

Chemometrics-enhanced simultaneous spectrophotometric determination of aluminum and bismuth with hematoxylin in vegetables and water using multivariate calibration

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ABSTRACT

A simple, novel and sensitive spectrophotometric method was described for simultaneous determination of aluminum and bismuth mixtures. All factors affecting the sensitivity were optimized, and the linear dynamic range for determination of aluminum and bismuth found. The simultaneous determination of aluminum and bismuth and use of spectrophotometric methods is a difficult problem due to the spectral interference. The multivariate chemometrics methods were used for this study. Chemometrics methods such as continuous wavelet transform (CWT), wavelet orthogonal signal correction-partial least squares (WOSC-PLS) and least squares-support vector machine (LS-SVM) methods were used without prior separation steps. The method is based on the colored complexes formed by aluminum and bismuth with hematoxylin in the presence of cetyltrimethylammonium bromide as a surfactant at pH 5.8. In this study, absorption spectra were recorded in the wavelength range 400–650 nm. The results showed that aluminum and bismuth could be determined simultaneously in the range of 0.1–11.0 and 0.1–7.0 $\mu\text{g mL}^{-1}$, respectively. The validation of the developed methods was tested by analyzing various synthetic mixtures of the aluminum and bismuth. The proposed methods have been applied to the simultaneous determination of aluminum and bismuth in vegetables and different water samples.

Key words | aluminum, bismuth, chemometrics, determination, vegetable, water

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INTRODUCTION

While metals are perhaps the oldest known toxic agents, they still hold interest and, in recent years, knowledge regarding their potential toxic effects has increased (Gutiérrez *et al.* 2007). Metals such as iron, copper, and zinc are considered essential for human beings, while heavy metals such as lead and cadmium are considered toxic environmental contaminants in food (Frías *et al.* 2008). Some metals such as aluminum and bismuth are not as toxic as heavy metals, but they can accumulate in organisms' tissues and lead to some diseases. Food and water provide the main portion of the total daily intake of trace elements by humans. Vegetables and cereals are some of the most common foods in the

human diet around the world. Aluminum is present in almost all foods, whereas bismuth levels in foods are usually low. Aluminum is one of the most common elements in the earth and relatively high levels are found in vegetables, which could have a potential impact on the environment, animals and man. It does not appear in nature in a pure form, always being associated with other elements, forming oxides, phosphates, sulfates, etc. (Tontrong *et al.* 2012). Bismuth is an element present in the earth's crust at a trace concentration ($8 \mu\text{g kg}^{-1}$). Bismuth-containing pharmaceuticals have long been used for different medical purposes, especially for the treatment of gastrointestinal tract

disturbances such as gastritis and peptic ulcers (Moyano *et al.* 2001). As the use of bismuth in medicine has increased, it has spread in the environment and the chance of exposure of organisms to bismuth has increased. The determination of trace metals in complex matrices remains one of the most complicated areas of analytical chemistry. In the early 1960s, molecular absorption spectrophotometry of colored metal chelates (colorimetry) used to be the only available method for the determination of traces of metal ions. With the development of more selective techniques such as atomic spectrophotometry and voltammetry, colorimetry lost its role as the central technique, since the new instrument techniques were more selective, and thus operations such as masking or isolating the analyte could be avoided. The simultaneous determination of several components in a mixture can be a difficult task, especially when their analytical characteristics are not very different. Quantitative simultaneous spectrophotometry has been greatly improved by the use of multivariate calibration techniques such as continuous wavelet transform (CWT) and partial least squares (PLS) methods. PLS modeling is a powerful multivariate statistical tool that can be performed with easily accessible statistical software (Geladi & Kowalski 1986; Brereton 2000). The basic concept of PLS was originally described by Gerlach *et al.* (1979) and Wold (1982). Wold *et al.* (1998) introduced orthogonal signal correction (OSC) as a pre-processing step that improves the calibration model by filtering strong structured (i.e. systematic) variation in X that is not correlated to Y . Therefore, one can be certain that important information regarding the analyte is retained. On the other hand, one of the techniques of the signal preprocessing is wavelet orthogonal signal correction (WOSC). This will be used to eliminate undesirable background effects and enhance the subsequent PLS regression model, by a combination of wavelet prism (WP) transformation of data into a set of frequency-domain spectra, and OSC preprocessing on the frequency-domain data to remove signals uncorrelated to the target property, followed by reconstruction of the filtered spectra from the processed frequency components (Feudale *et al.* 2005). A wavelet transform (WT) is the representation of a function by wavelets. The wavelets are scaled and translated copies or daughter wavelets of a finite-length or fast-decaying oscillating waveform (Alsberg *et al.* 1997). Wavelet transform contains two distinct parts,

