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An analytical method for the quantitative determination of iron ion chelating capacity: development and validation

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ABSTRACT. Iron is a fundamental microelement for human life; however, deficiencies or excesses of these metal ions can cause severe complications and mortality. Chelators are compounds that bind and inhibit iron. Ultraviolet-visible (UV-vis) spectrophotometric methods are key analytical tools in the identification of chemical entities, with the benefits of having good precision and accuracy, and the equipment being easily available as well as quick and simple to implement. In this study, we aimed to provide an alternative, cheaper method for the quantification of iron ion chelation by substituting ferrozine for gallic acid and validating its use with UV-vis according to official ANVISA and ICH guidelines. The parameters assessed were specificity, linearity, precision, accuracy, robustness, and finally, the percentage of iron ions chelating was calculated. The results demonstrated that this method was accurate, simple, specific, selective, precise, and reproducible, and was successfully validated for the determination of iron ions chelating. The percentage of iron ions chelating, promoted by the standard chelator EDTA, was 45% and 47% for Fe^{2+} and Fe^{3+}, respectively. It is concluded that this new method is beneficial in terms of its simplicity, rapidness, low cost, and the fact that it produces very low levels of dangerous residues.

Keywords: alternative method; human health; gallic acid; green analytical chemistry; foods.

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Introduction

Iron is a fundamental microelement for human life, being essential for cellular energy metabolism, O_2 transport, and DNA synthesis (Aisen, Inns, & Wessling-Resnick, 2001; Kontoghiorghes & Kontoghiorghe, 2020). However, it is highly reactive when in free form, with the concentration being tightly controlled by the cell. The physical state of the iron atom affects its absorption, existing as insoluble ferric (Fe^{3+}) or absorbable ferrous (Fe^{2+}) ions. Iron overload can occur due to excessive iron consumption, frequent transfusions, and chronic hepatitis. Deficiencies or excesses of these metal ions, as well as abnormalities in their metabolism, may cause the formation of free radicals leading to severe complications and even mortality (Kontoghiorghes & Kontoghiorghe, 2020; Malik, Firke, Patil, Shirkhedkar, & Surana, 2020).

Ultraviolet-visible (UV-vis) spectrophotometric methods are important analytical tools in the identification of chemical entities, including iron, which, in addition to being simple, quick, and easily available, have good precision and accuracy (Malik et al., 2020). The quantification of iron ions in food samples is highly important due to the implications for human health. At present, the conventional methodologies of quantification involve the use of expensive reagents and equipment such as ferrozine or atomic absorption. Such investigations have already been conducted for drinking water (Viollier, Inglett, Hunter, Roychoudhury, & Van Cappellen, 2000), cow and buffalo milk (Jaiswal, Bajaj, Mann, & Lata, 2015), wine (Nguyen & Waterhouse, 2018), buttermilk solids (Wong & Kitts, 2001), yogurt, kefir and cream cheese (Unal, 2012), in addition to brown polymeric macromolecules (melanoidins) (Morales, Fernández-Fraguas, & Jiménez-Pérez, 2005) and contaminated soils (that may be subsequently transferred into food crops) (Zhang et al., 2013).

Among the compounds that help in the inhibition of free iron in food products are the natural and synthetic compounds known as chelators. The word chelate is of Greek origin '*chele*', which means to clamp

or claw, and this action occurs through the sharing of electrons between a metal and a binder in the chelating agent (Kratzer, 2018). The ability of plant extracts or other chemical compounds in chelating transition irons is of paramount importance for the removal of iron (Santos, Brizola, & Granato, 2017); these ligands can be polysaccharides (Wang, Zhang, Zhang, & Li, 2008), proteins (Ribeiro, Batista, & Fernandes, 2020), peptides (Wu et et al., 2020), amino acids (Ashmead, 2001), organic acids (Takatera, Miyake, Hiramitsu, Inoue, & Katagiri, 2012), flavonoids (Leopoldini, Russo, Chiodo, & Toscano, 2006; Omololu, Rocha, & Kade, 2011), and phenolic acids (Rainey et al., 2019). The determination of iron chelation typically uses the reagent, ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine), which produces color upon reaction with free iron and is measured in terms of color intensity or absorbance (Wu et al., 2017). However, this reagent is expensive, making it an unviable option for implementation in some laboratories with less financial resources. Currently, the price of ferrozine is about 14 times higher than that of gallic acid (3,4,5-trihydroxybenzoic acid), which has been used in a great number of tests recently, such as in assays to determine the total phenolic compounds and antioxidant activity in samples (Mahindrakar & Rathod, 2020), quantification by Ultra-Performance Liquid Chromatography (UPLC) (Kamal et al., 2020), as well as for pharmaceutical and cosmeceutical applications (Khan et al., 2018).

