

Analytical Methods

# Simultaneous determination of calcium, magnesium and zinc in different foodstuffs and pharmaceutical samples with continuous wavelet transforms

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Received 1 October 2007; received in revised form 13 December 2007; accepted 27 December 2007

## Abstract

This work presents novel and very simple spectrophotometric methods by ratio spectra–continuous wavelet transformation for the simultaneous determination of ternary mixtures of calcium, magnesium and zinc without prior separation steps. The methods are based on the complexation reaction of these elements with bromopyrogallo red (BPR) at pH 9.4. The results showed that calcium, magnesium and zinc could be determined simultaneously in the range of 0.1–3.5 mg L<sup>-1</sup>, 0.2–3.2 mg L<sup>-1</sup> and 0.5–3.8 mg L<sup>-1</sup>, respectively. Mexican hat and Morlet from the family of continuous wavelet transforms were selected and applied under the optimal conditions for multi-component determinations. In this study for improving the sensitivity the normalized spectra of divisors were used instead of standard spectrum of divisor. The method was tested by analyzing various synthetic ternary mixtures of Ca<sup>2+</sup>, Mg<sup>2+</sup> and Zn<sup>2+</sup>. Under the working conditions, the proposed methods were successfully applied to simultaneous determination of elements in cows' milk, powder milk, pharmaceutical product and tea samples.

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*Keywords:* Ca<sup>2+</sup>; Mg<sup>2+</sup>; Zn<sup>2+</sup>; CWT; Ratio spectra; Cows' milk; Powder milk; Pharmaceutical products

## 1. Introduction

Trace metals in biological and pharmaceutical products play an important role in normal human and animal physiology, and trace metal status may also contribute to several diseases. Calcium, magnesium, and zinc are three of the most important minerals the body needs to gain and maintain good health. One to two percent of your body weight is calcium. It is the most abundant mineral in the body. Calcium is needed for growth and bone density, plus it keeps the heart pumping, muscles moving, and nerves communicating. Magnesium activates over 100 enzymes and helps nerves and muscles function. Zinc is essential part of more than 200 enzymes involved in digestion, metabolism, and reproduction and wound healing. So, zinc

is an essential element needed to support the body's immune system.

Calcium, magnesium and zinc may help to reduce the likelihood of kidney stones, reduce high blood pressure and the advent of mitral valve prolapse, help to treat arrhythmia and tachycardia, help to treat coronary artery spasm and other types of heart problems, reduce premenstrual syndrome (PMS) or menstrual cramps, reduce age-related eye diseases, reduce insomnia, reduce anxieties, chronic constipation, hyperactivity, various bone and periodontal diseases, sleep disturbances, mental health/depressive disorders and some forms of cardiovascular disease (McKenna, Ilich, Andon, Wang, & Matkovic, 1997; Spencer, Norris, & Williams, 1994; Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997).

However, if it is in excess, these metal ions can also play an important role in the progression of several damages to human body, including disturbances in energy metabolism

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or increasing in oxidative stress. Atherosclerosis varies among arterial regions, and it is possible that regional variation in tissue concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  may influence susceptibility to atherosclerosis. Depression can be related to high and low level of calcium and/or magnesium also, with low levels being often times associated with anxieties as well (Fitzgerald, Ports, & Yock, 1992; Hunt, Gallagher, Johnston, & Lykken, 1995; Mordes & Wacker, 1977; William, 2004).

Zinc deficiency, marked by severe growth retardation and arrested sexual development. Copper deficiency is rare, but can be caused by excess zinc from supplementation. Growth retardation, altered immune response, prematurity pregnancy, weight loss, anorexia, etc. are on other effect of the  $\text{Zn}^{2+}$  deficiency consequences. The most important and common causes in  $\text{Zn}^{2+}$  deficiency are related to the nutritional causes, thus, identifying the Zn content in different samples is very important (Wardlaw, 1999; Whitney & Rolles, 1996).

All of examples show that maintaining a balance of cations in the body is essential to human health. Trace minerals do not exist by themselves but in relationship to one another. Too much of one trace element can lead to imbalances in others resulting in disease, rather than the absence of disease for it is necessary to differentiate effect of each metal. Hence accurate determination of in foodstuffs such as different milks and tea samples or pharmaceutical products is important since intake of even low concentrations of metal ions can cause serious toxic effects. Accurate determination may help to elucidate the role and function of the trace metals in the different areas.

The determination of trace metals in complex matrices remains one of the most complicated areas of analytical chemistry. In the literature, due to a strong environmental and biological impact, trace metal ion determination have received particular attention in the last years.

Flame atomic absorption spectrometric methods (FAAS), (López-García, Viñas, Blanco, & Hernández, 1999; Shang & Hong, 1997) and inductively coupled plasma mass spectrometry (ICP-MS) (Forte et al., 2005; Yang & Swami, 2007) were used for determination of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  in mixtures. Some times is typically a necessity when using AAS or ICP methods to remove sample matrix interferences and/or preconcentrate metals of interest using ion exchange sorbents, often on-line prior to analysis, and many have been synthesized and evaluated for specific metals and applications (Pohl & Prusisz, 2007). Thienpont, Van Nuwenborg, and Stöckl (1994) used ion chromatography to the determination of calcium and magnesium in human serum. Alvarez, Marcó, Arroyo, Greaves, and Rivas (2003) used X-ray fluorescence spectroscopy to the determination of calcium, potassium, manganese, iron, copper and zinc levels in representative samples of two onion cultivars. They used expensive instruments that may not be available in each laboratory. First and second-derivative spectrophotometry for simultaneous determination of calcium and magnesium (Benamor & Agueressif, 2007) was also reported.

The use of the ratio spectra has firstly initiated by Blanco, Gene, Itrriaga, and Maspocho (1987) for the spectral analysis. After that the ratio spectra have combined with derivative method by Salinas and co-workers (1990), and they applied the ratio spectra derivative method to the resolution of binary mixture. In the following study, this method has modified as the ratio spectra derivative-zero crossing method by Berzas Nevado, Cabanilas, and Salinas (1992). An unwanted effect of the derivative methods is the decrease in *S/N*. This decrease follows from the fact that noise always contains the sharpest features in the spectrum.

The wavelets are scaled and translated copies or daughter wavelets of a finite-length or fast-decaying oscillating waveform (Alsberg, Woodward, & Kell, 1997). Wavelet transform have discontinuities and sharp peaks advantages. Continuous wavelet transform (CWT), can be applied for multi-component analysis. CWT has been applied for the signal processing and identification of overlapping peaks. WT to overcome the problem of noisy and incomplete data has been successfully put on a sound statistical basis by Donoho and co-workers (1994a, 1994b). WT was applied to Denoising (Barclay & Bonner, 1997; Mittermayr, Nikolov, Hutter, & Grasserbauer, 1996) and compressing of signals (Afkhami, Abbasi-Tarighat, & Bahram, 2007; Walczak & Massart, 1997; Zarei & Atabati, 2006).

