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# DETERMINATION OF IRON(II) IN POLYVITAMIN COMPOUNDS BY USING 6-[2'-(5-BROMOTHIAZOLYLAZO)]-3,5- DIHYDROXY-1,2-BENZENEDISOLFONIC ACID AS COMPLEXANT (BR-TDB)

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## ABSTRACT

**Objective**: The goal of this work was to determine iron in pharmaceutical formulations and the formed species during complexation with 6-[2'-(5-bromothiazolylazo)]-1,2-dihydroxy-3,5-benzenedisulfonic reagent (Br-TDB). **Material and Methods**: pharmaceutical preparations were purchased from drugstore, subjected to humid digestion and analysis by ultraviolet and ICP-OES. **Results**: The log β values for the formed complex species $ML_2H_2^{4-}$, $ML_2H^{5-}$ and $ML_2^{6-}$ (M = Fe(II) and L = Br-TDB$^{4-}$) were 29.26 ± 0.03, 25.11 ± 0.04 and 16.7 ± 0.11. The stoichiometry of the complex is 1:2. The complex absorbs at 528 nm. The limits of quantification and detection were equal to 0.008 and 0.027 mg $L^{-1}$, respectively. The analytical curve presented an unusual pattern with negative angular coefficient. The pattern is intrinsic to the complexation system with Br-TDB, once the same behavior was observed in determination of manganese, lead, cadmium and iron ions by UV-Vis. The methodology was validated by ICP OES. **Conclusion**: The concentration of iron in analyzed drugs samples was coincidente with manufactorer's. Moreover, both UV-Vis and ICP OES yielded similar results at a confidence level $\rho<0.05$.

**KEYWORDS:** Complexometry, Br-TDB, UV-VIS, thizolylazo.

## 1. INTRODUCTION

Iron (Fe) is an essential micronutrient for almost all organisms (Morel and Price, 2003). It is involved in metabolic processes, such as chlorophyll synthesis, nitrate reduction, detoxification of reactive oxygen species and electron transport in photosynthesis and respiration (Sunda and Huntsman, 1995). Fe is the fourth most abundant element in the Earth's crust, after oxygen, silicon and aluminum (Wedepohl 1995), with an abundance of 5~6% (Taylor, 1964; Laglera et al., 2013).

On the other hand, excess amounts of iron can result in toxicity and even death (Corbett, 1995). Toxicology considerations are important in terms of iron deficiency (anemia) and accidental acute exposure and chronic iron overload due to idiopathic hemochromatosis or as a consequence of excess dietary iron or frequent blood transfusions. The immediate cause of death from the inorganic compounds of Fe in animals is respiratory failure (Ahmed and Roy, 2009).

Many analytical methods had been developed to determine Fe species concentrations such as spectrophotometry,[8,9] atomic absorption spectrometry (AAS),[10,11] inductively coupled plasma-mass spectrometry (ICP-MS)[12,13] chemiluminescence[14-16] and fluorescence,[17] among others. Several complexing agents have been used to accumulate Fe complexes onto electrode surfaces, such as 1-(2-piridylazo)-2-naphthol (PAN),[18] 1-nitroso-2-naphthol (NN)[19] and 2, 3-dihydroxynaphthalene (DHN).[20] However, the chelating reaction times reported for these agents are relatively long, with many service restrictions. UltraViolet-Visible Spectroscopy (UV-Vis) is another efficient tool in determining elements from an array of samples since it presents high sensitivity and precision, besides being simple, cheap, versatile and easily available.[21-23]

In the present work, the validation of iron (II) determination by UV-Vis using 6-[2'-(5-bromothyazolylazo)]-3,5- dihydroxy-1,2-benzenedisulfonic acid (Br-TDB) as complexant in commercial multivitamin compounds was performed.

## 2. MATERIALS AND METHODS

### 2.1 Apparatus

Spectrophotometric measurements were made in a Varian Cary 50 UV-visible spectrophotometer with 1.00 cm glass cells. The pH measurements were carried out with an ANALYSER 300 pH meter.

### 2.2 Reagents and Solutions

All reagents used in this work had analytical purity and degree. The solutions were prepared with ultrapure water

by deionization in a Permution purifier, followed by reverse osmosis purification using a Millipore Simplicity 18S - 18 mΩ $cm^{-1}$.

