provide you with results that are not experimentally measurable at our current level of technology. Simulated in this part are two cases of standard addition datasets for two-component systems.

Case I. There are selective regions in spectral mode for two components, namely the analyte, X and interferent, Y. The simulated spectral profiles are shown in Fig. 2b. It is clear that there are selective regions at 200-260 nm and 340-400, in spectral profiles of two components, X and Y, respectively. The concentrations of both components in both cases are shown in Fig. 2a. As clearly seen, the concentration of analyte is increasing while the concentration of the interferent (Y) is constant.

Case II. In the second simulated case, only the analyte has selective region in the spectral mode as visualized in Fig. 2c. The recorded data of case I and II are shown in Figs. 3a and 3b, respectively.

THEORY

Principal Component Analysis

Principal component analysis (PCA) is a widely used multivariate data analysis method for explorative data analysis and regression purposes [18]. It is used to transform a set of correlated variables into a new set of independent (uncorrelated) variables or principal components (PCs). Linear combination of the original

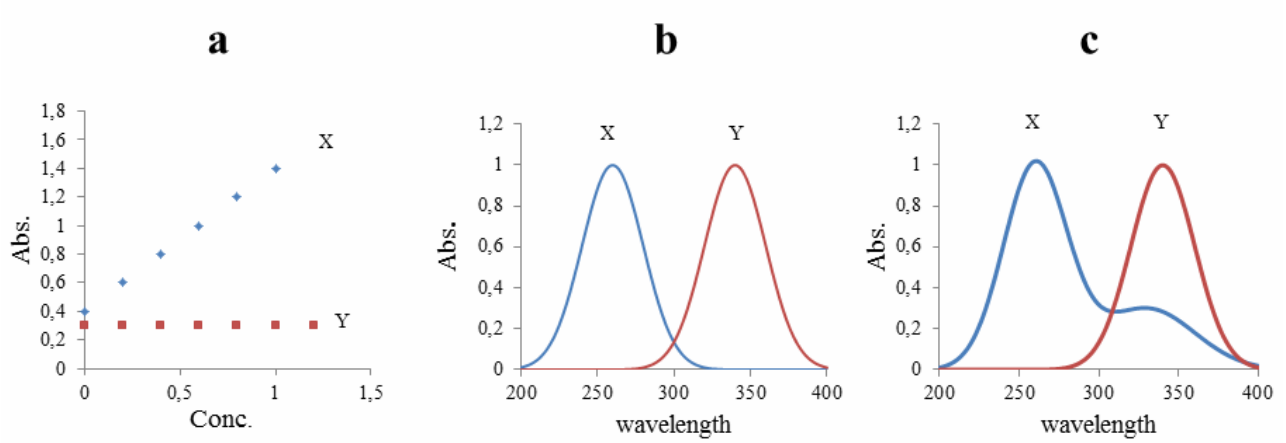

Fig. 2. The simulated concentration profiles (a) and the spectral profiles of case I and case II are b and c, respectively.