Recently used a plant rich in gallic acid for the quantification of iron ion chelation. The ability of gallic acid to bind iron ions and, through doing so, generate colored compounds could be an alternative option for the quantification of iron concentration in certain samples (Rattanakit & Maungchang, 2019). Thus, this study aimed to assess an iron ion chelation quantification method by UV-vis, in which the use of ferrozine was substituted with a reaction of gallic acid and acetate buffer. Furthermore, to verify its applicability, the method was validated following the Resolution of the Collegiate Board of Directors (RDC) nº 166 of the Brazilian Health Regulatory Agency (*Agência Nacional de Vigilância Sanitária* [ANVISA]) (Brasil, 2017) and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (European Medicines Agency [EMEA], 2005).

Material and methods

Materials

A UV–vis spectrophotometer Varian (Cary-1E) was used for all absorbance measurements. Ethylenediaminetetraacetic acid (EDTA; ⩾98.0%), gallic acid (⩾98.0%), iron (Fe^{2+}) sulfate heptahydrate ($FeSO_4$; ⩾99.0%), and iron (Fe^{3+}) chloride ($FeCl_3$; ⩾98.0%) were obtained from Sigma-Aldrich (USA). Two brands of glacial acetic acid (⩾99.5%; Nuclear and Anidrol, Brazil) and sodium acetate trihydrate (⩾99.0%; Nuclear and Neon, Brazil) were also purchased.

Method development

The colorimetric method was validated according to official guidelines (EMEA, 2005; Brasil 2017). The parameters studied were specificity, linearity (limit of detection - LOD, and limit of quantification - LOQ), precision, accuracy, and robustness. The percentage of iron ions chelating (PIC%) was also calculated.

Determination of wavelength of maximum absorption

Individual standard stock solutions of Fe^{2+} and Fe^{3+} ions (1 mg mL^{-1}) were prepared in volumetric flasks using deionized water. From the standard stock solution, secondary stock solutions of 600 and 300 µg mL^{-1} were prepared. The secondary stock solutions (0.4 mL) were added to tubes containing 0.8 mL of deionized H_2O. After 10 min at room temperature, 1.5 mL acetate buffer (0.2 M, pH 5.6) and 1.5 mL gallic acid (1%) were added. After 10 min. of reaction at room temperature, visible region spectroscopy scanning (700 - 350 nm) was performed using the respective solvent as a blank to determine the maximum wavelength (λ_{max}) of the iron complexes.

Specificity

The specificity of the UV-vis method was assessed by testing analytical interferences from solvents/reagents. This parameter was determined by comparing the visible absorption spectra obtained from the gallic acid and acetate buffer solution without the iron ions compared to the solution with the iron ions. The spectra were obtained in the range of 350 to 700 nm, and the overlap of absorption bands was evaluated.