### 2.3 Synthesis of Br-TDB

The 6-[2'-(5-bromothyazolylazo)]-3,5-dihydroxy-1,2-benzenedisulfonic acid (Br-TDB) was synthetized via diazotization of 2-amino-5-bromothiazole (Sigma-Aldrich) at 0-5ºC. The diazonium salt formed was coupled with 1,2-hydroxybenzene-3,5-disulfonic acid – Tiron (Sigma-Aldrich) and the precipitate was recrystallized in ethanol (VETEC).[21]

### 2.4 Determination of formation constants of Fe(II) - Br-TDB complex

The Br-TDB solution was prepared using water at 1.00 × $10^{-3}$mol $L^{-1}$. Aliquots of this solution were diluted in ultrapure water to a final volume of 15.00 mL, to obtain Br-TDB at 3.80 × $10^{-5}$ mol $L^{-1}$. The iron solution was prepared daily in water using a standard solution (1000 mg $L^{-1}$) for AAS (FLUKA).

An aliquot of this solution was added to the system using a concentration of 1.20 × $10^{-5}$ mol $L^{-1}$ to a final volume of 15.00 mL. The ionic force was maintained at 0.01 mol $L^{-1}$ in NaCl at 25 ºC. A solution of 0.9996 mol $L^{-1}$ NaOH was used for titring. The pH was measured using a potentiometer Digimed DM-20 equipped with a combined Ag/AgCl/glass electrode. The electrode was calibrated with buffer solutions (Merck) at pH 4.00 and 7.00. Spectrophotometric and pH measurements were carried out after addition of each titring aliquot, using spectrophotometer Varian model Cary 50. The data within 320 to 680 nm of wavelength and pH 1.85 to 10.45 were analyzed with the software SQUAD.[24]

#### 2.4.1 Method of continuous variation

The method of continuous variation of Job's method was used to determine the stoichiometry of Fe(II)-Br-TDB complex.[25] For that, we used aliquots of stock solution of Br-TDB and standard solutions of Fe(II) at 5,0 × $10^{-5}$ mol $L^{-1}$to a final volume of 1000 µL with variation in the volume ratio, inasmuch as the molar fraction of species varied from 0.1 to 0.9. We added 3.0 mL of acetate buffer (pH = 4.5) and completed with ultrapure water to a final volume of 10.0 mL. Afterwards, the solutions were used in spectrophotometric analysis at 528 nm.

### 2.5 Preparation of pharmaceutical samples

Different multivitamin supplements (pills, droplets, suspension and bran) were obtained in pet health stores in Jequié, state of Bahia, northeastern Brazil. The mineralization of samples was accomplished by humid decomposition in open systems. In each samples, the mass or the volume equivalent to 0.1 mg of analyte was mixed to 4.00 mL of nitric acid at 65% (VETEC), 2.00 mL of hydrogen peroxide P.A. (Êxodo) and 3.0 mL of deionized water. The mixture was placed in a 25.00 mL beaker and digested in a heating plate (Tecnal) for 5 h at 120 ºC.[26] The material was then transferred into a 10.00 mL volumetric flask and stored at 4 ºC. In the moment of analysis, the pH was adjusted with sodium hydroxide.

### 2.6 Determination of Fe(II)

Aliquots of 1.0 mL of digested material were used in analyses. Subsequently, 500 µL of Br-TDB (1.00 × $10^{-3}$ mol $L^{-1}$), 3.00 mL of acetate buffer pH 4.5 and water to a final volume of 10.00 mL were added. All analyses were performed in triplicates.

### 2.7 Analysis of interferents

After determining the best conditions for complexation between Fe(II) and Br-TDB, putative interferentes were studied by changing the concentration in order to verify whether the ion concentration could influence the determination of the selected analyte or not. Therefore, concentrations of metallic ions ranging from 0.1 to 5.0 mg $L^{-1}$ were tested in each system. Afterwards, the analysis by UV-Vis at 528 nm was carried out.

