First-Order Derivative Spectrophotometry Method with Triapine as Chelating Agent for Simultaneous Determination of Barium(II) and Lead(II)

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A simple, selective and sensitive first-order spectrophotometric method was used for the simultaneous determination of Ba(II) and Pb(II) with triapine as chelating agent. When triapine reagent was mixed with Ba(II) and Pb(II) solutions, it produces a light green colour in a basic medium. Maximum peaks for both Ba(II) and Pb(II) were observed at pH 8.5 (basic buffer solution) between 350 and 395 nm. Ba(II)-triapine has a molar absorbance of 5.276×10^6 L mol^{-1} cm^{-1} and a Sandell's sensitivity of 0.000189 g/cm^2. Similarly, they are 9.421×10^5 L mol^{-1} cm^{-1} and 0.00106 g/cm^2 for Pb(II)-triapine, respectively. The stability constants of Ba(II)-triapine and Pb(II)-trapine complexes are 3.207×10^4 and 5.118×10^4, respectively. The effects of Ba(II)-triapine and Pb(II)-triapine concentrations on amplitude were also studied. The detection and quantification limits for Ba(II)-triapine are 0.0882 g/mL and 0.746 g/mL, respectively. Similarly, detection and quantification values were 0.089 g/mL and 0.745 g/mL for Pb(II)-triapine, respectively. The developed or proposed method was used to examine biological samples of carrots, onions, beans, paints, milk powder and alloys. The sample results were close to the certified reference values for the ICP-AES, FDA and AAS methods.

Keywords: First-order spectrophotometric, Triapine, Ba(II)-triapine, ICP-AES.

INTRODUCTION

Since some trace metals are actually nutrients, knowing which ones are dangerous and which ones aren't is essential. Trace elements including iron, manganese, copper, chromium, zinc, and cobalt are essential for the survival of plants, animals, and humans [1-3]. However, these metals can become harmful when the homeostatic mechanisms determine their physiological limit. On the other hand, heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (Ar), nickel (Ni) and barium (Ba) are dangerous even at low concentrations [4,5]. Among them, Pb(II) and Ba(II) are the most toxic heavy metal ions in drinking water and surface soils. It can build up in the body and turn into a neurotoxin, which hurts the bones and kidneys [6]. Also, soluble Ba(II) compounds could affect the nervous system and cause heart problems, tremors, weak-ness, anxiety, shortness of breath and paralysis [7]. The sources of Ba(II) and Pb(II) contamination of drinking water are typically erosion of natural deposits, metal refinery discharges and drilling waste discharges. World Health Organization (WHO) revised its global drinking water guideline for Ba(II) from 0.7 to 2 mg/L and Pb(II) to 0 mg/L [8].

Many analytical methods in the literature can be used to test for trace amounts of heavy metals in mixed solutions [9-14]. Among the methods, the atomic absorption spectrophotometer (AAS) is a well-known method because it has great qualities such as sensitivity, reliability, flexibility, accuracy and precision. But the deployment of AAS is limited by the need for a high level of technical expertise, large costs and ongoing mainten-ance costs. Therefore, some other methods, namely voltammetry [15], inductively coupled optical emission spectroscopy (ICP-OES) [16,17] and inductively coupled plasma mass spectroscopy (ICP-MS) [18,19], are used in the process of determining many metals simultaneously. These methods require a high level of expertise as well as access to costly equipment and chemicals that are sometimes unattainable in countries with weak econ-omic infrastructures. The UV-Vis spectroscopy [20,21], a simple, inexpensive and environmentally friendly method of determ-ining heavy metal content in a sample is widely used in many laboratories.

Triapine, a heterocyclic carboxaldehyde thiosemicarbazone which fights cancer. Triapine blocks ribonucleotide reductase, an enzyme needed to make DNA. Triapine has been studied for its biological effects and analytical uses [22,23]. In addition, it has been used in fluorometry, atomic absorption spectro-photometry and as an indicator [24,25]. Triapine is known to chelate efficiently with alkaline earth metals, transition metals and their analogs, which are effectively coordinated with endogenous metals such as Ba, Pb, Cu and Fe [25,26]. Triapine was used as a chelating as well as chromogenic agent to estimate magnesium and titanium at the same time in alloyed steel, minerals, medical samples and food samples using a UV-Vis spectrophotometer [27].