Linearity

The linearity of the analytical procedure was executed for six different concentrations of the chelating agent, EDTA (6.2, 12.5, 25.0, 50.0, 100.0, and 200.0 µg mL^{-1}; delimited by preliminary tests, aiming for the lowest concentration detected in the UV-vis) in the chelation of 600 µg mL^{-1} iron ions. In the test, 0.8 mL EDTA solution was added to 0.4 mL of the iron ions for 10 min at room temperature. Then, 1.5 mL acetate buffer (pH 5.6) and 1.5 mL gallic acid (1%) were added for 10 min at room temperature. The absorbances were obtained in the visible spectrum at 570 nm. The experiment was performed in triplicate for both iron ion solutions. The obtained data were used to plot the linearity curves so that the regression equation and correlation coefficient equation could be determined. The limit of detection (LOD) and limit of quantification (LOQ) of the proposed visible method were calculated using the standard deviation of the response and the slope of the corresponding curve using Equations 1 and 2:

$$LOD = (\sigma/S)*3.3 \quad (1)$$

$$LOQ = (\sigma/S)*10 \quad (2)$$

Where; σ represents the standard deviation of absorbance of the sample and S represents the slope of the calibration curve.

Precision

The precision of the analytical procedure was evaluated in terms of intra-day and inter-day variations (intermediate precision). Precision levels were determined for three different concentrations of EDTA (6.2, 50.0, and 200.0 µg mL^{-1}) in the chelation of 600 µg mL^{-1} iron ions. For determining intra-day precision, the absorbance of the three concentrations was measured three times a day in triplicate. For inter-day precision, the absorbance was determined daily for 2 days in triplicate conducted by two analysts. The results were expressed as the relative standard deviation (RSD%) of the analytical measurements using the following Equation 3:

$$RSD\% = (SD/AAC)*100 \quad (3)$$

Where; SD is the standard deviation of the spectrophotometric measurements, and AAC is the average absorbance concentration determined.

Accuracy

Accuracy was determined based on the recovery of known amounts of iron ions added to reagents at the levels of 1, 25, and 100% of the sample concentration, calculated as the percentage of the analyte recovered together with the relative standard deviation (RSD%) between the measurements. For the calculation of the concentration recovery (CR) and recovery percentage (RP), Equations 4 and 5 available in RDC nº 166 (Brasil, 2017) were used:

$$CR = (AT/AE)*EAC \quad (4)$$

$$RP = (EAC/TC)*100 \quad (5)$$

Where; AT is theoretical absorbance; AE is experimental absorbance; EAC is the experimental average concentration; TC is theoretical concentration.

Robustness

The robustness of the procedure was determined through small modifications in the established analytical conditions. In this case, the experiments were conducted by testing minor variations in the selected wavelength for analysis (568 and 572 nm) and two different manufacturers for the glacial acetic acid (Solution A) and the sodium acetate (Solution B) used to make the acetate buffer (pH 5.6) as shown in Table 1. For this test, three different concentrations of EDTA (6.2, 50.0, and 200.0 µg mL^{-1}) were used in the chelation of 600 µg mL^{-1} iron ions.

Table 1. Combinations of the different solvent and reagent brands in the preparation of the acetate buffer (pH 5.6).

Variations	$Solution^{BI}$	$Solution^{BII}$
$Solution^{AI}$	C^{I}	C^{II}
$Solution^{AII}$	C^{III}	C^{IV}

Percentage of iron ions chelating (PIC%)

The absorbance was converted into a percentage of the iron ions chelating (PIC%) for both Fe^{2+} and Fe^{3+}, using Equation 6 (Kong et al., 2010), in order to obtain a more applicable result of the analytical measurements.

$$PIC\% = ((AC_{570}-AS_{570})/AC_{570})*100 \quad (6)$$

Where; AC_{570} is the control absorbance (iron ions + H_2O) at 570 nm, and AS_{570} is the absorbance of the samples (iron ions + EDTA) at 570 nm.

Statistical analysis

The experiments were set up and performed in a completely randomized design, and the results are presented as the mean ± standard deviation (SD) and the relative standard deviation (RSD%). The data were analyzed using Statistica 10 (StatSoft Inc., Tulsa, OK) software. ANOVA and Tukey's post-hoc tests were used to evaluate the statistical significance between groups in the experiments. The results were considered statistically significant for values of $p \leqslant 0.05$.