### 2.8 Experimental Design

Linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), specificity and robustness of the method were determinated according to RE # 166/2017 of ANVISA (Agência Nacional de Vigilância Sanitária).[27]

## 3. RESULTS AND DISCUSSION

### 3.1 Spectrophotometric determination

The complex was analyzed by UV-Vis in a Varian Cary 50 spectrophotometer using quartz cuvettes with optic path of 1 cm within the wavelength of 300 to 800 nm. The free Br-TDB has a maximum absorption at 432 nm, while the highest absorbance in the complex form with Fe occurs at 528 nm, as shown in Figure 1. Moreover, there is a loss of molar absorptivity of free species when compared to that observed in the presence of metal. Such loss is intrinsic to the reaction between the cromophore Br-TDB reagent and the metallic ion. This loss is intrinsic to the reaction between the Br-TDB chromophore reagent and the metal ion.[21,28]

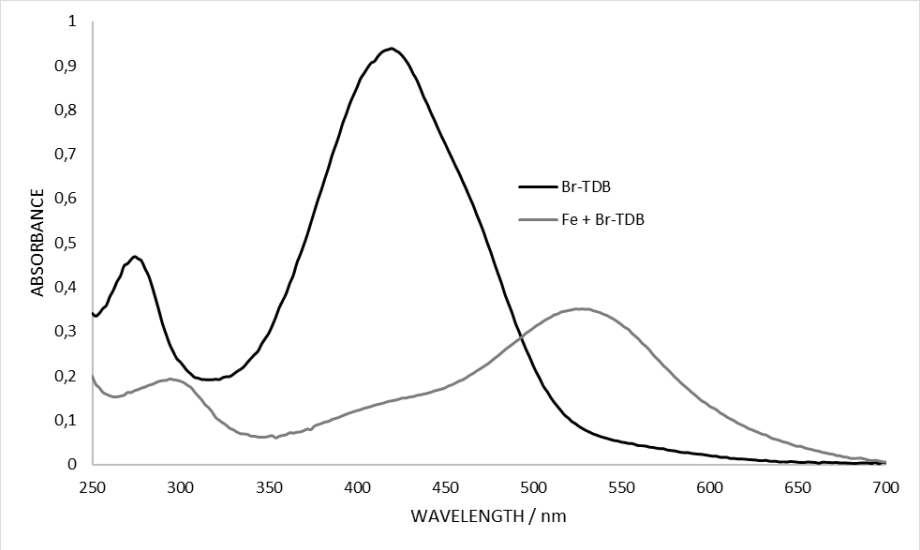


**Figure 1: Spectrum of absorption for Br-TDB and Br-TDB-Fe(II).**

### 3.2 Determination of the constants of formation of complex Fe (II) - Br-TDB

The formed species proposed during the titring of Br-TDB in the presence of Fe(II) and designed by $M^{2+}$, is presented in Table 1, which also includes the logarithm values of formation constants of complex species, represented as:

$p[H^+] + q[M^{2+}] + r[\text{Br-TDB}^{4-}] \leftrightarrow [(H^+)_p(M^{2+})_q(\text{Br-TDB}^{4-})_r]^{p+2q+r}$ (Eq. 1)

**Table 1: Logarithm of formation constants in Br-TDB species and complex Br-TDB and Fe(II) species.**

| Species | *p, q, r* | Formulea* | Log β |
|---|---|---|---|
| 1 | 2, 0, 1 | $H_2L^{2-}$ | 13.05 ± 0.03 |
| 2 | 1, 0, 1 | $HL^{3-}$ | 8.57 ± 0.01 |
| 3 | 2, 1, 2 | $ML_2H_2^{4-}$ | 35.63 ± 0.05 |
| 4 | 1, 1, 2 | $ML_2H^{5-}$ | 28.43 ± 0.03 |
| 5 | 0, 1, 2 | $ML_2^{6-}$ | 19.65 ± 0.02 |
| *L= Br-TDB$^{4-}$, M = $Fe^{2+}$ | | | |

### 3.2.1 Determination of complex stoichiometry

Taking into account an equilibrium in which several complexes can be formed, it is important to establish the molar relationship between metal/ligand. Different processes can be used to determine this proportion, such as the method of continuous variation of Job's (Figure 2) and modified by Vosburgh and Cooper.[29] Table 2 lists the values related to ligand and metal volumes to a final volume of 10.00 mL, the values of $X_{Ligand}$.

