EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 3
68-74
Year: 2020

DETERMINATION OF MANGANESES IN FOOD BY COMPLEXOMETRY

Vitor Hugo Migues*

*Centro Universitário UniRuy | Wyden. Departamento de Saúde, Av Luis Viana Junior, 3230, Salvador, Ba, Brasil, CEP 40230-000.

***Corresponding Author: Vitor Hugo Migues**

Centro Universitário UniRuy | Wyden. Departamento de Saúde, Av Luis Viana Junior, 3230, Salvador, Ba, Brasil, CEP 40230-000.

Article Received on 05/01/2020 **Article Revised on 25/01/2020** **Article Accepted on 15/02/2020**

ABSTRACT

Manganese (Mn) is one of the most abundant elements in biosphere, corresponding to 0.098% of elements on Earth. This metal is an essential micronutrient in animal diet, being responsible for activation of several enzymes in metabolism. In the present work, the validation of manganese (II) determination by UV-Vis using 6-[2'-(5-bromothyazolylazo)]-3,5-dihydroxy-1,2-benzenedisulfonic acid (Br-TDB) as complexant in food was performed. The reaction between the reagent Br-TDB and Mn (II) has a maximum absorption at 502 nm. The system obeys Beer's law 0.1-3,0 mg L^{-1} having LD = 0.009 and LQ = 0.020 mg L^{-1}, and RSD 0-1.2%. The accuracy was assessed by comparing the results with those obtained by ICP OES. After validation, the procedure was applied to the determination of manganese food samples . The results had no significant differences at a confidence level of 95%.

KEYWORDS: *Manganese, Br-TDB, UV-Vis.*

INTRODUÇÃO

Manganese (Mn) is both an essential and a toxic trace element, mainly depending on its levels in human tissues.[1] Elevated Mn levels may induce neurotoxic effects, including a Parkison-like disease called manganism.[2] The main dietary sources are cereals and tubers, fruits and vegetables, followed by meat, fish and seafood.[3] Other foods with high Mn content include nuts, dried fruits and seeds, chocolate and tea leaves.[3,5] Moreover, intestinal absorption (generally <10% of the ingested Mn) is influenced by iron storage status and intake[6], calcium or phosphorus intake[7], and by the use of dietary supplements[8], including soy formula.[9] Besides dietary intake, Mn exposure may arise from its release into the air, soil and water from industries manufacturing products containing Mn such as pesticides and Mn alloys, from mining activities, and from automobile exhausts, leading to environmental contamination of potential public health concern.[10,11]

Distinct techniques have been employed to quantify Mn, such as: Flame Atomic Absorption Spectroscopy (FAAS)[12], Electrothermal Atomic Absorption Spectroscopy (ETAAS)[13,14] and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES).[15] 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.[16]

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

MATERIALS AND METHODS

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}.

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).[17]

Preparation of samples

Different foods were obtained markets 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[18]. 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.

Determination of Mn(II)

Aliquots of 1.0 mL of digested material were used in analyses. Subsequently, 500 µL of Br-TDB (1.00 x 10^{-3} mol L^{-1}), 3.00 mL of borate buffer pH 10.0 and water to a final volume of 10.00 mL were added. All analyses were performed in triplicates.

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).[19]

Analysis of interferents

After determining the best conditions for complexation between Mn(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 502 nm was carried out.

RESULTS AND DISCUSSION

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 442 nm, while the highest absorbance in the complex form with Mn occurs at 522 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.[17]