Few reports have been published on the application of wavelet transforms for simultaneous determination of chemical species. Recently, the combined use of CWT and zero-crossing has firstly formulated by Dinç and Baleanu (2003a, 2003b, 2004a, 2004b) for the quantitative resolution of two-component mixtures. The simultaneous use of the CWT and zero-crossing technique was applied to the resolution of various binary mixtures (Dinç & Baleanu, 2003c; Dinç & Baleanu, 2007; Dinç, Baleanu, Ustundag, & Aboul-Enein, 2004; Dinç, Kanbur, & Baleanu, 2007; Dinç, Kaya, Doganay, & Baleanu, 2007; Dinç, Ozdamir, & Baleanu, 2005a, Dinç, Özdemir, & Baleanu, 2005b). In the next study, the ratio spectra-CWT approach has proposed by Dinç and Baleanu (2004a) for the spectral analysis of binary mixtures. This combined approach was used for the analysis of the veterinary formulation (Dinç, Kanbure, & Baleanu, 2005). After modification of the above approaches, Dinç et al. (2005a, 2005b, 2006) used the ratio spectra-CWT with zero-crossing technique for the multi-component spectral analysis of ternary mixtures of three active compounds with overlapping spectral.

In this study, combination of ratio spectra and continuous wavelet transformation for determination of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  based on their complexes with boromopyrogallol red (BPR) was developed. The applicability of different continuous mother wavelet was tested on different synthetic data. In previous methods the optimal concentration of divisor were used. In this work we suppressed the dependency on divisor concentration by using the normalized spectrum of the standard solution of divisor. The methods were validated by simultaneous determination of

Ca<sup>2+</sup>, Mg<sup>2+</sup> and Zn<sup>2+</sup> in ultra high temperature treated (UHT) cows' milk, powder milk, multivitamine tablet and tea samples. To the best of our knowledge, this is the first report on the simultaneous spectrophotometric determination of calcium, magnesium and zinc.

## 2. Theory

### 2.1. Wavelet transform

Continuous wavelets are functions which used by the continuous wavelet transform. The original signal can be reconstructed by suitable integration over all the resulting frequency components after projection of given signal on a continuous family of frequency bands. The subspace of scale  $a$  or frequency band is generated by the functions

$$\psi_{(a,b)}(t) = \frac{1}{\sqrt{a}} \psi\left(\frac{t-b}{a}\right) \quad (1)$$

where  $a$  is positive and defines the scale and  $b$  is any real number and defines the shift.

Mexican hat wavelet in mathematics and numerical analysis

$$\psi(t) = \frac{1}{\sqrt{2\pi\sigma^3}} \left(1 - \frac{t^2}{\sigma^2}\right) e^{-\frac{t^2}{2\sigma^2}} \quad (2)$$

is the normalized second-derivative of a Gaussian function. It is a special case of the family of continuous wavelets known as Hermitian wavelets. Morlet is another mother wavelet in mathematics, which was originally formulated as a constant  $\kappa_\sigma$  subtracted from a plane wave and then localized by a Gaussian

$$\psi_\sigma(t) = c_\sigma \pi^{-\frac{1}{4}} e^{-\frac{1}{2}t^2} (e^{i\sigma t} - \kappa_\sigma) \quad (3)$$

where  $\kappa_\sigma = e^{-\frac{1}{2}\sigma^2}$  is defined by the admissibility criterion and the normalization constant  $c_\sigma$  is

$$C\sigma = \left(1 + e^{-\sigma^2} - 2e^{-\frac{3}{4}\sigma^2}\right)^{-\frac{1}{2}} \quad (4)$$

### 2.2. Ratio Spectra-CWT

Consider a ternary mixture of three metal ions,  $M_1$ ,  $M_2$  and  $M_3$  which the absorbance value of this ternary mixture at  $\lambda_i$  can be written as follows:

$$A_{\lambda_i} = \alpha_{M_1} C_{M_1} + \alpha_{M_2} C_{M_2} + \alpha_{M_3} C_{M_3} \quad (5)$$

where  $A_{\lambda_i}$  is the absorbance of the ternary mixture at wavelength  $\lambda_i$  and the coefficients  $\alpha_{M_1}$ ,  $\alpha_{M_2}$  and  $\alpha_{M_3}$  are absorption coefficients of  $M_1$ ,  $M_2$  and  $M_3$ , respectively. By dividing the Eq. (5) by the spectrum of a standard solution of one of the components (for example,  $M_1$ ) the following equation will be obtained:

$$\frac{A_{\lambda_i}}{\alpha_{M_1} C_{M_1}^0} = \frac{\alpha_{M_1} C_{M_1}}{\alpha_{M_1} C_{M_1}^0} + \frac{\alpha_{M_2} C_{M_2}}{\alpha_{M_1} C_{M_1}^0} + \frac{\alpha_{M_3} C_{M_3}}{\alpha_{M_1} C_{M_1}^0} \quad (6)$$

If CWT was applied on Eq. (6), the following equation can be obtained

$$\text{CWT} \left[ \frac{A_{\lambda_i}}{\alpha_{M_1} C_{M_1}^0} \right] = \text{CWT} \left[ \frac{\varepsilon_{M_2} C_{M_2}}{\alpha_{M_1} C_{M_1}^0} \right] + \text{CWT} \left[ \frac{\varepsilon_{M_3} C_{M_3}}{\alpha_{M_1} C_{M_1}^0} \right] \quad (7)$$

Because the CWT of constant is zero, at the zero cross of  $M_2$ , the last equation rewritten in

$$\text{CWT} \left[ \frac{A_{\lambda_i}}{\alpha_{M_1} C_{M_1}^0} \right] = \text{CWT} \left[ \frac{\varepsilon_{M_3} C_{M_3}}{\alpha_{M_1} C_{M_1}^0} \right] \quad (8)$$

If Eq. (5) is divided by  $\alpha_{M_1}$  corresponding to the absorption coefficients of  $M_1$  in ternary mixture, the ratio spectrum is obtained in the form of Eq. (9) (for possibility of dividing operation, the zero values of  $\alpha_{M_1}$  should not be used in the divisor):

$$\frac{A_{\lambda_i}}{\alpha_{M_1}} = \frac{\alpha_{M_1} C_{M_1}}{\alpha_{M_1}} + \frac{\alpha_{M_2} C_{M_2}}{\alpha_{M_1}} + \frac{\alpha_{M_3} C_{M_3}}{\alpha_{M_1}} \quad (9)$$

By application of CWT on ratio spectra, Eq. (9) gives

$$\text{CWT} \left[ \frac{A_{\lambda_i}}{\alpha_{M_1}} \right] = \text{CWT} \left[ \frac{\alpha_{M_2} C_{M_2}}{\alpha_{M_1}} \right] + \text{CWT} \left[ \frac{\alpha_{M_3} C_{M_3}}{\alpha_{M_1}} \right] \quad (10)$$

At the zero point of  $M_2$ , the last equation rewritten in

$$\text{CWT} \left[ \frac{A_{\lambda_i}}{\alpha_{M_1}} \right] = \text{CWT} \left[ \frac{\alpha_{M_3} C_{M_3}}{\alpha_{M_1}} \right] \quad (11)$$

The Eq. (11) indicates that ratio-CWT spectra of sample only depended on the concentration of  $M_3$  and independent from  $M_2$  and  $M_1$  concentration of zero cross of  $M_2$ . Eq. (7) shows that ratio-CWT spectra of sample at zero cross of  $M_2$  not only dependent on the concentration of  $M_3$  but also dependent on  $M_1$  concentration.