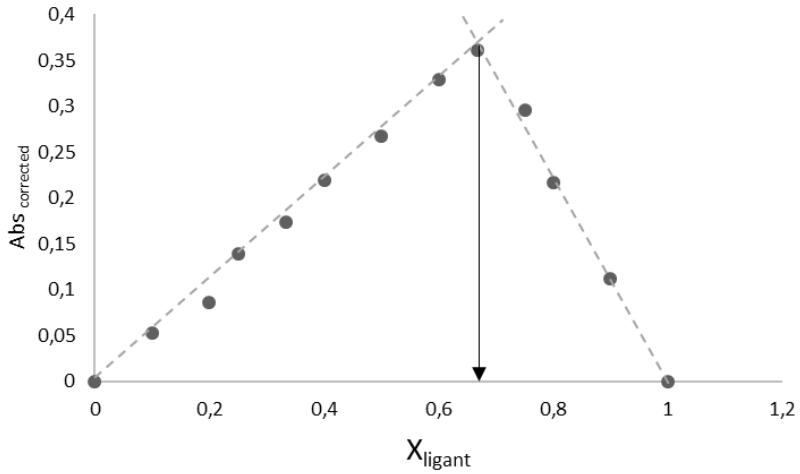


**Figure 2: Determination of stoichiometry of the complex between Br-TDB and iron (II).**

**Table 2: Solutions for the Job's method and their values of maximum absorption to obtain the graphs $A_L$ (free Br-TDB), $A_{ML}$ (complex $[FeL_2]^{2-}$), $Abs_{(corrected)}$ versus $X_{Ligand}$ (molar fraction of Br-TDB).**

| Sample | Vol. Br-TDB | Vol. Metal | $X_{Ligand}$ | $A_L$ | $A_{ML}$ | $Abs_{corrected}$ |
|---|---|---|---|---|---|---|
| Free ligand | 1000 µL | 0 | 1 | 1 | 0.9809 | 0 |
| L:M (1:9) | 100 µL | 900 µL | 0.100 | 0.1 | 0.9574 | 0.9041 |
| L:M (1:4) | 200 µL | 800 µL | 0.200 | 0.2 | 0.7512 | 0.665 |
| L:M (1:3) | 250 µL | 750 µL | 0.250 | 0.25 | 0.6391 | 0.5004 |
| L:M (1:2) | 333 µL | 667 µL | 0.333 | 0.333 | 0.605 | 0.4314 |
| L:M (1:1,5) | 400 µL | 600 µL | 0.400 | 0.4 | 0.6208 | 0.401 |
| L:M (1:1) | 500 µL | 500 µL | 0.500 | 0.5 | 0.6445 | 0.3774 |
| L:M (1,5:1) | 600 µL | 400 µL | 0.600 | 0.6 | 0.6562 | 0.3276 |
| L:M (2:1) | 667 µL | 333 µL | 0.667 | 0.667 | 0.5777 | 0.2163 |
| L:M (3:1) | 750 µL | 250 µL | 0.750 | 0.75 | 0.453 | 0.1574 |
| L:M (4:1) | 800 µL | 200 µL | 0.800 | 0.8 | 0.3791 | 0.1628 |
| L:M (9:1) | 900 µL | 100 µL | 0.900 | 0.9 | 0.1893 | 0.0769 |
| Free metal | 0 | 1000 µL | 0 | 0 | 0 | -0.0146 |

The standard deviation and the square sum in absorbance were equal to $8.30 \times 10^{-3}$ and $5.44 \times 10^{-2}$, respectively, for the complex Br-TDB and Fe(II) model in equation 1 and values presented in Table 2. These values can be considered acceptable for a confidence level at 95%. The thiazole compounds form colored complexes with several metals, resulting in stable chelates, particularly when coupled with transition metals. The metals form complexes with ligands in a proportion of 1:1 or a mixture of complexes at 1:1 and 1:2 ratios when in acid or slightly acid solutions. On the other hand, the equilibrium is often deviated to the complex in 1:2 ratio in alkaline solutions. The slow complexing reaction with some transition elements is caused by the slow substitution of aquo-complexes in these metals. The less electropositive elements tend to form more stable complexes because of covalent bonds.[30,31] Some reports indicate that Fe(II) complexes with organic molecules to form compounds with co-ordination number 4 or 6[32] of square-planar and octahedral geometries, respectively. Since the formation constants of complex species (Table 1) were high, it suggests that Br-TDB is linked to Fe(II) ions as a trident chelateas proposed in Figure 3.