discrete and continuous. CWT plays an important role in the signal processing technique and identification of overlapping peaks. The use of WT to overcome the problem of noisy and incomplete data has been successfully put on a sound statistical basis by Donoho & Johnstone (1994a, 1994b). Few reports have been published on the application of wavelet transforms to denoising of data (Barclay *et al.* 1997), compressing of signals (Afkhami *et al.* 2008) and simultaneous determination of chemical species (Dinç *et al.* 2005; Dinç & Baleanu 2007). In recent years, the combined use of CWT and zero-crossing was firstly formulated by Dinç & Baleanu (2003, 2004) for the simultaneous determination of compounds. The support vector machine (SVM) was introduced as a valuable tool for solving pattern recognition and classification problem (Cortes & Vapnik 1995; Vapnik 1998). SVMs can be applied to regression problems by the introduction of an alternative loss function. As a simplification, Suykens & Vandewalle (1999) proposed a modified version of SVM called least squares-SVM (LS-SVM), which resulted in a set of linear equations instead of a quadratic programming problem, which can extend the applications of the SVM.

In this study, different chemometrics methods based on spectrophotometric data were applied for the simultaneous determination of binary mixtures consisting of aluminum and bismuth compounds. To succeed in obtaining a reliable result by the CWT, PLS, WOSC-PLS and LSSVM methods, different parameters such as wavelet functions, decomposition level and the number of PLS factors were tested. The results obtained were compared and successful results were reported. The methods were validated by simultaneous determination of aluminum and bismuth in some synthetic samples.

MATERIALS AND METHODS

Partial least squares

Partial least squares is a method for building regression models on the latent variable decomposition relating to two blocks, matrices X and Y , which contain the independent, x , and dependent, y , variables, respectively. These matrices can be simultaneously decomposed into a sum of

f latent variables, as follows:

$$X = TP^T + E = \sum t_f p'_f + E \quad (1)$$

$$Y = UQ^T + E = \sum u_f q'_f + F \quad (2)$$

in which T and U are the score matrices for X and Y , respectively; P and Q are the loadings matrices for X and Y , respectively, and E and F are the residual matrices. The two matrices are correlated by the scores T and U , for each latent variable, as follows:

$$u_f = b_f t_f \quad (3)$$

in which b_f is the regression coefficient for the f latent variable. The matrix Y can be calculated from u_f , as Equation (4), and the concentration of the new samples can be estimated from the new scores T^* , which are substituted in Equation (4), leading to Equation (5):

$$Y = TBQ^T + F \quad (4)$$

$$Y_{\text{new}} = T^*BQ^T \quad (5)$$

In this procedure, it is necessary to find the best number of latent variables, which is normally performed by using cross-validation, based on determination of the minimum prediction error (Niazi & Yazdanipour 2007).

Wavelet transform

Wavelet transform is a newfound signal processing technique developed from Fourier transform, which is represented as a series of functions that relate to each other by simple scaling and translation. All of the basic functions are generated from the original WT function, which is called the mother wavelet. Let us choose a mother wavelet (Walczak 2000) by $\Psi(\lambda)$. By scaling and shifting $\Psi(\lambda)$, a set of functions denoted by $\Psi_{a,b}(t)$ is obtained as follows:

$$\psi_{(a,b)}(t) = \frac{1}{\sqrt{a}} \psi\left(\frac{t-b}{a}\right) \begin{cases} a, b \in R \\ a \neq 0 \end{cases} \quad (6)$$

where (a) is positive and denotes the scale parameter, which is a variable used to control the scaling, (b) demonstrates the translation parameter controlling the translation, and R is the domain of real numbers. The CWT of a given signal denoted by $f(t)$ is defined as:

$$C(a, b) = \int_{-\infty}^{+\infty} f(t) \psi_{a,b}^*(t) dt = \langle f(t), \psi_{a,b} \rangle \quad (7)$$

where the superscript $*$ represents the complex conjugate and $\langle f(t), \Psi_{a,b} \rangle$ denotes the inner product of function $f(t)$ onto the wavelet function $\Psi_{a,b}(t)$. The wavelet Ψ is called invertible if it fulfills the following condition:

$$C\omega = \int_{-\infty}^{+\infty} \frac{|\psi(\omega)|^2}{\omega} d\omega < \infty \quad (8)$$