In this work, a first-order spectrophotometric derivative technique is applied for the simultaneous detection of Ba(II) and Pb(II) using triapine as chelating ligand. The main benefit of the proposed method is that it can be used in real biological samples without having to treat them first and more sensitive and selective than existing methods. The second-order models in ribonucleotide reductase inhibitor medium were also considered and discussed from both equilibrium and kinetic stand points to evaluate the determination of Ba(II) and Pb(II).

EXPERIMENTAL

Standard solutions of Ba(II) and Pb(II) were prepared using analytical grade chemicals $BaCl_2 \cdot 2H_2O$ and $Pb(NO_3)_2$, respectively. To prepare a stock solution (1000 mg/L) of Ba(II), 0.610 g of $BaCl_2 \cdot 2H_2O$ was dissolved in a 25 mL Erlenmeyer flask with double-distilled water. A Pb(II) stock solution (1000 mg/L) was also prepared by dissolving 0.828 g of $Pb(NO_3)_2$ in 10 mL of concentrated HNO_3 and diluting the solution with deionized water to 1000 mL. Basic buffer solutions are made from NaOH, NH_4OH and NH_4Cl. Acidic buffer solutions are made from HCl, acetic acid and sodium acetate. The triapine (chelating agent/chromogenic) ligand was acquired from Hi-Media Laboratory and used exactly as received. An exact amount of triapine was dissolved in methanol to make a 0.001 M triapine reagent solution. The melting point of triapine is between 230-234 °C.

Preparation of standard derivative spectrum: A standard derivative spectrum experiment was performed according to the reported procedure [27]. Briefly, 1.5 mL of Ba(II) and Pb(II) from stock solution, 4 mL of 0.001 M triapine and 4 mL of buffer solution (pH 8.5) were placed in a 25 mL Erlenmeyer flask and filled up to the mark with double-distilled water. The wavelength of these solutions was monitored in the range of 350-420 nm compared to a reagent blank using a Shimadzu-2600 (Japan), spectrophotometer. A digital pH meter (Systronics) equipped with glass electrode was used for pH measurement. The complexation formed after the addition of triapine with metal(II) ions is represented in **Scheme-I**.

Biological, food and alloy sample preparation

Biological or food sample: In 250 mL Erlenmeyer flask, 20 g of food or a biological sample was added and 25 mL of concentrated HNO_3 was added and rapidly stirred in a heating condition until a fine emulsion formed. Heating was maintained by gradually adding 6% H_2O_2 (20 mL) to this mixture. An aqueous phase was later transferred to a beaker. The extraction was performed twice, each time adding 25 mL of conc. HNO_3 and 20 mL of 6% H_2O_2 and then the extracted mixture was evaporated to absolute dryness. After dilution with dil. HCl, the residue was transferred in proportions to a 50 mL Erlenmeyer flask. The ingredients are diluted with double-distilled water if necessary.

Alloy: A steel sample (alloy) of 0.1 to 0.5 g (0.6×10^{-5} M) was dissolved in 3 mL of conc. HCl and 15 mL of conc. HNO_3. The resulting solution was evaporated and reduced to a half of its volume followed by the addition of 5 mL of 1:1 solution (H_2O and H_2SO_4) and left at room temperature for further evaporation and drying. The remaining residue was then mixed with 15 mL of distilled water and filtered through Whatman No. 41 filter paper. The filtrate is put into an Erlenmeyer flask with 100 mL of water and diluted to the right volume. If necessary, the solution was further diluted.