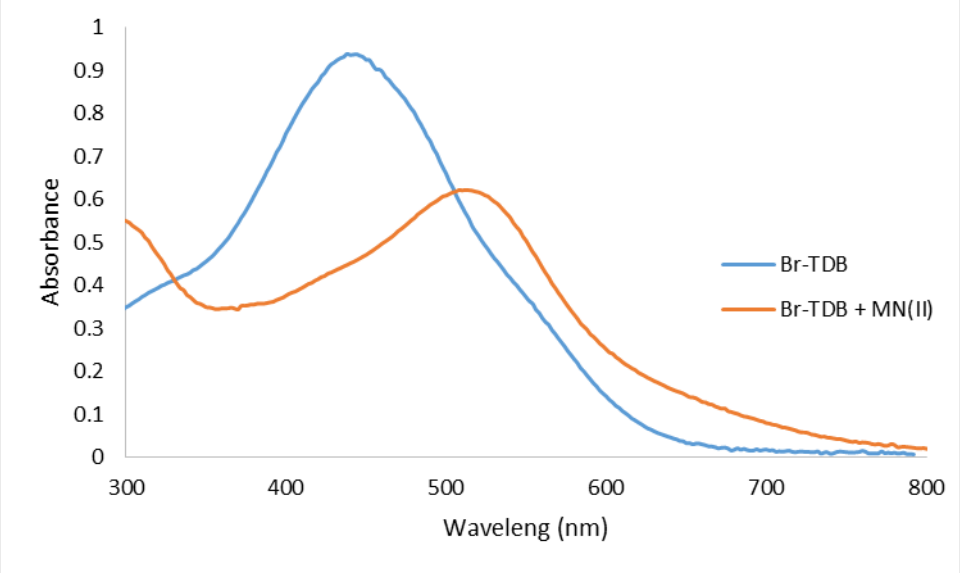

Figure 1: Spectrum of absorption for Br-TDB and Br-TDB-Mn(II).

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.[20] A coefficient of correlation higher than 0.999 is regarded as evidence of perfect adjustment of data in linear regression.[21] ANVISA.[22] recommends a correlation coefficient of 0.99 and INMETRO[23] values above 0.90 based on calibration curves of, at least, five different concentrations.[24]

The standard curve for the select method was built from six concentrations ranging from 0.1 to 3.0 mg L^{-1}. The equation obtained by linear regression based on minimum square method (y = - 0,0072x + 0,645 R^2 =0,9991) indicates there is a correlation between areas and concentration of metallic ions, i.e., the data are properly adjusted to the linear regression.[25] In some cases, the absorbance is inversely proportional to changes inconcentration of analytes.[26] For instance, some reports about determination of fluor using SPADNS method showed a similar pattern of absorptivity loss as the analyte concentration increased.[27,28] The negative angular coefficient in calibration curve has also been reported in other chemical analyses such as quantification of α-

polylysine[29], which presented a high correlation between absorbance and concentration of samples in analyses. In this methodology, the negatively-charged stain interacts with a polycationicion to form the α-PDL complex, leading to a quantitative precipitation and decreasing the intensity of blue color in the supernatant.[30]

Limits of detection and quantification

The limit of detection (LOD) represents the lowest concentration of analyte that can be reliably detected in na experiment.[20] The limit of quantification (LOQ) represents the lowest concentration of analyte that can be reliably quantified using an specific level of precision.[31] 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 Mn(II) in the present analyses, ten negative controls were evaluated, resulting in LOD of 0.0009 and LOQ of 0.0020 mg L^{-1}, with a relative standard deviation (RSD) of 0-1.2%.

Selectivity

The effect of interferent metallic ions like Na(I), K(II), Fe(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 2). 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.

The only exception was observed in relation to the presence of iron, cupper, cadmium and lead. Cupper was the metal that caused the highest interference followed by iron, cadmium and lead. The presence of Cu, Fe, Cd and Pb at concentrations of 2; 2.5; 4 and 5 times, respectively, higher than the concentration of Mn determined spectral overlapping and relative errors of 5.53; 5.72; 6.05 and 7.81% for Cu, Fe, Cd and Pb.

These results show that the complexation between manganese and Br-TDB is highly selective. On the other hand, depending on the conditions of reaction, this reagent might be used in multielement techniques involving this metallic ion inasmuch as the selected pH reduces the errors and the spectral interference of complex ions.