Results and discussions

In Figure 1, a schematic model depicts the possible reaction that occurs in the method proposed by this study. In step 1, the chelating agent, EDTA, is in contact with iron ions for 10 min, forming the chelated iron complex (iron ions + EDTA). In step two, iron ions that are not chelated by EDTA (free) become linked to the gallic acid added to the reaction in acetate buffer, forming a purple-colored complex (iron ions + gallic acid), which can then be quantified by UV-vis. The proportions of bonds between gallic acid and iron ions (formation of complexes; 1:1, 1:2, or 2:2) may vary depending on the conditions. In comparison, ferrozine has been found to form a complex with EDTA at a 3:1 molar ratio (Stookey, 1970). According to the literature, the ferric (Fe^{3+}) ion is the relatively biologically inactive form but can be reduced to the active Fe^{2+} form by reducing agents, such as polysaccharides or phenolic compounds of plants, which in turn catalyzes degradative reactions (Wong & Kitts, 2001).

Chelate rings with five/six members, as formed with the EDTA, are usually extremely stable when the central ion is bivalent/trivalent (Mg^{2+}, Ca^{2+}, Fe^{3+}, etc.) (Knepper, 2003). EDTA is a chelate ligand with a high affinity constant to form metal - EDTA complexes, and is deliberately added to sequester metal ions in the detergent, cosmetics, foodstuffs, and pharmaceutical industries, as well as for water treatment (Oviedo & Rodríguez, 2003), however, its biodegradation is very slow *in nature*.

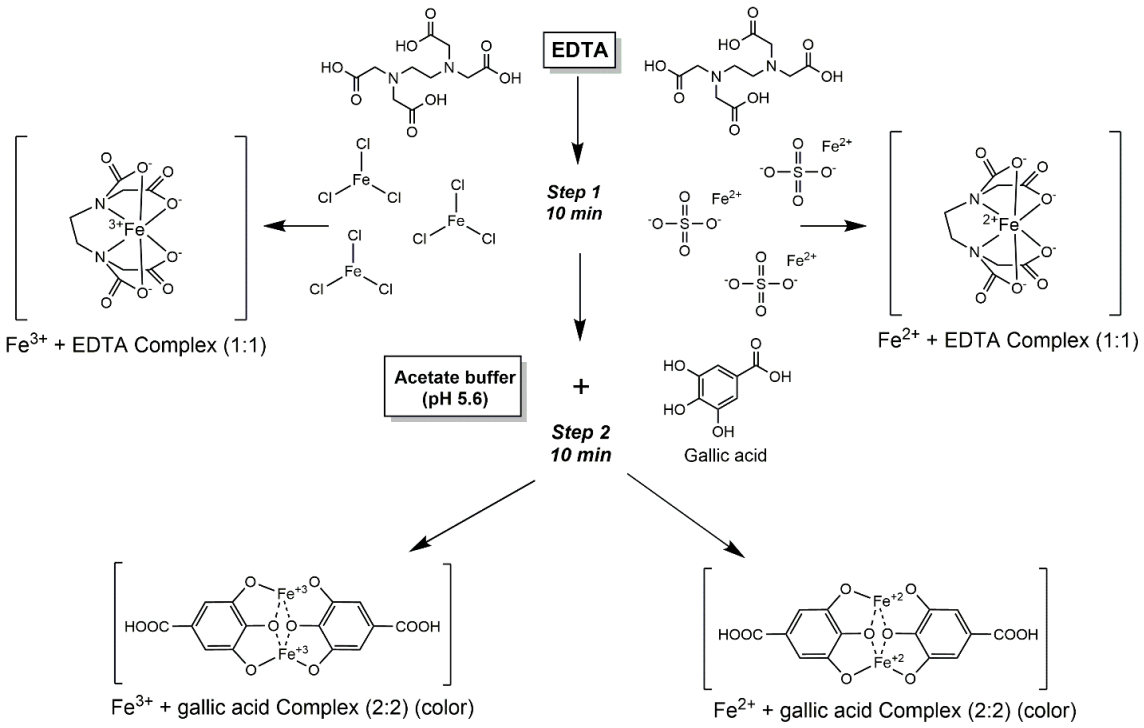

Figure 1. Reaction scheme in the analytical validation of the use of gallic acid in the quantification of iron ion chelation with EDTA.