RESULTS AND DISCUSSION

Derivative spectra of Ba(II) and Pb(II)-triapine complexes: Due to the similarity of zero-order species in these spectral profiles, the zero-order spectrum of a mixture containing both Ba(II) and Pb(II) in the presence of triapine yields only one peak at 384 nm; simultaneous determination is not possible because no resolution occurs (Fig. 1a). An advanced analytical advantages offered by derivative spectrophotometry. A mixture containing Ba(II) and Pb(II) was analyzed without prior separation. To determine the derivative order, the first-order derivative spectra of Ba(II) and Pb(II) were examined (Fig. 1b). Based on spectra, the first-order derivative spectra (350-420 nm) had the best resolution, sensitivity and lowest detection limit. The peaks of both metal chelate systems resolved clearly to form
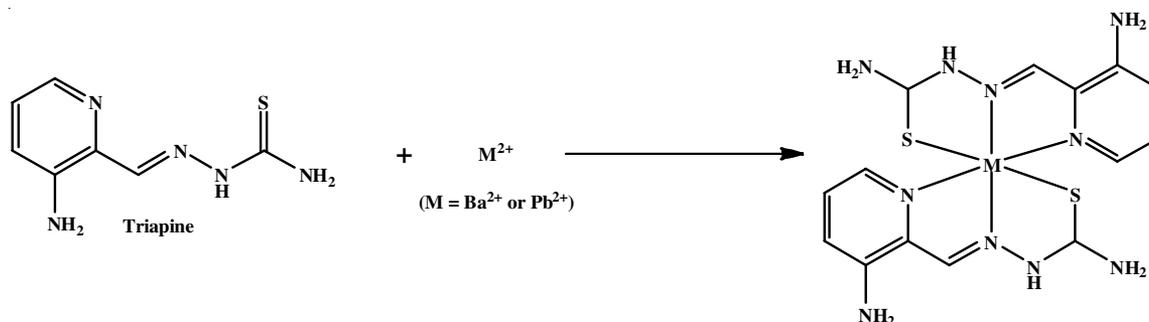
Scheme-I: Reaction between metal ions and chelating agents to form complexes
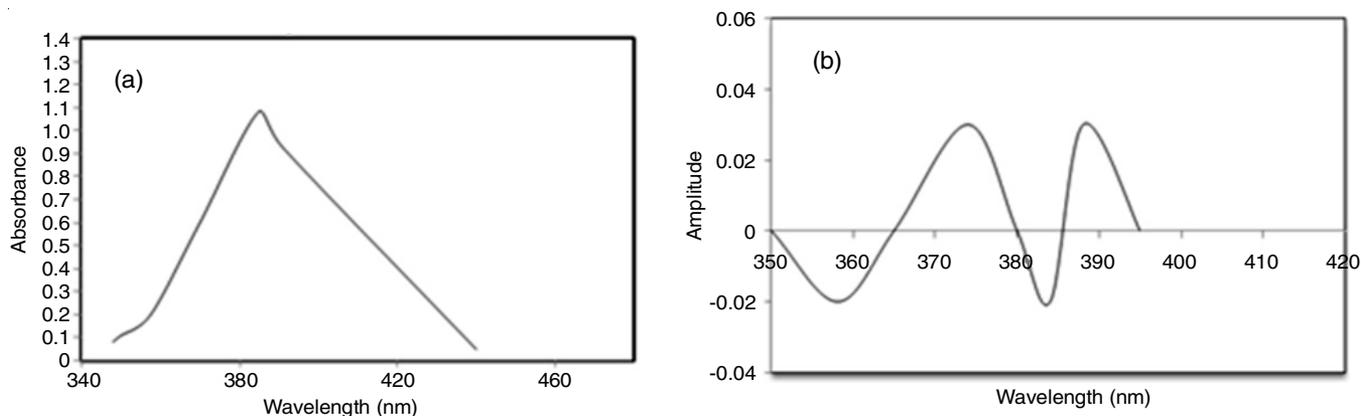
Fig. 1. (a) Zero-order spectrum of Ba(II) + Pb(II) in the presence of triapine is as follows: [Ba(II)] = [Pb(II)] = 1 × 10⁻⁵ M; triapine = 0.16 × 10⁻⁴ M; pH = 8.5; (b) Ba(II) + Pb(II) in the presence of triapine [Ba(II)] = [Pb(II)]=1 × 10⁻⁵ M; pH=8.5; triapine = 0.16 × 10⁻⁴ M

two distinct peaks. Furthermore, the first-order spectra was recorded where Ba(II) concentrations was constant while Pb(II) concentrations vary (Fig. 2a). In alternative case, the concentration of Pb(II) remains constant, while the concentration of Ba(II) changes (Fig. 2b). To improve the sensitivity, the plots of Ba(II) concentration *versus* peak amplitude and Pb(II) concentration *versus* peak amplitude were used. In both cases, the linear plots are obtained (Fig. 3a-b). Therefore, the first-order derivative spectrometry was used to determine Ba(II) in the presence of Pb(II) and *vice-versa*.