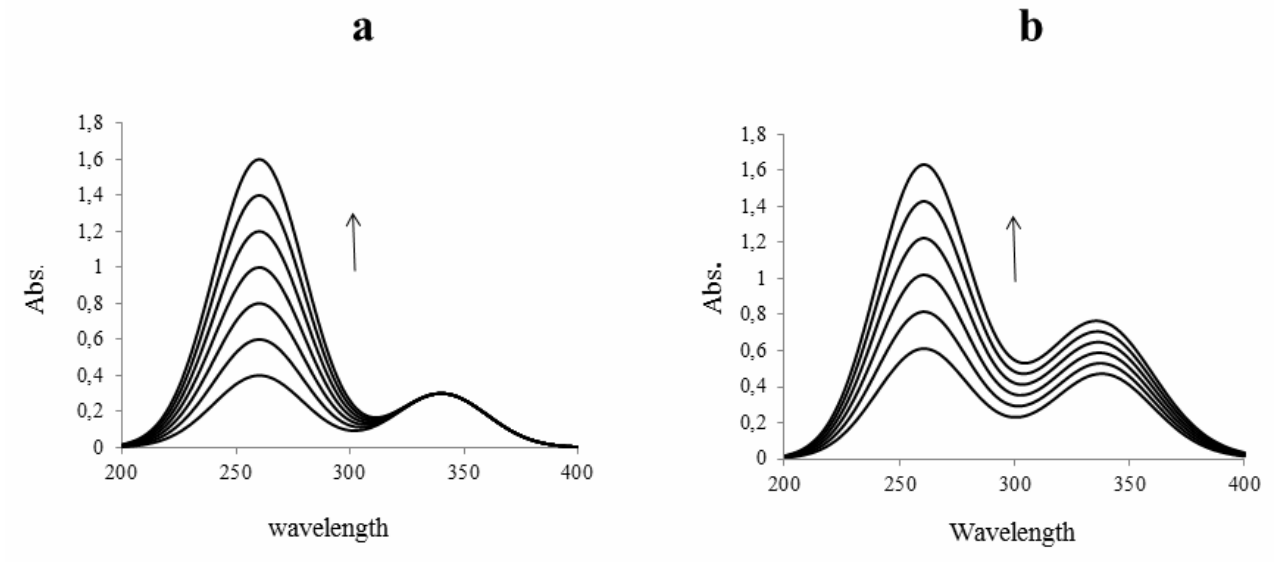

Fig. 3. The simulated data set I and II are visualized in a and b, respectively.

variables are chosen in such a way that the first PC explains most of the variance in the data, the second one explains most of the variance not accounted for and orthogonal to the first one, and so on. With PCA, it is possible to build an empirical mathematical model of the data as described by Eq. (1):

$$A = T_k V_k^T + \varepsilon \quad (1)$$

where T_k is the n × k matrix of principal component scores, and V_k is the m × k matrix of eigenvectors. The eigenvectors in V_k can be used to form a set of orthonormal row basis vectors for A. The eigenvectors are also called "loadings" indicating that the vectors form a basis set for the row space of A. The columns of T_k are called "scores" and are mutually orthogonal but not normalized. They can be used to form a set of column basis vectors for A [19]. PCA on a two-component system results in determining scores (n × 2) and loadings (2 × m), basis set of row space and column space, respectively.

Often a few components account for a large portion of total variance, and as such, they can be used to represent the original observations [20]. Plotting the data on the plane formed by the first two PCs, a two-dimensional representation of the data is obtained which can give a reasonable idea of the original multidimensional structure. Score and loading plots are used for this purpose. Score plot shows the position of the objects (or samples) in the new space; *i.e.*, the projection of data onto the subspace. Loading plot is the plot demonstrating the relation between original variables and subspace dimension. It can be obtained by plotting the first loading vector against the second one. Loading plots are of useful means to visualize multivariate datasets. This useful plot has an amazing property: for two-component systems, the linear regions with zero intercept on this graph represent correlated variables or those variables with the same direction in subspace. As an example, we can consider spectrophotometric data for which the linear part with zero intercept in the loading plot represents wavelength regions where there are pure compounds or selective region for that analyte [21]. This concept can be used to find selective regions in a two-component system. However, another reason that can generate this linear region in the loading plot is local rank deficiency. This phenomenon can cause this linear region in the complex cases. In this work, the main assumption is the absence of local rank deficiency.

Spectral Ratio Plot (SRP)

The absorbance of a mixture of two components, X and Y, measured at wavelength λ_i can be written as in Eq. (2):

$$A_{mix,\lambda_i} = \alpha_{X,\lambda_i} C_X + \alpha_{Y,\lambda_i} C_Y \quad (2)$$

where $A_{mix}\lambda_i$ is the absorbance of the binary mixture at wavelength λ_i, $\alpha_{X,\lambda i}$ and $\alpha_{Y,\lambda i}$ are absorptivities of X and Y, respectively, whose concentrations are represented by C_X and C_Y, respectively. The spectrum of the standard sample X with concentration C'_X can be expressed by Eq. (3):

$$A_{X,\lambda_i} = \alpha_{X,\lambda_i} C'_X \quad (3)$$

Dividing Eq. (2) by Eq. (3), the spectrum ratio is obtained as:

$$A_{X,\lambda_i} = \alpha_{X,\lambda_i} C'_X + \alpha_{X,\lambda_i} C'_X \quad (4)$$

Equation 4 can be simplified as follows:

$$A_{X,\lambda_i} = \alpha_{X,\lambda_i} C'_X + C'_X \quad (5)$$

The values of α_{Y,λ_i} will be zero within the selective region of wavelengths for component X. So, the plot of A_{X,λ_i} *vs.* λ_i represents a line at a slope and intercept values equal to zero and C_X / C'_X, respectively. In other words, the linear region at zero slopes in $A_{mix,\lambda_i} / A_{X,\lambda_i} - \lambda_i$ plot represents the selective zone of spectral profile of the analyte [22].