Least squares-support vector machine

In principle, LS-SVM always fits a linear relation ($y = wx + b$) between the regression (x) and the dependent variable (y). The best relation is the one that minimizes the cost function (Q) containing a penalized regression error term:

$$Q = \frac{1}{2} w^T w + \frac{1}{2} \gamma \sum_{i=1}^N e_i^2 \quad (9)$$

subject to:

$$y_i = w^T \varphi(x_i) + b + e_i \quad i = 1, \dots, N, \quad (10)$$

where φ denotes the feature map. The first part of this cost function is a weight decay, which is used to regularize weight sizes and penalize large weights. Due to this regularization, the weights converge to a similar value. Large weights degrade the generalization ability of the LS-SVM because they can cause excessive variance. The second part of the cost function is the regression error for all training data. The relative weight of this part is compared to the first part indicated by the parameter γ , which has to be optimized by the user. Similar to other multivariate statistical models, the performances of LS-SVMs for quantitative analysis depends on the combination of several

parameters. The attainment of the kernel function is cumbersome and it will depend on each case. However, the kernel function which is used more is the radial basis function (RBF), $\exp(-(\|x_i - x_j\|^2)/2\sigma^2)$, a simple Gaussian function, and polynomial functions $(x_i, x_j)^d$, where σ^2 is the width of the Gaussian function and d is the polynomial degree, which should be optimized by the user to obtain the support vector. For σ of the RBF kernel and d of the polynomial kernel, it should be stressed that it is very important to carry out a careful model selection of the tuning parameters, in combination with the regularization constant γ , in order to achieve a good generalization model (Niazi *et al.* 2007).

Instrumentation and software

A Hewlett-Packard 8,453 diode-array spectrophotometer controlled by a computer and equipped with a 1 cm path length quartz cell was used for UV-Visible spectra acquisition. Spectra were acquired between 400 and 650 nm (2 nm resolution) with 0.2 s integration time and 0.5 s cycle time. These integration and cycle times allow the acquisition of relatively low noise spectra at a relatively fast rate. A Metrohm 692 pH-meter furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH 3.00 and 9.00. The HP Personal Computer (1 GB RAM) equipped with the Windows Vista operating system and MATLAB (Version 8.1) was used. The PLS evaluations were carried out using the PLS program from PLS-Toolbox Version 4.0 and CWT were performed by wavelet toolbox in MATLAB. The LS-SVM optimization and model results were obtained using the LS-SVM lab toolbox (Matlab/C Toolbox for Least-Squares Support Vector Machines).

Chemicals and reagents

All reagents used were of analytical reagent grade, and deionized water was used throughout. Stock standard solutions of aluminum and bismuth were prepared from commercial salts (nitrate) and standardized titrimetrically. Standards of working solution were made by appropriate dilution daily as required. The ethanolic hematoxylin solution (4×10^{-3} mol L⁻¹) was prepared by dissolving reagent (Merck) in 100 mL ethanol. Cetyltrimethylammonium

bromide (CTAB), 0.01 mol L⁻¹, was prepared by dissolving CTAB in 100 mL of water. A universal buffer solution (pH 5.8) was prepared (Lurie 1987). All laboratory glassware was cleaned with a 5% (v/v) HNO₃ solution, and then rinsed with double distilled deionized water.

General procedure

Standards or samples were prepared in 5 mL standard flasks by taking the required volume of the solution to be analyzed to obtain aluminum and bismuth concentrations over their respective linear determination ranges (0.1–11.0 µg mL⁻¹ for aluminum and 0.1–7.0 µg mL⁻¹ for bismuth) and then 0.4 mL of hematoxylin, 2 mL of CTAB solution, and the volume was completed with a buffer of pH 5.8. After 20 minute the spectra was recorded from 400 to 650 nm against a reagent blank. A blank solution was also run under the same conditions without adding the analyte. In order to show the performance of the proposed method, real samples were analyzed under optimum conditions.

Real sample preparation

The vegetable samples were prepared in triplicate by ashing 1.0 g portions of oven-dried ground plant tissue in porcelain crucibles for 2.5 h at 500 °C, and dissolving the residue in 2 mL of 6 mol L⁻¹ HCl. The resulting solutions were evaporated and the residues were again dissolved in 10 mL of 2 mol L⁻¹ HCl. The resulting solutions were heated and filtered. The residues were then washed again with 10 mL of the 2 mol L⁻¹ HCl solution and 10 mL of water, and the filtrates were collected into 50 mL volumetric flasks and completed to volume (Williams & Campbell 1971). Mineral water and river water samples were collected from Hamedan, Iran. The impurities of collected water were first removed by filter paper. The concentration ranges of aluminum and bismuth were added to the 0.1–11.0 and 0.1–7.0 µg mL⁻¹ of sample waters, respectively.