Effect of pH: Triapine forms a light green complex when it reacts with B(II) and Pb(II). The effect of reagent amounts on Ba(II) and Pb(II) complexes was investigated by measuring absorbance values at 380 nm and 390 nm for solutions containing a specific amount of Ba(II) and Pb(II), respectively. As shown in Fig. 4, the coloured solution showed maximum absorbance in the pH range 7-10 at 380 nm and 390 nm for Ba(II) and Pb(II), respectively. Furthermore, pH 8.5 has the highest absorbance for both Ba(II) and Pb(II) and the results are reproducible at this pH. So, a solution with a pH of 8.5 was chosen for more
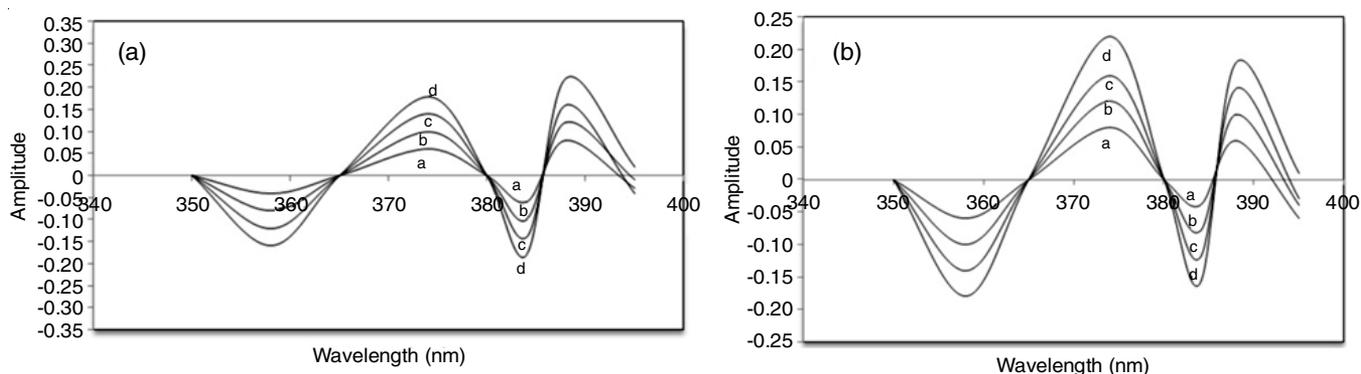
Fig. 2. (a) First order derivative spectrum of Ba(II)+Pb(II) in the presence of triapine, where Ba(II) is kept constant by varying concentration of Pb(II) concantrations, [Ba(II)] = [Pb(II)] = 1 × 10⁻⁵ M; pH = 8.5; triapine = 0.16 × 10⁻⁴ M; Ba(II) = a-d: 0.5 2.0 mL, Pb(II) = a-d: 1.0-2.5 mL; (b) Pb²⁺ is kept constant by varying concentration of Ba²⁺. [Ba(II)] = [Pb(II)] = 1 × 10⁻⁵ M; pH = 8.5; triapine = 0.16 × 10⁻⁴ M; Pb(II) = a-d: 0.5-2.0 mL, Ba(II) = a-d: 1.0-2.5 mL
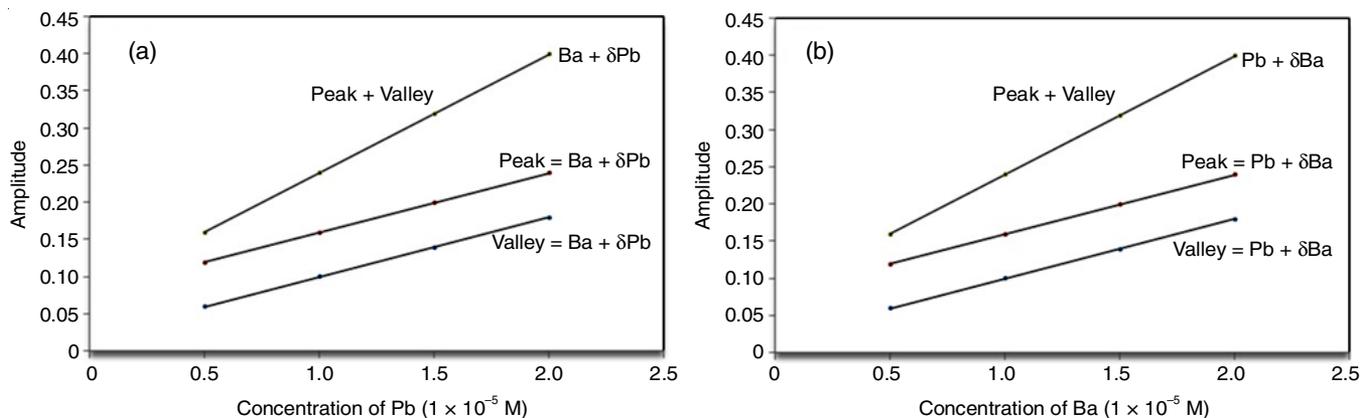
Fig. 3. (a) First order derivative amplitude *vs.* concentration of Pb(II), pH = 8.5; (b) Ba(II), pH = 8.5
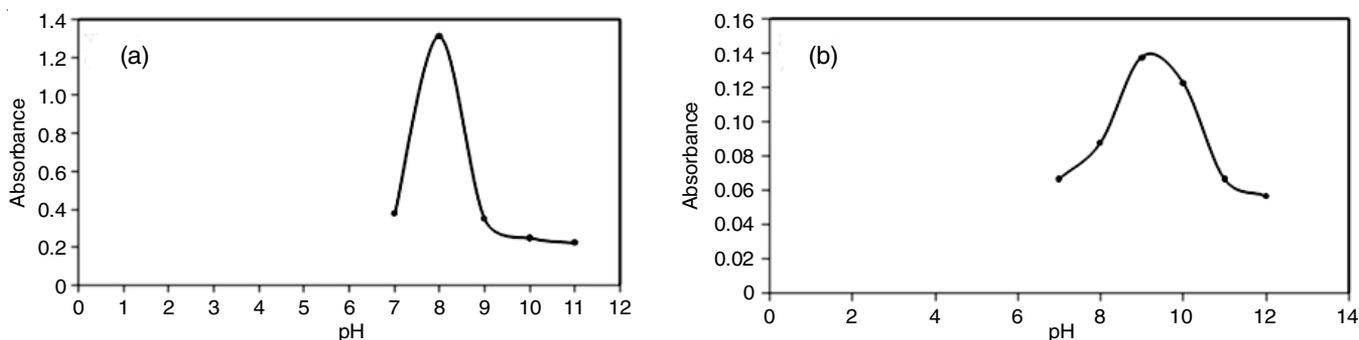
Fig. 4. Effect of pH on the absorbance of (a) Ba(II)-triapine, Ba(II) = 1.2×10^{-5} M, triapine = 1.2×10^{-4} M; (b) Pb(II)-triapine Pb(II) = 1.2×10^{-5} M, triapine = 1.2×10^{-4} M