RESULTS AND DISCUSSIONS

In order to investigate the ability of LP and SRP to discover the selective zones, two simulated data sets and several experimental data sets were analyzed. The steps can be summarized as:

1) Recording the multivariate data: In order to get multivariate data, the spectral profiles of different samples must be recorded at a suitable wavelength range, and then this recording process must be performed for standard added solutions.

2) Discovering the selective spectral zones for the analyte: LP and SRP can be used to analyze data and find the corresponding selective region.

3) Using standard addition method to determine the concentration of analyte using interpolation: Standard addition method is used to remove matrix effect, while interpolation method is applied to reduce the bias error.

The Simulated Cases

By applying PCA on the simulated data sets (using SVD function in MATLAB), scores (left eigen vectors) and loadings (right eigen vectors) can be obtained. Loading plot can be established by plotting the first row of the loading matrix versus the second one. Figure 4a is the loading plot of the first simulated case and it is obvious that there are two linear regions with zero intercept in this plot. A

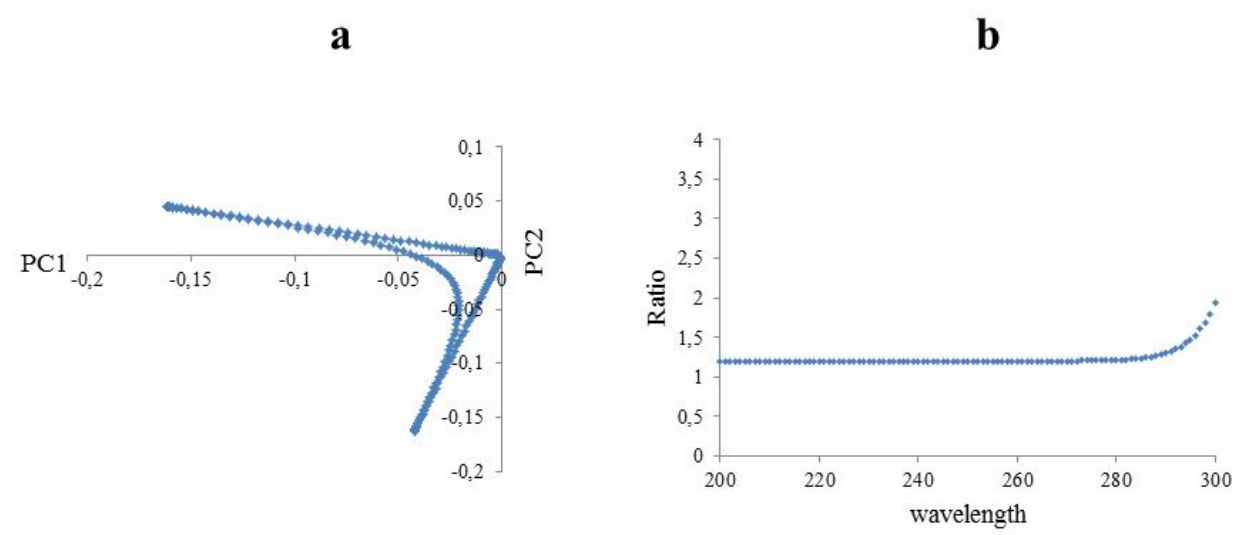

Fig. 4. The loading plot (a) and spectral ratio plot (b) of the first simulated case.