RESULTS AND DISCUSSION

The absorption spectra of aluminum and bismuth complexes in water at pH 5.8 at 400–650 nm intervals were

recorded. Sample spectra of aluminum and bismuth complexes at pH 5.8 in water are shown in Figure 1. As can be seen in Figure 1, the absorption spectra of aluminum and bismuth markedly overlapped. Due to this similarity, the absorption spectra of aluminum and bismuth are also similar and analysis of these cations is simply not possible with conventional methods. This problem was overcome by the use of chemometrics methods. For studying the optimum conditions, the influence of pH values on the spectrum of each complex at a constant concentration of each ion was studied. The complexes formed with aluminum and bismuth were affected differently with pH. In order to select the optimum pH value at which the minimum overlap occurs, the influences of the pH of the medium on the absorption spectra of aluminum and bismuth complexes were studied over the pH range 2.0–10.0. However, pH 5.8 was chosen as the optimum pH for this work, because both complexes have maximum absorbance and minimum overlap at this pH. The effect of concentration of the hematoxylin was also investigated, a reagent concentration of $3.2 \times 10^{-4} \text{ mol L}^{-1}$ was chosen because it ensures sufficient reagent excess.

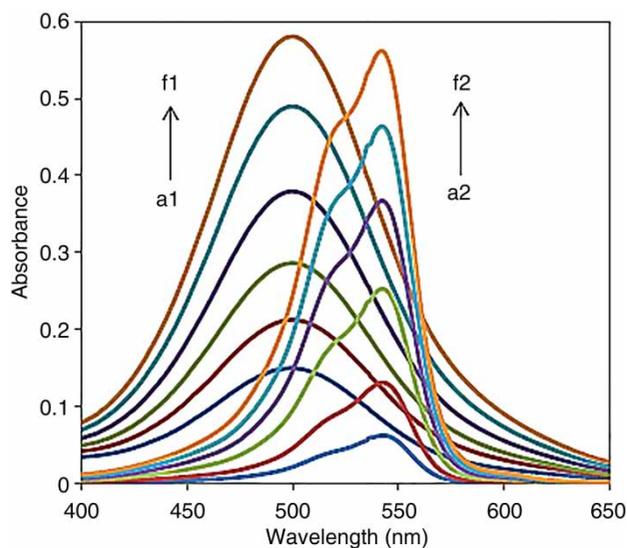


Figure 1 | Absorption spectra in the concentration range of aluminum: (a1) $0.1 \mu\text{g mL}^{-1}$, (b1) $3.0 \mu\text{g mL}^{-1}$, (c1) $5.0 \mu\text{g mL}^{-1}$, (d1) $7.0 \mu\text{g mL}^{-1}$, (e1) $9.0 \mu\text{g mL}^{-1}$, (f1) $11.0 \mu\text{g mL}^{-1}$ and bismuth: (a2) $0.1 \mu\text{g mL}^{-1}$, (b2) $1.0 \mu\text{g mL}^{-1}$, (c2) $2.5 \mu\text{g mL}^{-1}$, (d2) $4.0 \mu\text{g mL}^{-1}$, (e2) $5.5 \mu\text{g mL}^{-1}$, (f2) $7.0 \mu\text{g mL}^{-1}$.

Chemometrics study

In this study, continuous wavelet transforms in combination with the zero cross-point technique, partial least square, WOSC-PLS and LS-SVM methods were successfully applied to overcome the overlapping of spectra in the simultaneous determination of aluminum and bismuth. The main goal of this study is to show the chemometrics methods to quantitative simultaneous analysis of the amounts of aluminum and bismuth compounds in real samples. In order to obtain calibrations, the standard series was prepared in the concentration range of $0.1\text{--}11.0 \mu\text{g mL}^{-1}$ and $0.1\text{--}7.0 \mu\text{g mL}^{-1}$ for aluminum and bismuth, respectively. The absorption spectra were recorded over the wavelength range of $400\text{--}650 \text{ nm}$ against a blank. For the validation of different chemometrics methods, a recovery set of aluminum and bismuth mixtures were prepared.

Continuous wavelet transform method

The selection of the optimal mother wavelet is the most important step in the CWT method. It may be that two or more CWT methods give satisfactory results, so the most suitable CWT method has to be found for the problem. Various wavelet families with different scales were tested to find the optimal signal processing for obtaining desirable calibration graphs and reliable determination of investigated metal ions. Biorthogonal (bior2.4) ($a = 160$), Biorthogonal (bior3.9) ($a = 160$) and coiflet (coif3) ($a = 100$) were selected as optimal conditions, because they gave the highest sensitivity with large slope values in the linear regression equations for the two subjected compounds. Absorption spectra of standard solutions of aluminum and bismuth with different concentrations were recorded in the spectral region $400\text{--}650 \text{ nm}$ against a blank solution. The absorption spectra were transferred from Excel to the wavelet domain in a MATLAB environment. The absorption spectra of the standard solutions of aluminum and bismuth were transferred into the wavelet domain by bior2.4, bior3.9 and coif3, and CWT spectra were obtained by plotting Ca,b coefficients versus wavelengths, as can be seen in Figure 2. The zero cross-points were used for simultaneous determination of the metal