### 2.3. Normalization of the spectra

Consider the vector (spectrum)  $X$  as an ordered array of  $n$  elements

$$X = [x_1, x_2, \dots, x_n] \quad (12)$$

The length of each vector can be calculated by Eq. (13)

$$\|X\| = \sqrt{\left(\sum_{i=1}^n x_i^2\right)} \quad (13)$$

where  $n$  is the number of each vector elements. Normalization of an arbitrary vector  $X$  is obtained by dividing each of its elements by the norm  $\|X\|$  of the vector.

$$X_{\text{normalized}} = \frac{X}{\|X\|} \quad (14)$$

## 3. Experimental

### 3.1. Standard solutions

All solutions were prepared with analytical grade reagents. Stock solutions of Mg<sup>2+</sup>, Ca<sup>2+</sup> and Zn<sup>2+</sup>

(1000 mg L<sup>-1</sup>) were prepared by dissolving CaCl<sub>2</sub>, MgCl<sub>2</sub> · 6H<sub>2</sub>O and ZnCl<sub>2</sub> (Fluka) in water. A 5.0 × 10<sup>-3</sup> mol L<sup>-1</sup> boromopyrogallol red (BPR) (Merck) was prepared by dissolving it in minimum amount of ethanol (Merck) and diluting to the mark with doubly distilled water. A pH 9.40 buffer solution was prepared by mixing 95.0 mL of 0.05 mol L<sup>-1</sup> Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O and 5.0 mL of 0.1 mol L<sup>-1</sup> potassium dihydrogen phosphates (Dean, 1995).

### 3.2. Apparatus

Spectra were recorded and stored using a single beam UV-mini-WPA spectrophotometer with a 1-cm path length quartz cell. A model 713 Metrohm pH meter was used for the measurement of pH of the solutions. Calculations were performed using Toolbox for MATLAB 7.1 and EXCEL (XP windows 2003 operating system). A short program in MATLAB 7.1 was written for performing normalization of the data. Transformation of ratio spectra was performed in MATLAB 7.1 then the CWT ratio signals were transferred to EXCLE.

### 3.3. General procedure

Suitable amounts of each metal, 1 mL of 5.0 × 10<sup>-3</sup> mol L<sup>-1</sup> BPR solution and 1 mL of buffer solution were added to a 10 mL volumetric flask. The solution was diluted to the mark with water and allowed to stand for 3 min at room temperature. A portion of the solution was then transferred into a 1-cm glass cell to record the absorption spectra in the wavelength range 350–650 nm.

### 3.4. Preparation of multivitamin tablet sample

Ten tablets were weighted and powdered in a mortar. A tablet amount and 2 mL concentrated HNO<sub>3</sub> was transferred to a 50 mL flask and diluted to the mark with doubly distilled water. The content of flask was mechanically shaken for a period of 30 min and filtered. After that the developed methods were applied to the final solution.

### 3.5. Preparation of UHT cows' milk sample

In this work, non-fatted long life liquid ultra high temperature treated (UHT) cows' milk available on the local markets (Pegah shir) was purchased for investigation. For determination of total concentration of Mg<sup>2+</sup>, Ca<sup>2+</sup> and Zn<sup>2+</sup> cations in analyzed UHT milk, a 0.50 mL sample portion and 5 mL of HNO<sub>3</sub> was heated on a hot plate in the glass beaker to dryness. After that, the sample was cooled and 3.0 mL of H<sub>2</sub>O<sub>2</sub> (30%) was added and the heating was repeated to achieve about 0.50 mL sample solutions. The resulting solution was transferred into a 50.0 mL volumetric flask (Pohl & Prusisz, 2007) and diluted to the mark with water. The amounts of Mg<sup>2+</sup>, Ca<sup>2+</sup> and Zn<sup>2+</sup> in the sample was then determined as described in general procedure section.

### 3.6. Preparation of milk powder sample

A 0.5 g of sample was accurately weighted for determination and digested with HNO<sub>3</sub>–HClO<sub>4</sub> (9:1 v/v) to near dryness. Distilled water of 20 mL was added in the residue and evaporated to dry again for removing residual (Yuanqian, Jingmei, Jingguo, Bo, & Yuanqing, 2002). The resulting solution was transferred into a 50.0 mL volumetric flask and diluted to the mark with water. After that the developed method was applied to the final solution.

### 3.7. Preparation of tea leaves solution

A 0.1 g sample was taken in a beaker and dissolved in concentrated nitric acid (5 mL) with heating. The solution was cooled, diluted and filtered. The filtrate was made up to 100 mL with distilled water in a calibrated flask (Taher, 2000). This solution was applied for analysis.

## 4. Results and discussion

### 4.1. Preliminary investigations

The chromogenic reagent boromopyrogallol red (BPR) can be use as a reagent for the determination of trace amounts Ca<sup>2+</sup>, Mg<sup>2+</sup> and Zn<sup>2+</sup>. The compositions of complexes were determined by molar ratio method. The values of molar ratio indicated a 1:1, 1:2 and 1:2 metal:ligand ratio for Ca-BPR, Mg-BPR and Zn-BPR, respectively. The absorption spectra of Zn<sup>2+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup> complexes are shown in Fig. 1. As Fig. 1 shows, the spectra of the complexes and their ternary mixture which spectra of the complexes, overlap with each other, and therefore each compound interfere in the spectrophotometric determination of the others. But this system can be suitable for simultaneous determination of Ca<sup>2+</sup>, Mg<sup>2+</sup> and Zn<sup>2+</sup> using continuous wavelet transforms.