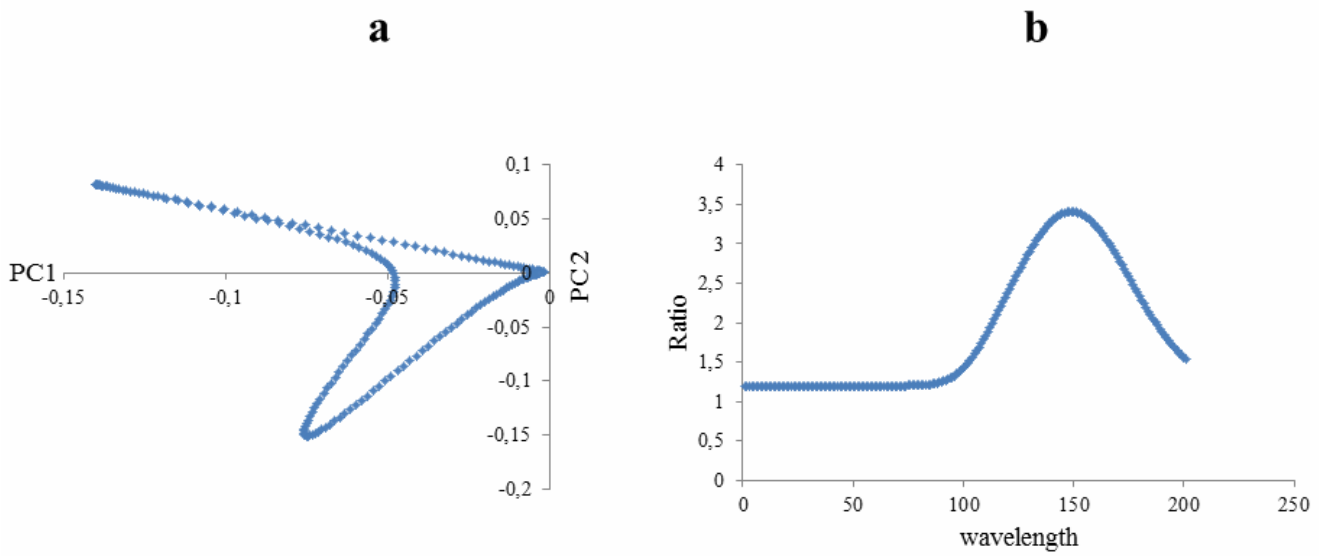

Fig. 5. The loading plot (a) and spectral ratio plot (b) of the second simulated case.

comparison of selective wavelengths specified in Fig. 2a with pure absorptive spectra of components X and Y will help recognizing the nature of these zones which depend to X or Y.

Another way to find the selective region of a data set in spectral mode is using the SRP which is employed in this contribution. Demonstration of the capabilities of the proposed plot requires access to unknown and pure absorption spectral profiles of the component X with a selective region. Figure 4b shows the spectral ratio plot of the analyte. It is clear that the wavelength range of 200-260 nm is a selective region for the analyte where a linear curve of zero slopes is observed.

In addition, the loading plot and spectral ratio plot can be calculated and visualized for the second simulated case, too (Fig. 5b). The loading plot of this data (case II) had a

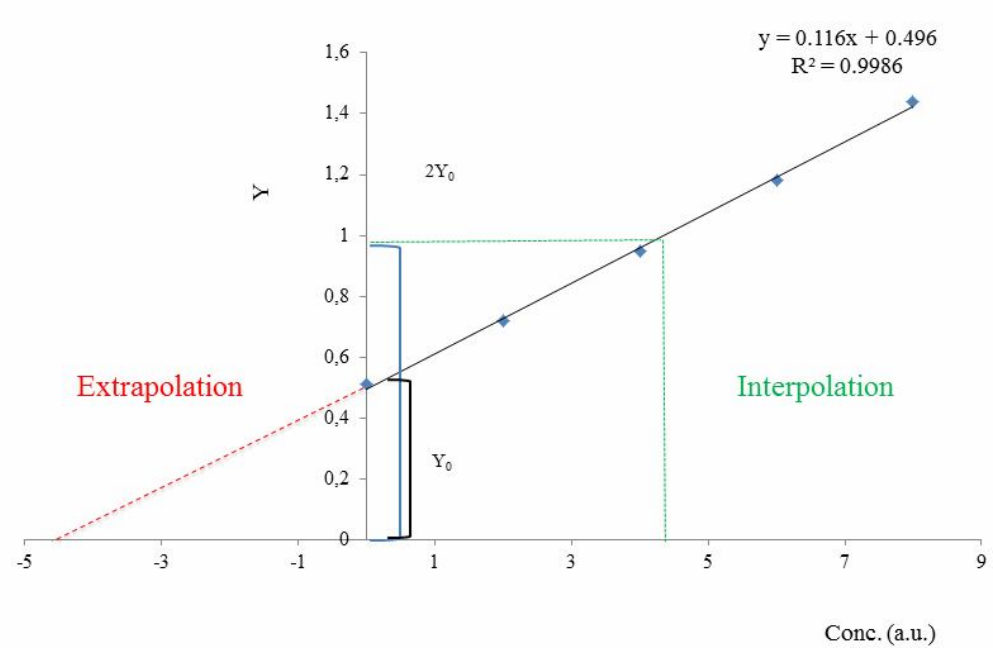

Fig. 6. Visualization of interpolation and extrapolation in the standard addition method.