in-depth research and testing of both metal ions at the same time. In addition, Ba(II) and Pb(II) do not form stable complexes in acidic media up to pH 6. This might be due to the hydrolysis of either the reagent or the complex itself. Strongly alkaline media (> pH 10) get cloudy slowly. This may be due to the generation of the stable hydroxides. A 15-fold excess of triapine reagent was found to be necessary for the maximum colour development with Ba(II) and Pb(II) ions. However, excess triapine showed no significant change in absorbance.

Linearity and limit of quantification: At concentrations ranging from 0.162 to 1.961 g/mL for Ba(II) and 0.037 to 0.215 g/mL for Pb(II), the effect of metal ion concentration on absorbance was investigated. The reagent concentration was kept constant at 1.2×10^{-4} M. Fig. 5 shows the absorbance values at 380 nm for Ba(II) and 390 nm for Pb(II) against a blank solution devoid of metal ions. Therefore, this method can be used to estimate the amount of barium in the range of 0.162 to 1.961 g/mL and the amount of lead in the range of 0.037 to 0.215 g/mL. The analytical parameters of Ba(II)-triapine and Pb(II)-triapine complexes were 5.276×10^6 L mol^{-1} cm^{-1}, 9.421 $\times 10^5$ L mol^{-1} cm^{-1} and 0.000189 g/cm^2 and 0.00106 g/cm^2, respectively.

The limit of blank (LOB) is the highest concentration of analyte that can be seen in replicates of a blank sample that has no analyte.

$$\text{LOB} = \text{Mean (blank)} + \text{Standard deviation (blank)} \quad (1)$$

The limit of detection (LOD) is the lowest concentration that is likely to be reliably distinguished from the LOB and at

which detection is possible. LOD is found by using both LOD and multiple tests of a low-concentration sample.

$$\text{LOD} = \text{LOB} + \text{Standard deviation (low concentration)} \quad (2)$$

The limit of quantitation (LOQ) is the lowest concentration at which the analyte can't be reliably found, but at which some predefined targets for bias and imprecision are met. The LOQ may eventually equal the LOD or be at a higher concentration.

$$\text{LOQ} = 10 \times \frac{\text{SD}}{\text{Slope of caliration curve}} \quad (3)$$

The limits of detection (LOD) and limit of quantification (LOQ) of Ba(II) and Pb(II) were 0.0882 µg/mL, 0.089 µg/mL and 0.746 µg/mL, 0.745 µg/mL, respectively. Since the value of R^2 is close to 1, it means that both the concentrations of reagents and metal ions are dependent variables. The colour reaction of Ba(II)-triapine and Pb(II)-triapine systems is instantaneous at room temperature. Since, the complex is stable for more than 2 h, it can be used in analytical applications for the real samples.