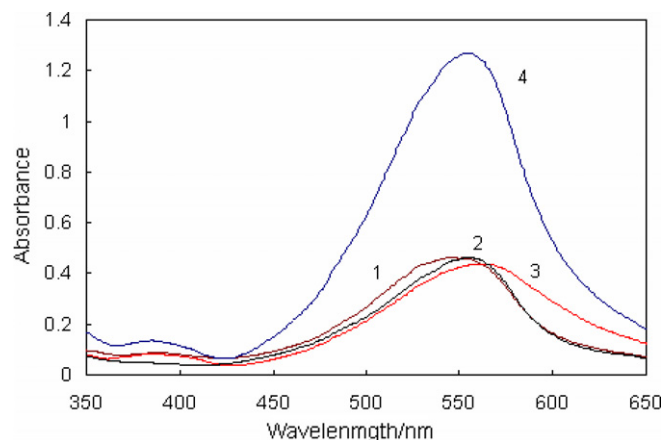


Fig. 1. Absorption spectra for the solution of (1) Zn<sup>2+</sup> (2 mg L<sup>-1</sup>); (2) Ca<sup>2+</sup> (1.5 mg L<sup>-1</sup>); and (3) Mg<sup>2+</sup> (2 mg L<sup>-1</sup>) (4) ternary mixture of three metal ions, in the presence of 5.0 × 10<sup>-4</sup> mol L<sup>-1</sup> BPR at pH 9.40.

#### 4.2. Optimization of the experimental conditions

In order to find the optimum pH, the effect of pH in the range 4–11 and different buffer solutions on the complex formation reactions were investigated. The complex formation reactions of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  with BPR depend on pH. Maximum colors of complexes were observed in the pH range 9–9.8. Therefore, pH 9.40 was used for further works. Borax–Potassium dihydrogen phosphate and ammonia (aqueous)–ammonium chloride buffer solutions of pH 9.40 were tested and the Borax solution was found as the best. A  $5 \times 10^{-4} \text{ mol L}^{-1}$  BPR was used as optimum concentration.

#### 4.3. Spectral characteristics and selection of appropriate mother wavelets

As Fig. 1 shows, the spectra of complexes of Ca-BPR, Mg-BPR and Zn-BPR at optimum conditions, show strong overlapping in the spectral region of 350–650 nm. Due to their mutual interference, the simultaneous determination of Ca-BPR, Mg-BPR and Zn-BPR in the same samples is not possible by using the classical analytical methodologies. On the other hand, the simultaneous dissolution of them is

impossible. Application of continuous wavelet transform and ratio spectra can provide successful determination of investigated elements without any separation. Absorption spectra in the linear concentration range  $0.1\text{--}3.5 \text{ mg L}^{-1}$ ,  $0.2\text{--}3.2 \text{ mg L}^{-1}$  and  $0.5\text{--}3.8 \text{ mg L}^{-1}$  for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ , respectively, in the spectral region 350–650 nm, were recorded. For selection of an appropriate divisor concentration, some divisor concentrations were tested and the concentrations of 0.80, 0.80 and  $0.50 \text{ mg L}^{-1}$  of  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ , respectively, were found as the best concentrations of divisors. The ratio spectra were obtained with  $\Delta\lambda = 1 \text{ nm}$ . Various wavelet families were tested to find the optimal signal processing for obtaining desirable calibration graphs and reliable determination of the investigated elements. Mexican hat (Mexh) and Morlet (Morl) were selected as optimal mother wavelets for transformation of absorption spectra. Simultaneous determinations of desirable metal ions were also performed using normalized spectrum of each species as divisors.

#### 4.4. Analytical parameters

The absorption spectra of the standard solutions of the  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with different concentrations were

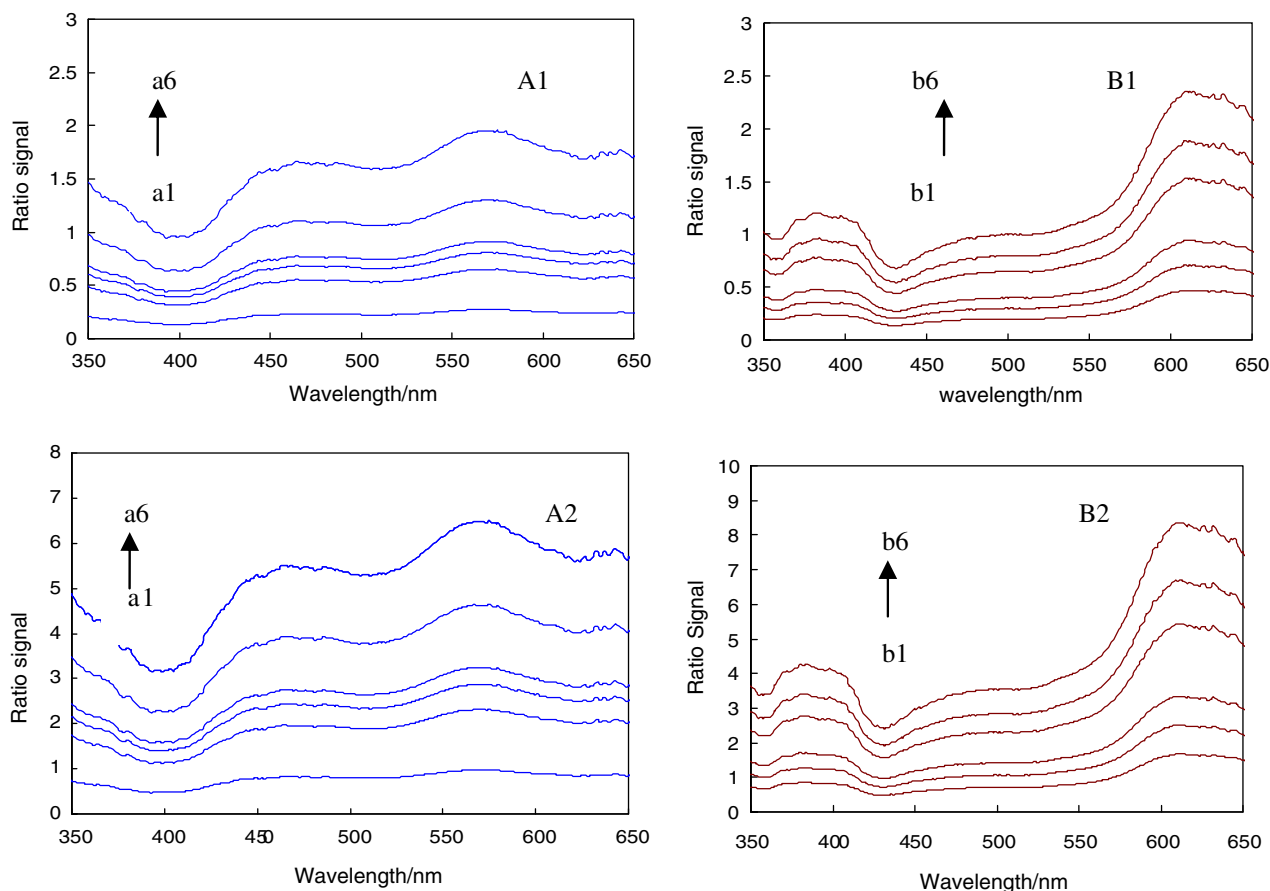


Fig. 2. The ratio spectra for Ca-BPR created by (A1)  $0.08 \text{ mg L}^{-1} \text{ Zn}^{2+}$  and (A2) using normalized spectrum of Zn as divisor;  $5 \times 10^{-4} \text{ mol L}^{-1}$  BPR and (a1) 0.2, (a2) 0.8, (a3) 1.0, (a4) 1.5, (a5) 2.0 and (a6)  $2.5 \text{ mg L}^{-1} \text{ Ca}^{2+}$  and for Mg-BPR created by (B1)  $0.08 \text{ mg L}^{-1} \text{ Zn}$  and (B2) by using normalized spectrum of  $\text{Zn}^{2+}$  as divisor;  $5.0 \times 10^{-4} \text{ mol L}^{-1}$  BPR and (b1) 0.20, (b2) 0.50, (b3) 0.70, (b4) 2.0, (b5) 2.8 and (b6)  $3.2 \text{ mg L}^{-1} \text{ Mg}^{2+}$ .