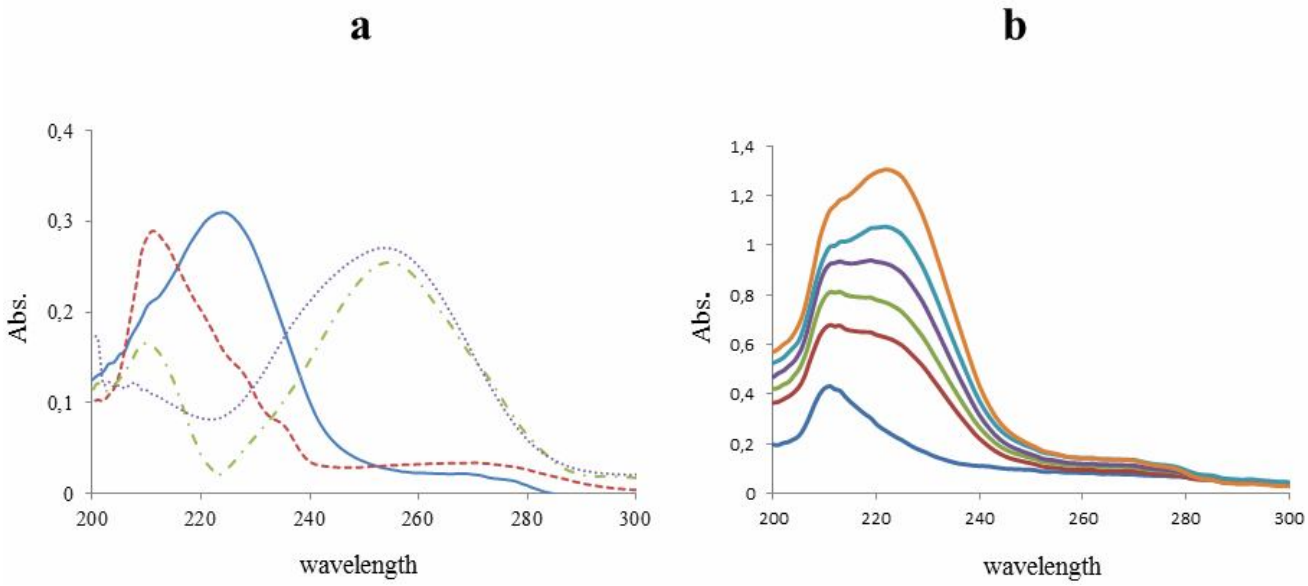

Fig. 7. a) The recorded spectral profiles of Benzoic acid, sorbic acid, methylparaben and saccharin, b) the recorded standard addition data of the yoghourt drink sample.

linear segment with zero intercept, because of the selectivity of the analyte (Fig. 2c). In addition, Fig. 5b shows the spectral ratio plot of the analyte. The selective region of the analyte can be found within wavelength range of 200-260 nm where a linear segment at zero slopes is observed.