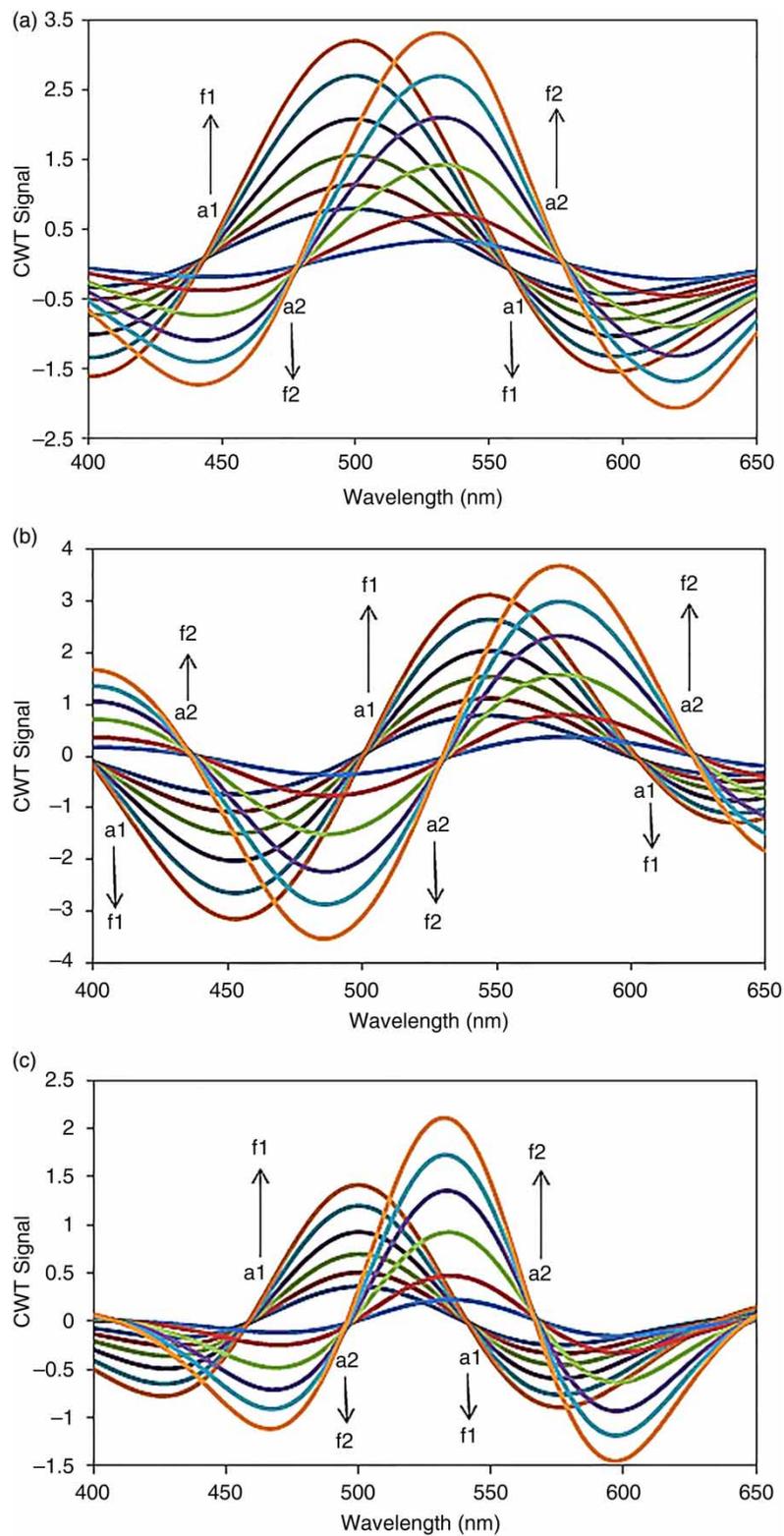


Figure 2 | CWT spectra of aluminum (a1) $0.1 \mu\text{g mL}^{-1}$, (b1) $3.0 \mu\text{g mL}^{-1}$, (c1) $5.0 \mu\text{g mL}^{-1}$, (d1) $7.0 \mu\text{g mL}^{-1}$, (e1) $9.0 \mu\text{g mL}^{-1}$, (f1) $11.0 \mu\text{g mL}^{-1}$ and bismuth (a2) $0.1 \mu\text{g mL}^{-1}$, (b2) $1.0 \mu\text{g mL}^{-1}$, (c2) $2.5 \mu\text{g mL}^{-1}$, (d2) $4.0 \mu\text{g mL}^{-1}$, (e2) $5.5 \mu\text{g mL}^{-1}$, (f2) $7.0 \mu\text{g mL}^{-1}$ by (a) bior2.4 (b) bior3.9 and (c) coif3.

ions, and the calibration graphs of each ion were obtained. The calibration graphs of aluminum for the wavelet methods were created by plotting the transformed signals versus the concentration of bismuth at the zero-crossing points, and contrariwise. The calibration graphs of bior2.4 were obtained by measuring the transformed signals at 477 and 576 nm for aluminum (corresponding to the zero crossing point of bismuth) and at 442 and 555 nm for bismuth (corresponding to the zero crossing point of aluminum). By using a similar method, the calibration graphs for bior3.9 and coif3 were created by measuring the signal domain at 435, 529, 622, 495 and 566 nm for aluminum and at 500, 400, 605, 452 and 540 nm for bismuth. Linear regression analysis and the statistical results are summarized in Table 1.