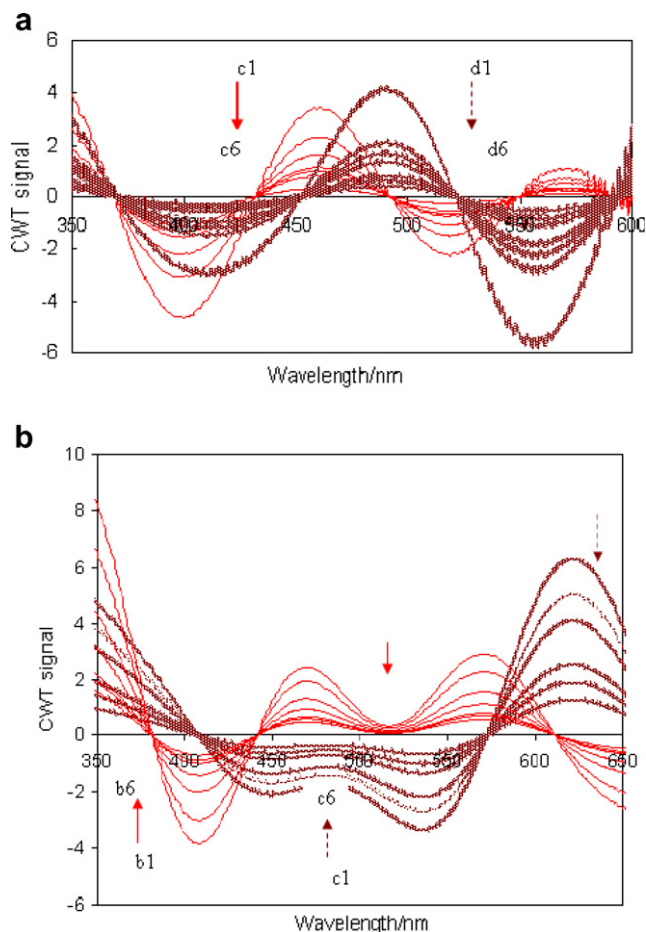


Fig. 3. (a) CWT-Mexh signal of Ca-BPR (c1) 0.2 mg L<sup>-1</sup>, (c2) 0.8 mg L<sup>-1</sup>, (c3) 1 mg L<sup>-1</sup>, (c4) 1.5 mg L<sup>-1</sup>, (c5) 2 mg L<sup>-1</sup>, (c6) 2.5 mg L<sup>-1</sup> and Mg-BPR; (d1) 0.2 mg L<sup>-1</sup>, (d2) 0.50 mg L<sup>-1</sup>, (d3) 0.70 mg L<sup>-1</sup>, (d4) 2.0 mg L<sup>-1</sup>, (d5) 2.8 mg L<sup>-1</sup>, (d6) 3.2 mg L<sup>-1</sup>; (b) CWT-Morlet signal of Ca-BPR (c1) 0.2 mg L<sup>-1</sup>, (c2) 0.8 mg L<sup>-1</sup>, (c3) 1 mg L<sup>-1</sup>, (c4) 1.5 mg L<sup>-1</sup>, (c5) 2 mg L<sup>-1</sup>, (c6) 2.5 mg L<sup>-1</sup> and Mg-BPR; (d1) 0.2 mg L<sup>-1</sup>, (d2) 0.50 mg L<sup>-1</sup>, (d3) 0.70 mg L<sup>-1</sup>, (d4) 2 mg L<sup>-1</sup>, (d5) 2.8 mg L<sup>-1</sup> and (d6) 3.2 mg L<sup>-1</sup>.

recorded in the wavelength range 220–380 nm and divided by the normalized spectrum of the Zn<sup>2+</sup> and the ratio spectra were obtained (Fig. 2). These ratio spectra were transferred from EXCLE to wavelet domain in MATLAB 7.1. The optimum value of scaling factor for determination of Ca<sup>2+</sup> and Mg<sup>2+</sup> in their ternary mixture was found as  $a = 40$ . Due to the higher amplitude of ratio spectra of normalized spectrum, the low value of scaling factor can improve the sensitivity of determination. As Fig. 3 shows, the graphs of CWT for calibration sets of Ca<sup>2+</sup> and Mg<sup>2+</sup> and their ternary mixtures, were obtained by Mexh ( $a = 40$ ) on the ratio absorption spectra at the same wavelength range as ratio spectra. Calibration graphs of Ca<sup>2+</sup> and Mg<sup>2+</sup> were obtained by measuring the CWT signals at 409 or 573 for Ca<sup>2+</sup> corresponds to zero crosses of Mg<sup>2+</sup> and 383 or 444 nm for Mg<sup>2+</sup> corresponds to zero crosses of Ca<sup>2+</sup>. Linear regression analysis and its statistical results are shown in Table 1. For the prediction of concentration of Ca<sup>2+</sup> and Mg<sup>2+</sup> in synthetic ternary mixtures the same procedure was used except that the spectra of the mixture were used instead of the spectra of standard solution of Ca<sup>2+</sup> and Mg<sup>2+</sup> and concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> in ternary mixtures were estimated by CWT-Mexh calibration equations at zero crosses. The estimated recoveries of Ca<sup>2+</sup> and Mg<sup>2+</sup> which obtained by proposed methods are given in Table 2.