Specificity

To observe the maximum absorbance wavelength for iron ions chelating and to estimate the interference of the matrix components in the visible spectrophotometric readings, a specificity study was carried out. Specificity is the ability of the method to measure the analyte, excluding any other components, that might interfere (Riley & Rosanske, 1996). For this, a comparison of the visible spectra for the chelation reaction of the iron ions Fe^{2+} and Fe^{3+} versus the reaction without the iron ions was made to determine whether there was any other analyte in the reaction that could produce absorbance and there by mask the true results.

No interference of the solvents/reagents was observed since no peak was detected after the analysis of the iron-free chelation reaction at set wavelengths (Figure 2A). The chelation reaction produced a decrease in the purple color, shown in Figure 2B, due to the iron ions complexing with EDTA, and therefore, fewer free ions interacting with the gallic acid. The choice of such potentially interfering materials should be based on sensible scientific judgment with a consideration of the interferences that could occur (EMEA, 2005).

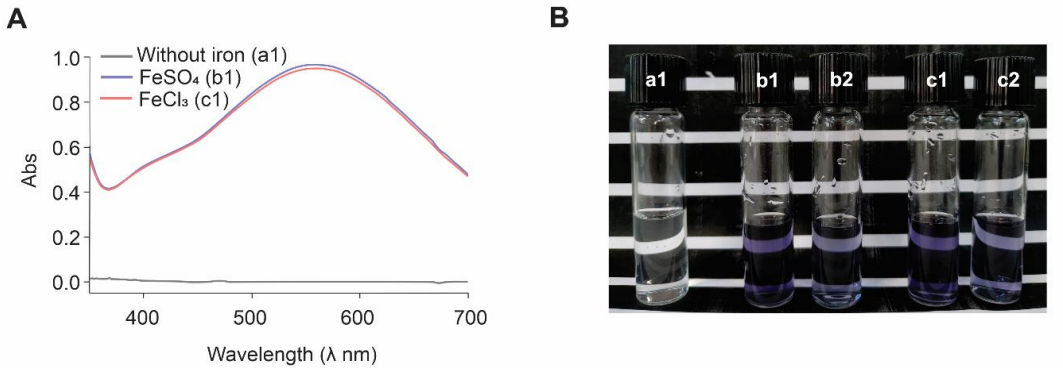

Figure 2. A) UV-vis spectroscopic spectra of specificity. **B**) Iron chelation reaction. Notes: Black line/a1: reaction excipients only, without iron ions; Blue line/b1: reaction containing excipients and the Fe^{2+} ion (600 µg mL^{-1}); b2: Fe^{2+} (600 µg mL^{-1}) chelation by EDTA (200 µg mL^{-1}); Red line/c1: reaction containing excipients and the Fe^{3+} (600 µg mL^{-1}); c2: Fe^{3+} ion (600 µg mL^{-1}) chelation by EDTA (200 µg mL^{-1}).

A UV-vis spectroscopic scanning run allows the selection of the best wavelength for the detection of a specific reaction (Wrasse-Sangoi, Secretti, Diefenbach, Rolim, & Sangoi, 2010). The highest absorbance in the scanning spectra was obtained at 570 nm, which is the same range of wavelengths applied in methodologies using ferrozine (Kong et al., 2010).

Linearity

The linearity of an analytical procedure is its ability to obtain results that are directly proportional to the concentration of an analyte in a sample (Chan, Lee, Lam, & Zhang, 2004). Regarding validation parameters, the linearity (Table 2 and Figure 3) resulted in regression equations for the iron ions chelating as y = 0.7966-0.0016x for Fe2+, and y = 0.7382-0.0017x for Fe3+. Pearson correlation coefficients (R2) of 0.9996 and (R) of 0.9998, for both Fe2+ and Fe3+, were obtained as the mean value for three plotted analytical curves, calculated by the least-squares method. The coefficient is considered linear when it is between 0.9990 and 0.9999 (Brasil, 2017). The results show that the methodology is linear for both iron ions.

Table 2. Linearity test parameters in the iron ion chelating quantification method.