Interpolation in Standard Addition Method

Usually extrapolation is using to find analyte

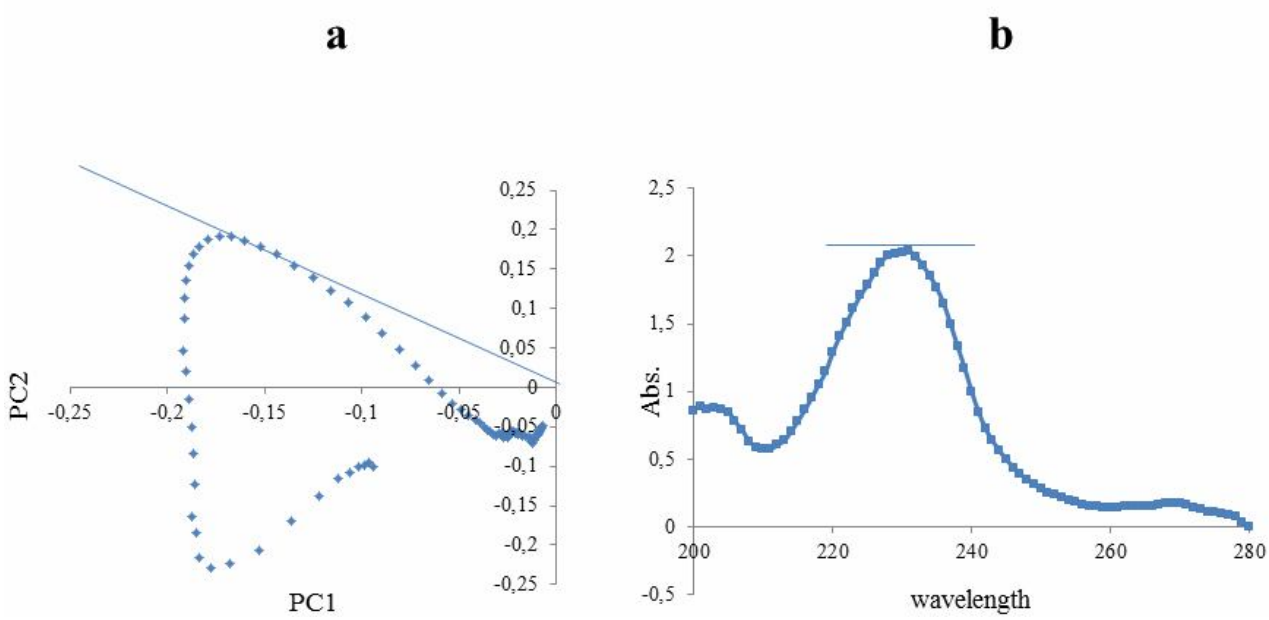

Fig. 8. The loading plot (a) and spectral ratio plot (b) of the yoghourt drink data.

Table 1. The Results of LP and SRP of Real Samples

Real samples	Analyte	Linear wavelengths (LP)	Linear wavelengths (SRP)	Selected wavelength	Found values (µg ml^{-1})	Recovery (%)
Toothpaste	Saccharin	220-225 nm	219-225 nm	222 nm	748.50 µg g^{-1}	105 ± %8
Eye drop	Methylparaben	241-252 nm	242-250 nm	245 nm	150.75 µg ml^{-1}	97 ± %3
Cola	Sorbic acid	253-262 nm	250-265 nm	260 nm	54.55 µg ml^{-1}	100
Moisturizing cream	Methylparaben	255-259 nm	253-259 nm	255 nm	1168 µg/g	99 ± %6
Juice	Benzoic acid	220-225 nm	218-224 nm	220 nm	103.32 µg ml^{-1}	101 ± %3
Yoghourt drink	Benzoic acid	228-232 nm	227-234 nm	230 nm	48.75 µg ml^{-1}	100 ± 4

concentration in standard addition method. However, Andrade *et al.* showed that this can be a suboptimal practice [16], because even a slight departure of calibration points from straight line could affect the prediction ability of SAM. So, it has been recommended to use the interpolation instead of extrapolation in the course of prediction. Using central part of regression line may minimize the bias in the prediction and the associated variance. Figure 6 illustrates the application of interpolation, instead of extrapolation, in SAM.

Experimental Data Sets

Finally LP and SRP coupled with standard addition method were used to quantify food additives in real samples. Benzoic acid, sorbic acid, methylparaben and saccharin were selected as given analytes. Figure 7a shows

electronic absorption spectra of Benzoic acid, sorbic acid, methylparaben and saccharin. Different real samples were selected and checked to find selective zones within their absorption spectra using spectral ratio plot and loading plot. Figure 7b shows the recorded standard addition data of the yoghourt drink sample in 200-400 nm before and after addition of different amounts of standard solution of benzoic acid.

Figures 8a and 8b show the loading plot and the spectral ratio plot of this real case (drink yoghourt), respectively. The linear region of the loading plot with zero intercept as well as the linear region of spectral ratio plot at zero slope represent wavelength range (227-233 nm) of the selective region of benzoic acid in the yoghourt drink sample. In the other words, benzoic acid is the only absorptive component in this region. After finding the selective region, standard addition curve was plotted at 230 nm and the concentration of benzoic acid was calculated using interpolation. The results are shown in Table 1.

In this work, several real samples were analyzed: toothpaste, eye drop, shampoo, cola, moisturizing cream, juice and yoghourt drink. For each sample, we tried to quantify a preservative as summarized in Table 1 Lp and SRP curves are plotted for each sample with the linear segment determined. Then, standard addition method within the selected wavelength range was used for quantification. The concentration of the additives in each sample is reported in the last column of Table 1. Spike method was used to validate the obtained results. The average of recoveries for three replications of different added amounts is summarized in Table 1.

CONCLUSIONS

Loading plot and spectral ratio plot were used to find the selective regions in the spectral direction in order to use standard addition method. Standard addition method was successfully used to predict the concentration of given analytes. Interpolation of standard addition curve was successfully employed to reduce any bias error. Benzoic acid, sorbic acid, methylparaben and saccharin, as food additives, were determined in different real samples like: tooth paste, shampoo, sterile eye drops, moisturizing cream, juice (Rani and Sunich), yoghurt and cola.

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