Partial least squares method

PLS regression is a suitable and simple technique, which presents a good recovery. A mixture design was used to statistically maximize the information content in the spectra. A training set of 25 samples was taken. The concentrations of aluminum and bismuth were between $0.1\text{--}11.0\ \mu\text{g mL}^{-1}$ and $0.1\text{--}7.0\ \mu\text{g mL}^{-1}$ varied in five

levels, respectively. The number of latent variables (factors) for each cation was determined by the cross-validation method. The prediction error sum of squares (PRESS) for cross-validated models was calculated. The cross-validation method was employed to eliminate only one sample at a time, and then PLS calibrated the remaining samples. By using this calibration, the concentration of the sample left out was predicted. This process was repeated until each standard had been left out once. One reasonable choice for the optimum number of factors would be the number which yielded the minimum PRESS. Notice that the optimum number of factors of the PLS model for aluminum and bismuth (N.F = 6) is larger than the expected value. Therefore, pre-processing of wavelet-OSC is carried out, and it caused a decrease in the number of factors for WOSC-PLS (N.F = 4), so they shifted to expected values. For the prediction set, five mixtures were prepared that were not included in the previous set as an independent test.

Preprocessing by wavelet transform

In order to enhance the predictive power of multivariate calibration methods, spectral data are often corrected

Table 1 | Statistical results of calibration graphs obtained by the CWT method

Mother wavelet	λ (nm)	Linear range ($\mu\text{g mL}^{-1}$)	Regression equation	R^{2a}	LOD ^b
Bior 2.4	477	0.10–11.00	$A = 0.1783C_{Al} + 0.4668$	0.9739	0.0072
Bior 2.4	576	0.10–11.00	$A = -0.0755C_{Al} - 0.2527$	0.9739	0.0172
Bior 2.4	442	0.10–7.00	$A = -0.2261C_{Bi} - 0.1593$	0.9993	0.0064
Bior 2.4	555	0.10–7.00	$A = 0.2860C_{Bi} + 0.2425$	0.9978	0.0051
Bior 3.9	435	0.10–11.00	$A = -0.1934C_{Al} - 0.4515$	0.9736	0.0067
Bior 3.9	529	0.10–11.00	$A = 0.1833C_{Al} + 0.4986$	0.9740	0.0070
Bior 3.9	622	0.10–11.00	$A = -0.0700C_{Al} - 0.2292$	0.9732	0.0185
Bior 3.9	500	0.10–7.00	$A = -0.4043C_{Bi} - 0.3156$	0.9986	0.0036
Bior 3.9	400	0.10–7.00	$A = 0.2191C_{Bi} + 0.1572$	0.9992	0.0066
Bior 3.9	605	0.10–7.00	$A = 0.2401C_{Bi} + 0.2161$	0.9970	0.0061
Coif 3	495	0.10–11.00	$A = 0.0988C_{Al} + 0.2536$	0.9748	0.0131
Coif 3	566	0.10–11.00	$A = -0.0564C_{Al} - 0.1740$	0.9742	0.0230
Coif 3	452	0.10–7.00	$A = -0.1211C_{Bi} - 0.0688$	0.9997	0.0120
Coif 3	540	0.10–7.00	$A = 0.2566C_{Bi} + 0.2163$	0.9979	0.0057

^aCorrelation coefficient.

^bLimit of detection.

prior to the data analysis. In this case, the pre-processing method (WOSC) was used. The WOSC-PLS method combines OSC, which effectively removes structured noise unrelated to the concentration, and WPT, which performs feature extraction, signal compression and denoising, as well as PLS, which provides a calibration model and reduces the dimension of the raw spectra. To succeed in obtaining a reliable result by the WOSC-PLS method, different parameters such as wavelet functions (Coiflet 1, 2... 5, Daubechies 2–8 and Symmlet 4, 5... 8), decomposition level ($L = 1-5$), the number of OSC components and the number of PLS factors, were tested. In this investigation, Coiflet 2, $L = 1$, number of OSC components = 2 and number of PLS factors = 4 were selected as optimal parameters.