Iron ion 600 (µg mL^{-1})	Linear range EDTA (µg mL^{-1})	R^2	R	Intercept	Slope	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Fe^{2+}	6.2-200	0.9996	0.9998	0.7966±0.0001	0.0016±0.0005	1.4039	4.2542
Fe^{3+}	6.2-200	0.9996	0.9998	0.7382±0.0016	0.0017±0.0010	1.9540	5.9214

±: standard deviation; n = 3; LOD: limit of detection; LOQ: limit of quantification.

The LOD is a concentration resulting in a signal that is significantly different, higher or lower, than that of the background, and LOQ is the highest and lowest concentrations of an analyte that have been demonstrated to be measurable with acceptable levels of bias (Lee et al., 2006). The calculated values of 1.4 and 4.2 µg mL-1 were verified for the LOD and LOQ for Fe2+, and 1.9 and 5.9 µg mL-1 for Fe3+, respectively. In a method using ferrozine to quantify the oxidation caused by iron in wine samples, the LOD and LOQ for Fe2+ were 20.0 and 60.0 µg mL-1, respectively (Nguyen & Waterhouse, 2018). The lower the results are, the better the limit of detection and quantification; thus, the LOQ and LOD of the proposed method could be considered superior to that using ferrozine.

The term accuracy expresses how close an individual measurement is to the real value (Stauffer, 2018). For a given method, the most important factors in the determination of repeatability and reproducibility are the laboratory, analyst, time, and instrumentation. The reproducibility is the expected maximal difference between results acquired by repeated application of the analytical procedure, and those obtained by different operators/laboratories on different days using the same method (Burgess, 2000).

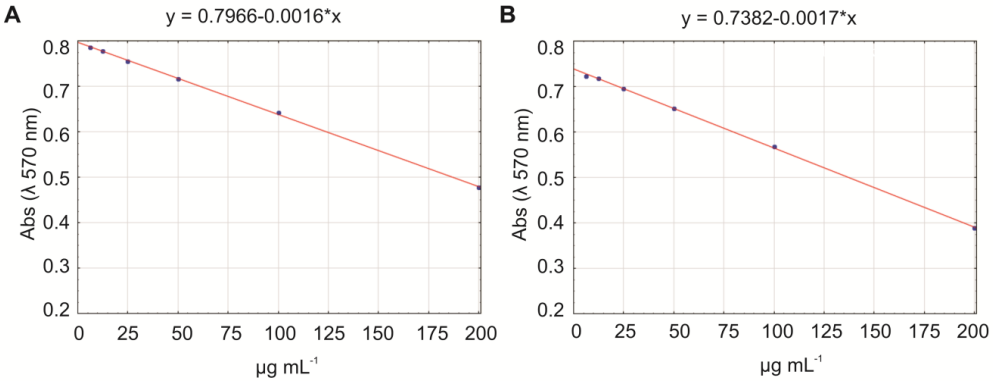

Figure 3. The analytical curve of linearity in iron ion chelation with straight-line equations; **A**) Fe^{2+}; **B**) Fe^{3+}.

Precision

The results of intermediate precision, repeatability, and reproducibility, both intra-day and inter-day, with values of the RSD% are summarized in Table 3. Bioanalytical assays with an RSD% of 5.0 or less characterize a very precise method, whilst between 5.0 - 10.0 is more common and represents an accepted industrial standard in terms of precision (Riley & Rosanske, 1996; Brasil, 2017). For both parameters, the values obtained for the RSD% were less than 5.0, showing reproducibility in the assay. Thus, an adequate precision with a high degree of agreement was obtained for the current analytical assay. Furthermore, the results showed no significant differences in the UV-vis measurements comparing analysis intra-day, inter-day, and between the two analysts ($p \leq 0.05$).

Table 3. Precision levels (intermediate precision, repeatability, and reproducibility) for the analytical validation of the iron ion chelating quantification method with EDTA.