Also by Morlet (as a mother wavelet) the transformation of ratio spectra of standards of Ca<sup>2+</sup> and Mg<sup>2+</sup> and ternary mixtures solutions were performed. Normalized spectrum of Zn<sup>2+</sup> was used as divisor. The optimum scaling value was found as  $a = 70$ . By measuring the amplitude of CWT signals at 452 or 521 nm for Ca<sup>2+</sup> (corresponding to zero crosses of Mg<sup>2+</sup>) and 434 or 494 nm for Mg<sup>2+</sup> (corresponding to zero crosses Ca<sup>2+</sup>), the calibration graphs of Ca<sup>2+</sup> and Mg<sup>2+</sup> were built. Linear regression analysis and its statistical results are shown in Table 1. By using the

Table 1  
Statistical results of calibration graphs obtained in zero cross points of Ca-BPR and Mg-BPR by proposed methods

Mother wavelet	Divisor	$\lambda$ (nm)	Linear range (mg L <sup>-1</sup> )	Regression equation	$r$	LOD (mg L <sup>-1</sup> )	LOQ (mg L <sup>-1</sup> )		
Mexh	Zn <sup>2+</sup>	411 <sup>a</sup>	0.1–2.5	$A = 0.3507 C_{Ca} + 0.3111$	0.9974	0.0042	0.0142		
		411 <sup>b</sup>	0.1–3.5	$A = 1.491 C_{Ca} + 0.4332$	0.9988	0.0010	0.0033		
		574 <sup>a</sup>	0.1–2.5	$A = 0.257 C_{Ca} + 0.2520$	0.9989	0.0058	0.0194		
		574 <sup>b</sup>	0.1–3.0	$A = -1.521 C_{Ca} - 0.5720$	0.9994	0.0009	0.0032		
		385 <sup>a</sup>	0.2–3.0	$A = 0.3405 C_{Mg} + 0.060$	0.9985	0.0044	0.0146		
		385 <sup>b</sup>	0.1–3.2	$A = 1.283 C_{Mg} + 1.016$	0.9996	0.0011	0.0038		
	Mg <sup>2+</sup>	448 <sup>a</sup>	0.3–3.0	$A = 0.541 C_{Mg} + 0.041$	0.9987	0.0028	0.0092		
		448 <sup>b</sup>	0.2–3.2	$A = -1.350 C_{Mg} - 1.230$	0.9989	0.0011	0.0038		
		Morlet	Zn <sup>2+</sup>	434 <sup>a</sup>	0.2–2.5	$A = 0.365 C_{Ca} - 0.3550$	0.9986	0.0041	0.0136
				434 <sup>b</sup>	0.1–2.8	$A = 1.999 C_{Ca} + 0.3660$	0.9997	0.0007	0.0025
				453 <sup>a</sup>	0.1–3.0	$A = -0.246 C_{Ca} 0.2390$	0.9984	0.0060	0.0203
				453 <sup>b</sup>	0.1–3.0	$A = -1.340 C_{Ca} - 0.2483$	0.9993	0.0011	0.0038
494 <sup>a</sup>	0.2–3.1			$A = 0.4267 C_{Mg} + 0.413$	0.9984	0.0035	0.0118		
494 <sup>b</sup>	0.2–3.2			$A = 1.280 C_{Mg} + 0.3400$	0.9992	0.0011	0.0040		
Mg <sup>2+</sup>	521 <sup>a</sup>	0.2–3.1	$A = -0.286 C_{Mg} - 0.200$	0.9989	0.0052	0.0174			
	521 <sup>b</sup>	0.3–3.2	$A = 0.152 C_{Mg} + 0.001$	0.9989	0.0099	0.0329			

<sup>a</sup> Using spectrum of optimal concentration of divisor.

<sup>b</sup> Using normalized spectrum of standard solution of divisor.

Table 2  
Mean recoveries and relative standard deviation for the simultaneous determination of  $Mg^{2+}$  and  $Ca^{2+}$  and  $Zn^{2+}$  in various synthetic mixtures by the proposed methods

	$Mg^{2+}$ as divisor		$Ca^{2+}$ as divisor		$Zn^{2+}$ as divisor	
	$Ca^{2+}$	$Zn^{2+}$	$Mg^{2+}$	$Zn^{2+}$	$Ca^{2+}$	$Mg^{2+}$
Mexh	$102.0 \pm 4.78^a$	$101.85 \pm 4.60^a$	$98.30 \pm 4.60^a$	$99.65 \pm 3.90^a$	$101.6 \pm 3.12^a$	$100.8 \pm 3.77^a$
	$99.26 \pm 3.84^a$	$102.38 \pm 4.24^a$	$101.0 \pm 2.18^a$	$103.0 \pm 2.16^a$	$100.27 \pm 0.90^b$	$101.2 \pm 0.98^b$
Morl	$102.0 \pm 1.32^b$	$101.3 \pm 0.980^b$	$102.5 \pm 0.98^b$	$102.0 \pm 1.28^b$	$99.34 \pm 3.86^a$	$101.6 \pm 4.92^a$
	$101.2 \pm 1.64^b$	$99.26 \pm 1.04^b$	$99.26 \pm 1.24^b$	$99.26 \pm 1.32^b$	$101.4 \pm 1.53^b$	$102.0 \pm 1.75^b$

<sup>a</sup> Using spectrum of optimal concentration of divisor.

<sup>b</sup> Using normalized spectrum of standard solution of divisor.

calibration equations,  $Ca^{2+}$  and  $Mg^{2+}$  by were determined in ternary synthetic mixtures. Mean recovery results presented in Table 2.

Same procedure for simultaneous determination of  $Ca^{2+}$  and  $Mg^{2+}$  in their ternary mixtures was applied using the  $0.80 \text{ mg L}^{-1} Zn^{2+}$  as an optimal divisor concentration. The ratio signals were obtained (Fig. 2). Fig. 2 shows that, in this case the amplitude of ratio spectra are less than the amplitude of ratio spectra which obtained by dividing to normalized spectrum of  $Zn^{2+}$ . It is due to the small values of normalized spectrum of  $Zn^{2+}$  and suppressing the concentration divisor dependency. Transformation of ratio spectra of standard spectra of  $Ca^{2+}$  and  $Mg^{2+}$  and ternary mixtures solutions were performed by Mexh ( $a = 65$ ) and Morl ( $a = 120$ ) in MATLAB 7.1 and CWT signal of ratio spectra were created. Linear regression analysis and its statistical results at zero crosses of calibration graphs for each metal ion are shown in Table 1. The contents of  $Ca^{2+}$  and  $Mg^{2+}$  in ternary synthetic mixtures were determined with calibration equations in zero crosses. Recovery results were presented in Table 2.

In the similar manner, simultaneous determination of  $Mg^{2+}$  and  $Zn^{2+}$  in ternary mixtures by creating ratio spectra of standard solution of them by dividing to normalized spectrum of  $Ca^{2+}$  was carried out. Transformation of these ratio spectra were performed by CWT-Mexh ( $a = 40$ ) and CWT-Morl ( $a = 120$ ). Amplitude of CWT-Mexh graphs yield straight lines at 554 or 566 nm for  $Mg^{2+}$  (correspond to zero crosses of  $Zn^{2+}$ ) and 570 nm for  $Zn^{2+}$  (corresponds to zero cross of  $Mg^{2+}$ ). Also two straight lines from amplitudes of CWT-Morl graphs were obtained at 418 and 577 nm for determination of  $Mg^{2+}$  and  $Zn^{2+}$ , respectively. Its regression coefficients and statistical results at zero crosses are shown in Table 3. Mean recoveries and the relative standard deviations were calculated and their results were given in Table 2. Simultaneous determination of  $Mg^{2+}$  and  $Zn^{2+}$  was also performed using  $0.80 \text{ mg L}^{-1} Ca^{2+}$  as an optimal concentration of divisor. The ratio spectra were created and transformed to MATLAB 7.1 for performing CW transformation. The zero crosses of each metal ion from the CWT-Mexh and CWT-Morl graphs were determined and used for concentration estima-