Least squares-support vector machines

LS-SVM was performed with the RBF as the kernel function. In the model development using LS-SVM and the RBF kernel, γ and σ^2 parameters were a manageable task, similar to the process employed to select the number of factors for PLS models, but in this case for a two-dimensional problem. For each combination of γ and σ^2 parameters, the root mean square error of cross-validation (RMSECV) was calculated and the optimum parameters were selected to produce the smaller RMSECV. These parameters were optimized, generating models with values of γ in the range of 1–1,000 ($\gamma = 100$) and σ^2 in the range of 1–1,000 ($\sigma^2 = 200$), with adequate increments.

Determination of aluminum and bismuth in synthetic mixtures

The predictive ability of different chemometrics methods was determined using five two-component aluminum and bismuth mixtures. The statistical results obtained by applying CWT, PLS, WOSC-PLS and LS-SVM to five synthetic samples are listed in Table 2. As can be seen, the statistical parameters were quite acceptable. For the evaluation of the predictive ability of models, the root mean square error of prediction (RMSEP) and relative standard error of

Table 2 | Mean recovery, RMSEP and RSEP(%) for the simultaneous determination of Al and Bi in various synthetic mixtures by CWT, PLS, WOSC-PLS and LS-SVM methods

Methods	Recovery (%) ^a		RMSEP ^b		RSEP (%) ^c	
	Al	Bi	Al	Bi	Al	Bi
bior 2.4	98.9	98.4	0.130	0.134	2.002	3.053
Bior 3.9	99.3	100.8	0.112	0.110	1.720	2.510
Coif 3	98.0	100.5	0.166	0.160	2.554	3.648
PLS	100.0	99.8	0.110	0.104	1.686	2.370
WOSC-PLS	98.6	101.6	0.046	0.060	0.701	1.366
LS-SVM	99.3	100.0	0.021	0.011	0.213	0.165

^aAverage of five determinations.

^bRoot mean squares error of prediction.

^cRelative standard error of prediction.

prediction (RSEP) can be used:

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (y_{pred} - y_{obs})^2}{n}} \quad (11)$$

$$\text{RSEP}(\%) = 100 \times \sqrt{\frac{\sum_{i=1}^n (y_{pred} - y_{obs})^2}{\sum (y_{obs})^2}} \quad (12)$$

where y_{pred} is the predicted concentration in the sample, y_{obs} is the observed value of the concentration in the sample and n is the number of samples in the validation set. RMSEP and RSEP (%) results for different chemometrics methods are summarized in Table 2. Good results were achieved in chemometrics models, with recovery ranges from 99.3–100.0 and 98.4–100.8% for aluminum and bismuth, respectively. As can be seen, the recovery was also quite acceptable.

Determination of aluminum and bismuth in real samples

In order to test the applicability and matrix interferences of the proposed method to the analysis of real samples, the method was applied in a variety of situations. For this purpose, vegetables and water samples were analyzed. Table 3 shows the results obtained using the CWT, WOSC-PLS and LS-SVM methods, and atomic absorption spectroscopy (AAS). The values of recoveries ranged from 97.7–100.7% and 99.0–101.5% for aluminum and bismuth, respectively,

Table 3 | Simultaneous determination of Al(III) and Bi(III) using CWT(Bior3.9), WOSC-PLS and LS-SVM and standard method in real samples ($\mu\text{g mL}^{-1}$)

Method	Sample	Added		Found ^a		Recovery (%)	
		Al	Bi	Al	Bi	Al	Bi
CWT	Spinach	–	–	7.83 ± 0.94	N.D.	–	–
		3.0	2.0	10.76 ± 1.02	2.01 ± 0.83	97.7	100.2
	Parsley	–	–	4.35 ± 0.86	N.D.	–	–
		3.0	2.0	7.31 ± 0.97	1.98 ± 0.54	98.7	99.0
	River water	–	–	7.69 ± 0.73	N.D.	–	–
		3.0	2.0	10.71 ± 0.56	2.01 ± 0.64	100.7	100.5
Mineral water	–	–	6.41 ± 0.90	N.D.	–	–	
	3.0	2.0	9.44 ± 0.78	1.99 ± 0.71	101.0	99.5	
WOSC-PLS	Spinach	–	–	7.97 ± 0.32	N.D.	–	–
		3.0	2.0	10.86 ± 0.46	2.01 ± 0.17	96.3	100.3
	Parsley	–	–	4.41 ± 0.29	N.D.	–	–
		3.0	2.0	7.39 ± 0.41	1.99 ± 0.33	99.3	99.5
	River water	–	–	7.68 ± 0.47	N.D.	–	–
		3.0	2.0	10.66 ± 0.52	2.01 ± 0.19	99.3	100.5
Mineral water	–	–	6.43 ± 0.71	N.D.	–	–	
	3.0	2.0	9.45 ± 0.33	1.98 ± 0.24	100.7	99.0	
LS-SVM	Spinach	–	–	7.95 ± 0.12	N.D.	–	–
		3.0	2.0	10.89 ± 0.23	2.03 ± 0.14	98.0	101.5
	Parsley	–	–	4.39 ± 0.18	N.D.	–	–
		3.0	2.0	7.28 ± 0.22	1.98 ± 0.13	96.3	99.0
	River water	–	–	7.72 ± 0.31	N.D.	–	–
		3.0	2.0	10.71 ± 0.29	2.03 ± 0.12	99.7	101.5
Mineral water	–	–	6.42 ± 0.42	N.D.	–	–	
	3.0	2.0	9.39 ± 0.28	2.02 ± 0.14	99.0	101.0	
Standard method (AAS) ^b	Spinach	–	–	8.01 ± 0.13	N.D.	–	–
		3.0	2.0	11.02 ± 0.11	1.99 ± 0.08	100.3	99.5
	Parsley	–	–	4.42 ± 0.09	N.D.	–	–
		3.0	2.0	7.41 ± 0.13	1.99 ± 0.07	99.7	99.5
	River water	–	–	7.70 ± 0.16	0.23 ± 0.13	–	–
		3.0	2.0	10.71 ± 0.12	2.24 ± 0.11	100.3	100.5
Mineral water	–	–	6.44 ± 0.14	0.15 ± 0.14	–	–	
	3.0	2.0	9.46 ± 0.12	2.16 ± 0.09	100.7	100.5	