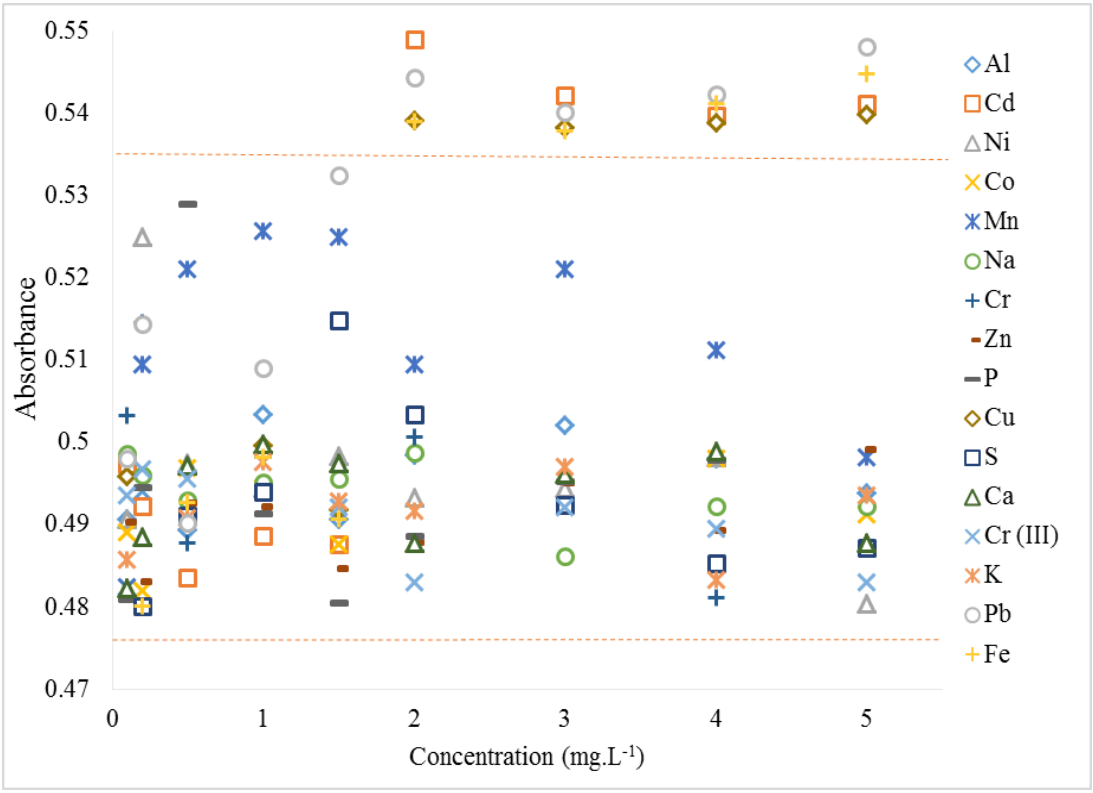

Figura 2: Study of interferents in the determination of Mn (II) ions by complexing with the Br-TDB reagent. [Mn (II)] = 1.0 mg L^{-1}; [Br-TDB] = 5.0 x 10^{-5} mol L^{-1}. The dotted lines represent the variation ± 5%.

Precision

Precision was expressed as repeatability between the absorbance signals of five determinations of solutions at a concentration of 1.00 mg L^{-1}, performed on the same day and by the same analyst (Table 1). The intermediate precision was obtained by analyzing the sample on two

different days, by two different analysts, the same being performed in triplicate, at a concentration of 1.00 mg L^{-1} (Table 2). From the results obtained, the standard deviation (SD) and the relative standard deviation (RSD) were calculated, and they were also compared using ANOVA.

Table 1: Intra-day repeatability data to determine Mn (II) at 1.00 mg L^{-1}.

Repetition	Absorbance morning	Absorbance afternoon	% Sample morning	% Sample afternoon
1	0.6378	0.6379	100.00	98.61
2	0.6376	0.6375	104.17	104.17
3	0.6376	0.6376	102.78	102.78
4	0.6378	0.6374	98.61	105.56
5	0.6377	0.6378	101.39	100.00
Mean	0.6377	0.6376	98.77	102,22
SD	0.0001	0,0002	1.39	2.880
RSD (%)	0.015	0.032	1.37	2.81

Table 2: Inter-day repeatability data to determine Mn (II) at 1.00 mg L^{-1}.