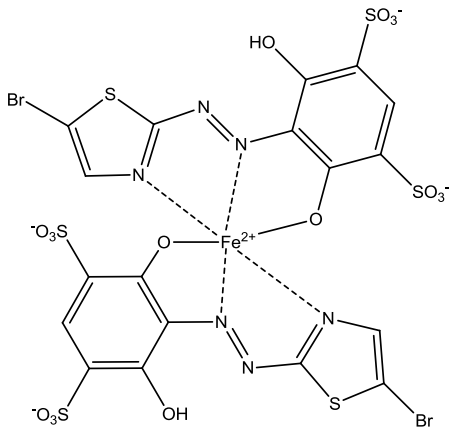


**Figure 3: Proposed structure for the complex $(Br\text{-}TDB)_2\text{-}Fe^{2+}$.**

### 3.3 Linearity and calibration curve

The linearity corresponds to the capacity of method in generating results directly proportional to the concentration of species within a certain variation.[33] A coefficient of correlation higher than 0.999 is regarded as evidence of perfect adjustment of data in linear regression.[34] ANVISA[27] recommends a correlation coefficient of 0.99 and INMETRO[35] values above 0.90 based on calibration curves of, at least, five different concentrations.[36]

The standard curve for the select method was built from six concentrations ranging from 0.1 to 2 mg $L^{-1}$. The equation obtained by linear regression based on minimum square method ($y = -0.0074x + 0.4870$, $R^2 = 0{,}9987$) indicates there is a correlation between areas and concentration of metallic ions, i.e., the data are properly adjusted to the linear regression.[37]

### 3.4 Limits of detection and quantification

The limit of detection (LOD) represents the lowest concentration of analyte that can be reliably detected in an experiment.[33] The limit of quantification (LOQ) represents the lowest concentration of analyte that can be reliably quantified using an specific level of precision.[27] LOQ and LOD are estimated as three and ten times, respectively, the standard deviation of analytical signal in a negative control samples (SBr) divided by the angular coefficient (b) of calibration curve.

To determine both LOQ and LOD of Fe(II) in the present analyses, 10 negative controls were evaluated, resulting in LOD of 0.008 mg $L^{-1}$ and LOQ of 0.027 mg $L^{-1}$, with a relative standard deviation (RSD) of 0-1.2%.

### 3.5 Selectivity

The effect of interferent metallic ions like Na(I), K(II), Mn(II), Co(II), Ni(II), Al(II), Cd(II), Cr(VI), Cr(III), P(II), Zn(II), Cu(II), S(II), Ca(II), Pb(II), V(IV) and V(V) was also evaluated (Figure 4). The other excipients that compose the studied drugs were not tested once these substances are degraded during the mineralization of samples and do not interfere in analyte determination.

Synthetical solutions containing 1 ppm of selected analyte and distinct quantities of other substances were analyzed in triplicates with a variation of concentration in potential interferents ranging from 0.1 to 5.0 mg $L^{-1}$. The tolerance limit was established as the concentration of foreigner ion that results in errors below 5% in determination of concentration of studied ion. This experiment revealed that most ions commonly found in polymineral and multivitamin formulae had no influence on determination of iron.

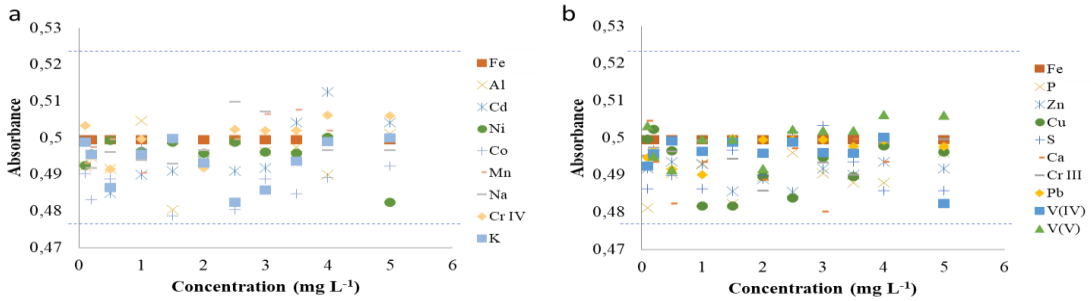


**Figure 4: Study of interferents in the determination of Fe (II) ions by complexing with the Br-TDB reagent. In (a) in the presence of Al (III), Cd (II), Ni (II), Co (II), Mn (II), Na (I), Cr (IV), K (I) and (b) P (II), Zn (II), Cu (II), S (II), Cr (III), Pb (II), V (IV) and V (V). The dotted lines represent the variation of ± 5%.**