^aAverage of three separate determinations ± standard deviation ($n = 3$).

^bAtomic absorption spectroscopy (AAS) as standard method.

demonstrating the applicability of the method. For comparison of the measurements obtained for both methods the t-test was used, and there was no significant difference between the two methods. The obtained results confirm the capability of the proposed analytical methods for simultaneous determination of aluminum and bismuth in vegetable and water samples.

Interference study

The interference due to several cations and anions was studied in detail. For these studies, different amounts

of the ionic species were added to a mixture of aluminum and bismuth containing $1 \mu\text{g mL}^{-1}$ of each. The starting point was $500 \mu\text{g mL}^{-1}$ of interference to a mixture of cations, and if interference occurred the ratio was progressively reduced until the interference ceased. The tolerated limits were taken as those concentrations causing changes no greater than $\pm 5\%$ in the absorption spectrum of complexes. The tolerance limits for different ions studied are listed in Table 4. As is shown, the method is relatively specific for aluminum and bismuth.

Table 4 | The maximum tolerance values of various cations and anions on the measurement of a mixture of 1 µg mL⁻¹ of aluminum and iron

Ions	Tolerance limit
Ca(II), Mg(II), Ba(II), Sr(II), Na(I), K(I), As(III), Mn(II), Li(I), Tl(I), Rb(I), NH ₄ ⁺ , Cs(I), Cd(II), CO ₃ ²⁻ , Br ⁻ , S ₂ O ₅ ²⁻ , IO ₄ ⁻ , IO ₃ ⁻ , CH ₃ COO ⁻ , Cl ⁻ , SCN ⁻ , WO ₄ ²⁻ , SO ₃ ²⁻ , I ⁻ , F ⁻ , SO ₄ ²⁻ , NO ₂ ⁻ , ClO ₄ ⁻ , N ₃ ⁻ , BrO ₃ ⁻ , C ₂ O ₄ ²⁻ , NO ₃ ⁻ ,	500
Hg(II), Ag(I), Pb(II)	100
Cu(II)	40
In(III), Ga(III), Th(IV)	20
Mo(VI)	10

CONCLUSIONS

In this study we successfully applied CWT, WOSC-PLS and LS-SVM methods for the simultaneous determination of aluminum and bismuth in mixtures and real samples. The purpose of this study is the application of chemometrics methods to binary mixtures and real samples consisting of subject compounds. CWT, WOSC-PLS and LS-SVM modeling can be applied with great success to complex mixtures. These chemometrics methods need to use an abstract mathematical content, and it is not easy to apply and understand the theory of these chemometrics techniques. The PLS method uses a mixture design for calibration, so requires many concentration sets, while CWT calibration does not need too many concentration sets; CWT uses a linear concentration range for preparation of the calibration curve. On the other hand, CWT is a good method for the pre-processing of data. The results imply that the WOSC method indeed removes information from spectrochemical data that is not necessary for the fitting of the concentration-variables. Also, in this study, the results obtained by CWT and WOSC-PLS are compared with results obtained by LS-SVM. By comparing the results obtained from the simultaneous determination of aluminum and bismuth by all of the methods used, we can conclude that chemometrics methods are acceptable, useful and rapid in application for the analysis of aluminum and bismuth. It was shown that the proposed procedure can be useful for the simultaneous spectrophotometric determination of aluminum and bismuth

in highly interfering chemical systems involving relatively complex samples such as vegetables and water.

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