Repetition	Absorbance Day 1	Absorbance Day 2	% Sanple Day 1	% Sample Day 2
1	0.6378	0.6381	100.00	95.83
2	0.6376	0.6372	104.17	108.33
3	0.6376	0.6378	102,78	100.00
4	0.6378	0.6388	98.610	97.22
5	0.6377	0.6377	101.39	101.39
Mean	0.6377	0.6376	101.39	100.56
SD	0.0001	0.0003	1.39	4.87
RSD (%)	0.015	0.054	1.37	4.84

The analysis of Tables 1 and 2 shows that there are no significant differences between the precision analyzes, since the relative standard deviation was below 5%. In addition, the concentration in the sample is in accordance with the standards required by Brazilian regulations.

Accuracy

The accuracy of the method is expressed in terms of percentage of recovery and defined as the amount of substance of interest present or added to the sample, which can be extracted and quantified. Accuracy can be assessed with reference to the addition of a standard reference substance or pure compound.[21]

Generally, the methods of analysis involve transferring the analyte from complex matrices to simpler solutions, thus enabling instrumental determination. However, this procedure results, in most cases, in loss of the analyte or retention of portions of it in the matrix itself after extraction, leading to erroneous quantification.[32]

Given the above, the recovery of the method must be evaluated in the expected concentration range for the substance of interest, which can be done by adding the substance in at least three different concentrations.[21] Table 3 contains the data obtained for accuracy, expressed as a percentage, assessed from the addition and recovery of known amounts of manganese (final concentrations of 0.5; 1.0; and 2.0 mg.L^{-1}) in the sample. The procedure was performed in triplicate.

Table 3: Recovery of standard solutions of Mn(II) added to the samples and analyzed by the proposed method (n = 3).

Concentration added (mg.L^{-1})	Concentration founded (mg.L^{-1})	Mean	RSD (%)	% Recovery
0.5000	0.4875	0.5007	2.46	97.50
0.5000	0.5120			102.40
0.5000	0.5025			100.50
1.0000	1.0456	1.0173	2.41	104.56
1.0000	1.0012			100.13
1.0000	1.0050			100.50
2.0000	1.9850	1.9768	1.13	99.25
2.0000	1.9513			97.57
2.0000	1.9940			99.70

In this study, it was observed that the recovery was in the range of 97.50 to 104.56%. The results are within the acceptable range of recovery, but percentages of recovery of the analyte close to 100% are desirable, but lower values are allowed, provided that the recovery is precise and exact according to RE 899 of 2003.[19] In addition, the values of relative standard deviation (SRD%) were below 5%.

Robustness

To assess the robustness of the methodology, the brand of the reagent sodium tetraborate (QUIMIS and VETEC) was varied, its concentration (0.1 mol.L^{-1} and 0.3 mol.L^{-1}) and the wavelength reading (518 and 526 nm). Robustness corresponds to the ability of a method to resist small changes in analytical parameters, indicating its confidence during normal use.[33] The modified analytical parameters did not show significant changes in the content of the sample and the SQR, and the variations were less than 1%, thus confirming the robustness of the analytical method.

Validation

The validation of methodology was performed by analyses of manganese ions by inductively coupled plasma optic emission spectroscopy(ICP OES) based on direct reading of samples after digestion. The equation of calibration curve was represented by $y = 1.00 \times 10^7 x - 135047$, $R^2 = 0.9991$ with linearity between 0 and 2.0 mg L^{-1} (N = 7). The obtained values are presented in Table 4.

Table 4: Concentration of Mn (mg kg^{-1}) determined in food samples using Br-TDB and ICP OES.

Sample	Br-TDB	ICP OES
Rice	1,191±0,007	1,198±0,001
Beans	2.003±0,005	2.064±0,001
Potato	2,493±0,003	2,502±0,003
Milk	0,054±0,007	0,057±0,006
Nut	18,211±0,011	18,274±0,009
Fruit	8,254±0,003	8,264±0,007

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 diferences ($\rho < 0.05$) between the determination of manganese (II) by UV-Vis spectrophotometry using Br-TDB or ICP OES were observed.