Table 3  
Statistical results of calibration graphs obtained in zero cross points by proposed methods

Mother wavelet	Divisor	$\lambda$ (nm)	Linear range ( $\text{mg L}^{-1}$ )	Regression equation	Correlation coefficient	LOD ( $\text{mg L}^{-1}$ )	LOQ ( $\text{mg L}^{-1}$ )
Mexh	$Ca^{2+}$	554 <sup>a</sup>	0.2–3.0	$A = -0.2374C_{Mg} - 0.208$	0.9988	0.0063	0.0200
		554 <sup>b</sup>	0.2–3.2	$A = 2.913C_{Mg} - 2.3063$	0.9994	0.0005	0.0017
		570 <sup>a</sup>	0.5–3.5	$A = -1.0499C_{Zn} + 0.630$	0.9979	0.0014	0.0048
		570 <sup>b</sup>	0.5–3.5	$A = 0.626C_{Zn} + 0.4724$	0.9986	0.0024	0.0079
Morl	$Ca^{2+}$	566 <sup>a</sup>	0.2–3.0	$A = 2.22C_{Mg} - 3.6120$	0.9987	0.0006	0.0022
		566 <sup>b</sup>	0.2–3.2	$A = 0.2718C_{Mg} + 0.286$	0.9992	0.0055	0.0184
		418 <sup>a</sup>	0.2–3.0	$A = -0.2374C_{Mg} + 0.280$	0.9988	0.0040	0.0133
		418 <sup>b</sup>	0.2–3.2	$A = 1.021C_{Mg} + 0.4258$	0.9993	0.0016	0.0048
		577 <sup>a</sup>	0.5–3.5	$A = 0.1254C_{Zn} - 0.5900$	0.9988	0.0066	0.0222
		577 <sup>b</sup>	0.5–3.8	$A = 0.321C_{Zn} + 0.7713$	0.9997	0.0047	0.0155
Mexh	$Mg^{2+}$	468 <sup>a</sup>	0.1–3.0	$A = 0.7720C_{Ca} + 0.7508$	0.9995	0.0019	0.0064
		468 <sup>b</sup>	0.1–3.0	$A = 1.235C_{Ca} + 0.4580$	0.9979	0.0012	0.0040
		578 <sup>a</sup>	0.5–3.5	$A = -0.6203C_{Zn} - 0.347$	0.9984	0.0024	0.0080
		578 <sup>b</sup>	0.5–3.8	$A = -1.3658C_{Zn} - 0.568$	0.9997	0.0010	0.0036
Morl	$Mg^{2+}$	417 <sup>a</sup>	0.1–3.0	$A = 0.4825C_{Ca} + 0.5057$	0.9986	0.0031	0.0103
		417 <sup>b</sup>	0.1–3.0	$A = 0.95471C_{Ca} + 0.324$	0.9987	0.0015	0.0052
		471 <sup>a</sup>	0.5–3.5	$A = 0.8233C_{Zn} + 0.4541$	0.9984	0.0018	0.0060
		471 <sup>b</sup>	0.5–3.5	$A = 1.478C_{Zn} + 0.5147$	0.9998	0.0006	0.0020
		459 <sup>a</sup>	0.5–3.0	$A = 0.4825C_{Zn} + 0.5057$	0.9995	0.0031	0.0103
		459 <sup>b</sup>	0.5–3.0	$A = 0.9624C_{Zn} + 0.3284$	0.9994	0.0015	0.0051

<sup>a</sup> Using spectrum of optimal concentration of divisor.

<sup>b</sup> Using normalized spectrum of standard solution of divisor.

tion of  $Mg^{2+}$  and  $Zn^{2+}$ . The recoveries of these determinations are presented in Table 2.

In the same way for quantitative determination of  $Ca^{2+}$  and  $Zn^{2+}$  in ternary mixtures the ratio spectra of standard series of  $Ca^{2+}$  and  $Zn^{2+}$  were obtained. Normalized spectrum of  $Mg^{2+}$  was used as a divisor. CWT signals of their ratio spectra by transformation with CWT-Mexh ( $a = 40$ ) and CWT-Morl ( $a = 70$ ), were created. Its regression coefficients and statistical results at zero cross of CWT graphs are presented in Table 3. Also mean recoveries and the relative standard deviations were calculated and their results were given in Table 2. Simultaneous determination of  $Ca^{2+}$  and  $Zn^{2+}$  also was performed using  $0.50 \text{ mg L}^{-1} Mg^{2+}$  as an optimal concentration of divisor. The recoveries of this determination presented in Table 2.

The limit of detection (LOD) (Miller & Miller, 2000) and limit of quantitation (LOQ) (Miller & Miller, 2000) of the method, were calculated and are given in Tables 1 and 3.

By comparing the results of Tables 1 and 3 it is clear that linear dynamic ranges will be independent on the divisor concentration by using the normalized standard spectrum of divisor instead of standard spectrum of divisor. Also the sensitivity and slope of calibration graphs were better than those obtained by optimal standard spectrum of divisor. Another advantage of normalized spectra instead of standard spectra cancels the optimizing process to find the best divisor concentration. So the method is a time saving method and reduces the analysis times. The obtained results show that two proposed mother wavelets are suitable for simultaneous determination of desirable metal ions.

#### 4.5. Interference study

The interference study was performed by analyzing solutions containing  $0.8 \text{ mg L}^{-1} Ca^{2+}$ ,  $0.8 \text{ mg L}^{-1} Mg^{2+}$  and  $1.0 \text{ mg L}^{-1} Zn^{2+}$  in the presence of different cations and anions. The tolerance limit was taken as the concentration of interfering ion caused less than 5% relative error in the determination of  $Ca^{2+}$ ,  $Mg^{2+}$  and  $Zn^{2+}$ . The results showed that the ions  $K^+$ ,  $Cl^-$  and  $Na^+$  did not interfere even when present in 1500-fold-excess over the  $Ca^{2+}$ ,  $Mg^{2+}$  and  $Zn^{2+}$ .  $ClO_3^-$  and  $SCN^-$  did not interfere up to 300 and interfered at higher concentrations.  $Ag^+$  did not interfere up to 100-fold-excess,  $I^-$  and  $Cr^{3+}$ ,  $Cd^{2+}$ ,  $SO_4^{2-}$  did not interfere up to 50-fold-excess.  $Al^{3+}$ ,  $Ni^{2+}$  and  $Si(IV)$  did not interfere up to 10-fold-excess, and  $Co^{2+}$ ,  $Fe^{2+}$ ,  $Cu^{2+}$  and  $NO_3^-$  did not interfere up to 5-fold-excess. Thiocyanate and thiourea did not interfere when present up to 50-fold-excess. The interfering effect of  $Cu^{2+}$ ,  $Co^{2+}$  and  $Fe^{2+}$  up to 5 and  $Ni^{2+}$  up to 10 was removed by the addition of 1.0 mL 1.5% thiourea and 1.0 mL 0.5% thiosyanate, respectively. Also interference of  $Cu^{2+}$  can be suppressed by using the ascorbic acid as a reducing agent at pH approximately 6 (pH values was controlled by adding appropriate amount of aqueous ammonia).