Composition and stability of complex: The stoichiometry and composition of Ba(II)-triapine and Pb(II)-triapine systems were studied using Job's method of continuous variation and molar ratio methods [28]. A 1:1 complex formation was investigated by both methods between Ba(II) and triapine, as well as between Pb(II) and triapine. The limiting logarithmic method [29] was used to figure out the formation/stability complex of Ba(II)-triapine and Pb(II)-triapine. The stability constants of Ba(II)-triapine and Pb(II)-triapine were found to be 3.207×10^4 and 5.118×10^4, respectively.
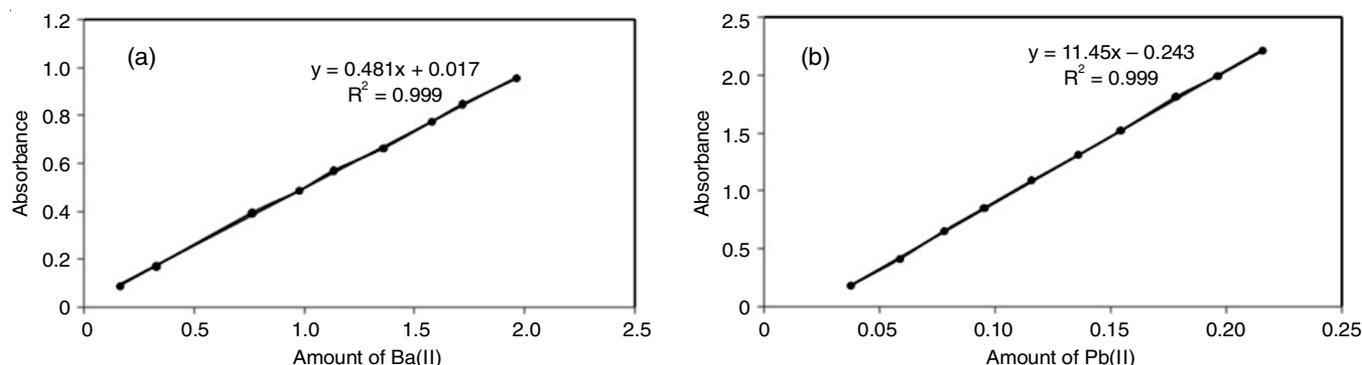
Fig. 5. Effect of concentration on absorbance, (a) Ba(II), λ_{max} = 380 nm, triapine = 1.2×10^{-4} M, pH = 8.0; (b) Pb(II), λ_{max} = 390 nm, triapine = 1.2×10^{-4} M, pH = 9.0

Effect of foreign ions: The influence of various foreign ions that interfere with the determination of Ba(II) and Pb(II) was investigated under the optimal conditions. The tolerance limit is thought to be the amount of foreign ions that cause a change in extinction of ±2%. The degree of interference with absorption by various ions such as sulfate, phosphate, fluoride, EDTA, oxalate, bromide, Ni, U, Zn, Mc, Cu and others was investigated by combining a constant amount of Ba(II) and Pb(II) with varying amounts of various ions. However, most ions did not interfere . Table-1 shows the calculated tolerance limits for different ions and cations. A similar nature was observed for the spectrophotometric determination of magnesium with triapine [30]. The analytical results of the derived method for Ba(II) and Pb(II) are summarized and listed in Table-2.