CONCLUSION

The Br-TDB, besides being soluble in water, has proved to be efficient to determine Mn(II) ions by complexometry. By comparing the results and methodologies available in literature[34,35] to that developed in the present work, the determination of Mn in food 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 food by UV-Vis or ICP OES had no significant differences at a confidence level of 95%.

REFERENCES

1. Guo T, Zhang L, Konermann A, Zhou H, Jin F and Liu W: Manganese superoxide dismutase is required to maintain osteoclast differentiation and function under static force. Sci Rep., 2015; 26(5): 8016.
2. Lucchini RG, Martin CJ and Doney BC: From manganism to manganese-induced parkinsonism: A conceptual model based on the evolution of exposure. Neuromolecular Med., 2009; 11(4): 311.
3. Williams M, Todd GD, Roney N, Crawford J, Coles C, McClure PR, Garey JD, Zaccaria K and Citra M: Toxicological Profile for ManganeseAgency for Toxic Substances and Disease Registry. Atlanta, GA: 2012.
4. Turconi G, Minoia C, Ronchi A and Roggi C: Dietary exposure estimates of twenty-one trace elements from a Total Diet Study carried out in Pavia, Northern Italy. Br J Nutr., 2009; 101: 1200.
5. Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, La Sébastien Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, et al: EFSA NDA Panel (EFSA Panel on Dietetic Products NaA: Scientific opinion on dietary reference values for manganese. EFSA J., 11: 34192013.
6. Meltzer HM, Brantsaeter AL, Borch-Iohnsen B, Ellingsen DG, Alexander J, Thomassen Y, Stigum H and Ydersbond TA: Low iron stores are related to higher blood concentrations of manganese, cobalt and cadmium in non-smoking, Norwegian women in the HUNT 2 study. Environ Res., 2010; 110: 497.
7. Institute of Medicine (US) Panel on Micronutrients (eds): ManganeseDietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium,

Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press; Washington, DC: 2001.
8. Greger JL, Davis CD, Suttie JW and Lyle BJ: Intake, serum concentrations, and urinary excretion of manganese by adult males. Am J Clin Nutr, 1990; 51: 457.
9. Cockell KA, Bonacci G and Belonje B: Manganese content of soy or rice beverages is high in comparison to infant formulas. J Am Coll Nutr, 2004; 23: 124.
10. Michalke B and Fernsebner K: New insights into manganese toxicity and speciation. J Trace Elem Med Biol., 2014; 28: 106.
11. Pavilonis BT, Lioy PJ, Guazzetti S, Bostick BC, Donna F, Peli M, Zimmerman NJ, Bertrand P, Lucas E, Smith DR, et al: Manganese concentrations in soil and settled dust in an area with historic ferroalloy production. J Expo Sci Environ Epidemiol, 2015; 25: 443.
12. Sahin, C. A.; Efeçinar, M.; Satiroğlu, N.; J. Hazar. Combination of cloud point extraction and flame atomic absorption spectrometry for preconcentration and determination of nickel and manganese ions in water and food samples. Mater., 2010; 176: 672.
13. Sarzanini, C.; Abollino, O.; Mentasti, E. Flow-injection preconcentration and electrothermal atomic absorption. Anal. Chim. Acta, 2001; 435: 343.
14. Tokman, N. The use of slurry sampling for the determination of manganese and copper in various samples by electrothermal atomic absorption spectrometry J. Hazar. Mater, 2007; 143: 87.
15. Ranjbar,L.; Yamini, Y.; Saleh, A.; Seidi, S.; Faraji, M.;. Ionic liquid based dispersive liquid-liquid microextraction combined with ICP-OES for the determination of trace quantities of cobalt, copper, manganese, nickel and zinc in environmental water samples, Microchim. Acta, 2012; 177: 119.
16. Dantas Filho, H. A.; Souza, E. S. O. N.; Visani, V.; Barrosa, S. R. R. C.; Saldanha, T. C. B.; Araújo, M. C.U.; Galvão, R. K. H.;. Simultaneous spectrometric determination of Cu^{2+}, Mn^{2+} and Zn^{2+} in polivitaminic/polimineral drug using SPA and GA algorithms for variable selection. J. Braz. Chem. Soc., 2005; 16: 58.
17. Migues, VH. Yamaki, RT. Determinação de Co(II) em Preparações Farmacêuticas e Estudo das Espécies Formadas Durante a Complexação por UV-Vis com o Reagente Br-TDB. *Rev. Virtual Quim*., 2014; *6*(6): 1533.
18. Krug, J. F.; Métodos de preparo de amostras: fundamentos sobre o preparo de amostras orgânicas e inorgânicas para análise elementar. Luiz de Queiroz, Piracicaba, 1ª ed., 2010.
19. ANVISA - Agência Nacional de Vigilância Sanitária; http://portal.anvisa.gov.br/wps/wcm/connect/4983b0004745975da005f43fbc4c6735/RE_899_2003_Determina+a+publica%C3%A7%C3%A3o+do+Guia+para+valida%C3%A7%C3%A3o+de+m%C3%A9todos+anal%C3%ADticos+e+bioanal%C3%ADticos.pdf?MOD=AJPERES.
20. Green, J. M.; Peer Reviewed: A Practical Guide to Analytical Method Validation, Anal. Chem., 1996; 68: 305.
21. Ribani, M.; Bottoli, C. B. G.; Collins, C. H.; Jardim, I. C. S. F.; Melo, L. F. C. Validação em métodos cromatográficos e eletroforéticos, Quím. Nova, 2004; 27: 771.
22. ANVISA - Agência Nacional de Vigilância Sanitária;http://portal.anvisa.gov.br/wps/wcm/connect/4983b0004745975da005f43fbc4c6735/RE_899_2003_Determina+a+publica%C3%A7%C3%A3o+do+Guia+para+valida%C3%A7%C3%A3o+de+m%C3%A9todos+anal%C3%ADticos+e+bioanal%C3%ADticos.pdf?MOD=AJPERES, Access in february 2020.
23. INMETRO - Instituto Nacional de Metrologia, Normalização e Qualidade Industrial; http://www.inmetro.gov.br/sidoq/arquivos/cgcre/doq/doq-cgcre-8_03.pdf, Access in february 2020.
24. Conselho Regional de Medicina do Estado de Santa Catarina; http://www.portalmedico.org.br/regional/crmsc/legislacao/gf99391a3.htm, Access in february 2020.
25. Shabir, G.; J. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization.. Chromatogr. A, 2003; 987: 57.
26. Marczenko, Z.; Balcerzak, M.; Analytical Spectroscopy Library, Book 10; Marczenko, Z.; Balcerzak, M., eds; Elsevier Science. 2000, cap. 2.
27. Motter, J.; Moyses, S. T.; França, B. H. S.; Carvalho, M. L.; Moysés, S. J.; Análise da concentração de flúor na água em Curitiba, Brasil: comparação entre técnicas. Rev. Panam. Salud Publica, 2011; 29: 120.
28. http://www.standardmethods.org, Access in february 2020.
29. Grotzky, A,; Manaka, Y.; Fornera, S.; Willeke, M.; Walde, P. Quantification of α-polylysine: a comparison of four UV/Vis spectrophotometric methods. Anal. Methods, 2010; 2: 1448.
30. Shen, W.; Yang, D. Ryser, H. J. P.; Colorimetric determination of microgram quantities of polylysine by trypan blue precipitation. Anal. Biochem, 1984; 2: 521.
31. ftp://ftp.fao.org/docrep/fao/007/w8420e/w8420e00.pdf, Access in february 2020.
32. AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, 2002, Access in february, 2020.
33. ANVISA - Resolução RE n. 899: Determina a publicação do Guia para validação de métodos analíticos e bioanalíticos, (indicado para determinação qualitativa, semi-quantitativa e/ou

quantitativa de fármacos e outras substâncias em produtos farmacêuticos), 2003.
34. Karpińska, J.; Kulikowska, M.; J. Simultaneous determination of zinc(II), manganese(II) and iron(II) in pharmaceutical preparations, Pharm. Biomed. Ana., 2002; 29: 153.
35. Sedaira, H. Simultaneous determination of manganese and zinc in mixtures using first- and second-derivative spectrophotometry, Talanta, 2000; 51: 39.