#### 4.6. Applications of the method to real samples

Results for the analysis of synthetic mixtures by the proposed methods (Table 2) showed satisfactory results for the simultaneous determination of  $Ca^{2+}$ ,  $Mg^{2+}$  and  $Zn^{2+}$ . To demonstrate the applicability of the optimized method to

Table 4

Simultaneous determination of  $Ca^{2+}$ ,  $Mg^{2+}$  and  $Zn^{2+}$  in different samples using CWT-ratio spectra zero crossing method by applying (I) optimal spectrum and (II) normalized spectrum of divisor and by standard method (FAAS)

Divisor type	Method	Sample	Mean $\pm$ SD			$t_{\text{calculated}}$			$t_{\text{theoretical}}$			
			$Ca^{2+}$	$Mg^{2+}$	$Zn^{2+}$	$Ca^{2+}$	$Mg^{2+}$	$Zn^{2+}$	$Ca^{2+}$	$Mg^{2+}$	$Zn^{2+}$	
I	Mexh	UHTcow's milk	75.8 $\pm$ 2.10	5.38 $\pm$ 0.00	1.30 $\pm$ 0.24	0.80	2.02	1.30	3.18 <sup>a</sup>	3.18 <sup>a</sup>	4.30 <sup>b</sup>	
		Milk Powder	28.8 $\pm$ 1.14	4.55 $\pm$ 0.01	1.14 $\pm$ 0.28	0.76	0.80	2.28				
		Multivitamin	4.28 $\pm$ 0.23	2.28 $\pm$ 0.01	0.30 $\pm$ 0.08	0.53	0.34	0.08				
		Tea	30.68 $\pm$ 2.1	6.18 $\pm$ 0.13	1.26 $\pm$ 0.16	0.33	2.70	0.58				
	Morl	UHTcow's milk	76.73 $\pm$ 1.8	5.04 $\pm$ 0.86	1.74 $\pm$ 0.19	1.85	1.94	1.50	3.18 <sup>a</sup>	2.13 <sup>c</sup>	4.30 <sup>b</sup>	
		Milk Powder	30.2 $\pm$ 1.01	4.35 $\pm$ 1.10	1.35 $\pm$ 0.68	2.38	1.30	1.59				
		Multivitamin	4.87 $\pm$ 0.94	2.61 $\pm$ 0.48	0.35 $\pm$ 0.04	1.22	1.60	1.00				
		Tea	28.2 $\pm$ 0.98	5.86 $\pm$ 0.58	1.22 $\pm$ 0.44	2.82	1.56	0.06				
	II	Mexh	UHTcow's milk	75.46 $\pm$ 0.48	5.13 $\pm$ 0.24	1.74 $\pm$ 0.20	1.22	2.70	1.41	3.18 <sup>a</sup>	3.18 <sup>a</sup>	4.30 <sup>b</sup>
			Milk Powder	28.84 $\pm$ 1.89	4.52 $\pm$ 0.58	0.78 $\pm$ 0.10	0.68	0.96	2.34			
			Multivitamin	4.20 $\pm$ 0.046	2.36 $\pm$ 0.08	0.32 $\pm$ 0.10	0.24	1.80	0.31			
			Tea	31.35 $\pm$ 1.63	5.85 $\pm$ 0.021	1.23 $\pm$ 0.02	0.28	2.50	0.34			
Morl		UHTcow's milk	75.4 $\pm$ 0.48	5.46 $\pm$ 0.44	1.8 $\pm$ 0.14	2.12	1.98	1.50	3.18 <sup>a</sup>	2.13 <sup>c</sup>	4.30 <sup>b</sup>	
		Milk powder	29.96 $\pm$ 1.06	4.15 $\pm$ 1.19	0.70 $\pm$ 0.07	1.47	1.08	1.84				
		Multivitamin	4.54 $\pm$ 0.50	2.41 $\pm$ 0.24	0.345 $\pm$ 0.5	1.13	1.14	1.00				
		Tea	29.68 $\pm$ 1.18	5.61 $\pm$ 0.53	1.12 $\pm$ 0.22	2.06	0.58	0.50				
FAAS		UHTcow's milk	74.84 $\pm$ 0.10	5.91 $\pm$ 0.10	1.95 $\pm$ 0.12							
		Milk powder	28.59 $\pm$ 0.11	4.80 $\pm$ 0.10	0.60 $\pm$ 0.10							
		Multivitamin	4.21 $\pm$ 0.10	2.31 $\pm$ 0.12	0.31 $\pm$ 0.08							
		Tea	31.0 $\pm$ 0.10	5.46 $\pm$ 0.10	1.2 $\pm$ 0.04							

<sup>a</sup> Calculated by  $n = 3$ .

<sup>b</sup> Calculated by  $n = 2$ .

<sup>c</sup> Calculated by  $n = 4$ .



real samples, it was applied to the simultaneous determination of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  cows' milk (Pegah shir), multivitamin tablet (Osvah Iran), milk powder (Humana) and tea leaves, which contain desired elements in different amounts. The results are summarized in Table 4. Flame atomic absorption spectrometry (FAAS) was used as reference method. The obtained values by proposed methods were found to be in good agreement with those obtained by FAAS. Statistical data obtained by using Student's *t*-test are given in Table 4. These values show that there are not significant errors in the simultaneous determination of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  by proposed methods.

## 5. Conclusion

In spite of, strong spectral overlapping of the absorption spectra of Ca-BPR, Mg-BPR, and Zn-BPR, simultaneous determination of these elements was successfully achieved by ratio spectra-CWT zero crossing method. The method was validated by determination of desirable elements in different complex mixtures. The proposed methods are simple, very sensitive, precise, and easy to understand and easy to use and apply. These methods do not decrease *S/N* ratio, do not need to use any separation steps, are very cheap and use original available reagents so can be use for routine analysis of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  in mixtures. Comparison of results obtained using the spectra of standard solutions as divisors with those obtained using normalized spectra of standard solution as divisor show that application of normalized spectra increases the amplitude of CWT graphs and therefore, improves the sensitivity and slope of CWT calibration graphs. High amplitude of CWT signals provides analysis of ternary mixtures with good sensitivity even by using low values of scaling factors and decreasing the time of analysis. The results were also demonstrated that, by using normalized spectrum as divisors, estimated concentration of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  in synthetic and real samples, are more close to real values and standard deviation of determinations are low.

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