### 3.6 Validation

The validation of methodology was performed by analyses of iron ions by inductively coupled plasma optic emission spectroscopy based on direct reading of samples after digestion. The equation of calibration curve was represented by $y = 0.223x - 0.0021$, $R^2 = 0{,}9994$ with linearity between 0 and 2.0 mg $L^{-1}$ (N = 7). The obtained values are presented in Table 3.

### 3.7 Analyses of real samples

The developed system was applied to determine Fe(II) in multivitamins. The results (Table 3) proved that this method is not affected by matrix effects and can be properly used to drug analyses.

**Table 3: Concentration of Fe (mg $g^{-1}$) determined in drug samples using Br-TDB and ICP OES.**

| Sample | Br-TDB | ICPOES | Relative error % |
|---|---|---|---|
| A | 0.1255 ± 0.0003 | 0.1252 ± 0.0001 | -0.24 |
| B | 0.0254 ± 0.0003 | 0.0261 ± 0.0002 | -1.91 |
| C | 0.0350 ± 0.0001 | 0.0347 ± 0.0002 | -0.82 |
| D | 0.0334 ± 0.0007 | 0.0319 ± 0.0001 | -1.77 |
| E | 0.5552 ± 0.0001 | 0.5551 ± 0.0001 | -0.03 |
| F | 0.0126 ± 0.0004 | 0.0122 ± 0.0002 | -3.28 |
| G | 0.0131 ± 0.0005 | 0.0141 ± 0.0003 | -1.89 |
| H | 0.2253 ± 0.0002 | 0.2240 ±0.0001 | -0.43 |
| I | 0.0620 ± 0.0002 | 0.0613 ± 0.0002 | -1.14 |
| J | 0.0229 ± 0.0003 | 0.0232 ± 0.0002 | -3.02 |
| L | 0.4316 ± 0.0003 | 0.4305 ± 0.0001 | -0.16 |
| M | 0.0700 ± 0.0005 | 0.0686 ± 0.0004 | -1.99 |
| N | 0.0071 ± 0.0005 | 0.0067 ± 0.0001 | -1.49 |
| O | 0.0252 ± 0.0004 | 0.0248 ± 0.0001 | -1.62 |
| P | 0.0125 ± 0.0003 | 0.0135 ± 0.0002 | 1.11 |
| Q | 0.1077 ± 0.0002 | 0.1068 ± 0.0002 | -0.44 |
| R | 0.1463 ± 0.0002 | 0.1454 ± 0.0003 | 0.80 |

### 3.8 Evaluation of methodology

The determination of metallic ions by UV-Vis spectrophotometry is a technique that relies on complexation to quantify the selected analyte. This technique was applied to monitor the formation of complex species according to pH. In general, as the pH of solution increased, the intensity of absorption by transference of charge in the regions of interest decreased, demonstrating that the electrons responsible for these transitions were no longer available. Moreover, it was possible to detect some differences in the obtained spectra of coupled material in relation to non-coupled ones, what confirmed the complexation of metals with Br-TDB.

The Student's t-test was applied to establish a comparison of the proposed method and validation by ICP OES assuming equivalent variables, resulting a high correlation between both results (0.999). Therefore, no significant differences ($\rho < 0.05$) between the

determination of iron (II) by UV-Vis spectrophotometry using Br-TDB or ICP OES were observed.

## 4. CONCLUSION

The Br-TDB, besides being soluble in water, has proved to be efficient to determine Fe(II) ions by complexometry, allowing us to understand the stoichiometric relationship of reactions. By comparing the results and methodologies available in literature to that developed in the present work, the determination of Fe in pharmaceutical formulae was efficient (limits of detection, precision and accuracy), even though the limit of detection in the proposed methodology was high. The results of analyses of metals in polyvitamin supplements by UV-Vis or ICP OES had no significant differences at a confidence level of 95%.

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