The current method is used to analyze both Ba(II) and Pb(II) in alloy and food samples at the same time. The method is validated for repeatability and precision, with an RSD range

TABLE-2
ANALYTICAL RESULTS OF Ba(II) AND
Pb(II) BY DERIVATIVE METHOD

Analytical constants	Ba(II)	Pb(II)
Sandell's sensitivity	0.000189	0.00106
Molar absorptivity	5.276×10^6	9.421×10^5
Stability constant	3.207×10^4	5.118×10^4
Range of detection	0.162-1.961	0.037-0.215
Linear regression coefficient	0.9995	0.9998
Composition of the complex	1:1	1:1
LOD	0.0882	0.0891
LOQ	0.746	0.745
RSD%	0.106	0.112

of 0.106% (Ba) to 0.112% (Pb) for 5 or 6 determinations. So, the developed method can be used to measure the two metal ions right after digestion, without the need for pretreatment or separation. The accuracy of the proposed method has been verified using certified reference materials from alloy and food samples (ASTM, ICP-AES, AES and FDA methods) [10-15, 17-19]. Tables 3 and 4 show the values calculated according to the proposed procedure and the determined values (n = 5 or n = 6) of the certified reference material were within the specified values.

Conclusion

First-order derivative spectrophotometric method was developed in simultaneous analysis of Ba(II) and Pb(II) using triapine as a chelating agent. It has been demonstrated that the proposed method can accurately determine Ba(II) and Pb(II) levels in the real samples such as alloys, biological samples and food samples. With this approach, no prior separation is required during analysis. The molar absorptivity and Sandell's sensitivity of Ba(II)-triapine and Pb(II)-triapine complexes, were 5.276 and 9.421×10^6 L mol^{-1} cm^{-1} and 0.000189 cm^2 and 0.00106 g/cm^2. Linearity was observed at concentrations ranging from 0.162 to 1.961 g/mL for Ba(II) and from 0.037 to 0.215 g/mL for Pb(II). The proposed method is better than

TABLE-1
TOLERANCE LIMIT OF FOREIGN IONS FOR THE
ESTIMATION OF Ba(II) (1.589 µg/mL) AND Pb(II) (0.184 µg/mL)

Foreign ion	Tolerance limit (µg/mL)	Foreign ion	Tolerance (µg/mL)
Thiourea	789	Cu(II)	103[a]
Tartrate	187	Mn(II)	254
Sulphate	75	Zn(II)	26
Phosphate	562	Ni(II)	156
Fluoride	121	Sr(II)	397
Chloride	431	Th(IV)	Interferes
Iodide	924	Ca(II)	172
Nitrate	273	Mg(II)	134
Oxalate	Interferes	K	867
EDTA	Interferes	Pb(II)	642
Bromide	322	Sn(II)	97
Citrate	621	Zr(IV)	89
Thiosulphate	109	Ti(IV)	534
Acetate	212	U(VI)	356
		Co(II)	146

pH = 8.5; λ_{max} = 384 nm; [a]Masked in presence of thiourea

TABLE-3
ANALYSIS OF Ba AND Pb ALLOY SAMPLES (µg) IN PRESENCE OF EACH OTHER

Sample	Amount found by present method		ASTM/AAS method		% Error (+/–)		RSD (%)
	Ba(II)	Pb(II)	Ba(II)	Pb(II)	Ba(II)	Pb(II)	
Human hair (when consumption of polluted water)	4.58	1.85	4.6	1.9	0.430	2.63	0.108
Pb-Ba alloys (UNS L50500–L50599)	0.48	988	0.5	990	4.000	0.20	0.111
Alloy of Ba, Pb and strontium	477	29	480	30	0.625	3.33	0.112
Gunshot residue	0.66	9.8	0.7	10	5.710	2.00	0.116

*Taken an average of five determinations.

TABLE-4
ANALYSIS OF BIOLOGICAL SAMPLES FOR BARIUM AND LEAD (µg) IN PRESENCE OF EACH OTHER

Sample	Amount found by present method		ASTM/AAS method		% Error (+/–)		RSD (%)
	Ba(II)	Pb(II)	Ba(II)	Pb(II)	Ba(II)	Pb(II)	
Barium oil paints (BaCO₃)	2.26	0.09	2.24	0.091	0.89	1.11	0.113
Heat reflecting paints (BaSO₄)	22.75	0.086	22.8	0.09	0.21	4.44	0.109
Cereal grains	22.1	0.19	22.0	0.20	0.45	5.00	0.114
River sediment	42.1	1.16	42.3	1.15	0.47	0.86	0.107
Light sandy soil	502	37.82	500	37.8	0.40	0.05	0.109

*Taken an average of six determinations.

traditional spectrophotometer in terms of precision, accuracy and repeatability for two different components. It also allows 36 estimates per hour to be made for digested biological and alloy samples.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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