Iron	EDTA µg mL^{-1}	Analyst^{I}				Analyst^{II}				Reproducibility RSD%
		Intra-day RSD%	1st day RSD%	2st day RSD%	Inter-day RSD%	Intra-day RSD%	1st day RSD%	2st day RSD%	Inter-day RSD%	
Fe^{2+}	6.2	1.07	0.76	1.07	0.92	0.44	1.36	0.74	1.05	0.87
	50.0	1.10	0.42	1.57	0.99	0.11	0.77	0.50	0.63	0.71
	200.0	1.17	0.86	1.78	1.32	1.50	1.31	0.34	0.83	1.20
Fe^{3+}	6.2	0.32	0.32	0.33	0.32	0.30	0.27	0.32	0.31	0.31
	50.0	0.40	0.40	1.06	0.73	0.63	1.42	1.22	0.92	0.67
	200.0	1.12	1.12	0.29	0.70	0.76	0.30	1.78	1.27	0.96

RSD: relative standard deviation (%); Intra-day: n = 3; Inter-day: n = 6. Iron ions at 600 µg mL^{-1}.

Accuracy

The recovered percentage value in the accuracy test must be between 90 to 110% (Brasil, 2017). Thus, the procedure was shown to be accurate, with variations in the recovery of Fe^{2+} from 96 to 100% and of Fe^{3+} from 99 to 106%. There was a greater RSD% for Fe^{2+}, possibly because it is in crystal form, and its relative weight varies, meaning it is a less homogeneous material compared to Fe^{3+} (Table 4). The results showed no significant differences in the UV-vis measurements in the accuracy tests, on three different days of analysis ($p \leq 0.05$).

Robustness

Robustness is a measure of the capacity of a method to remain unaffected by small, but deliberate, changes in parameters and indicates its reliability during normal run conditions (Lee et al., 2006). In the present study, the influence of wavelength variations at 568 and 572 nm, and the brands of solvents/reagents (different combinations: 2 brands of glacial acetic acid and 2 brands of sodium acetate in the preparation of the acetate buffer pH 5.6, given in Table 1), in the quantitative analysis of iron ions chelating, was investigated for three concentrations of EDTA. Table 5 indicates that there were no significant differences in the measurements between 568 and 572 nm, and the different brands of solvents/reagents analyzed ($p \leq 0.05$).

The results reveal that the method proved to be robust for the iron ion chelating quantification of Fe^{2+} and Fe^{3+}, as no significant differences could be observed ($p \leqslant 0.05$). The values obtained for the RSD% were less than 5.0, thus demonstrating an alternative robust quantification method of iron ion chelation.

Table 4. Accuracy levels for the analytical validation of iron ion chelating quantification method with EDTA.

Iron	Theoretical concentration	Concentration recovered (μg mL^{-1})					Recovered percentage (%)				
	EDTA	1st day	2st day	3st day	Mean	RSD%	1st day	2st day	3st day	Mean	RSD%
	6.2	6.22	6.22	6.16	6.20	0.52	100.32	100.32	99.47	100.04	0.49
Fe^{2+}	50.0	49.55	49.30	49.23	49.36	0.78	99.12	98.58	98.47	98.72	0.77
	200.0	194.91	194.14	195.46	194.84	2.27	97.45	97.07	97.73	97.42	2.27
	6.2	6.25	6.25	6.24	6.25	0.06	100.64	100.74	100.81	100.73	0.08
Fe^{3+}	50.0	48.75	50.87	49.14	49.58	2.28	97.49	101.74	98.28	99.17	2.26
	200.0	207.56	215.36	213.59	212.17	1.93	103.78	107.68	106.79	106.08	1.93

RSD: relative standard deviation (%); n = 9.

Table 5. Robustness for the analytical validation of the iron ion chelating quantification method with EDTA, by changing wavelength, and brand of reagent/solvent.

Iron	EDTA μg mL^{-1}	λ 568 nm	λ 572 nm	C^{I}	C^{II}	C^{III}	C^{IV}	Reproducibility
		RSD%	RSD%	RSD%	RSD%	RSD%	RSD%	RSD%
	6.2	0.05	0.05	0.06	0.05	0.06	0.05	0.06
Fe^{2+}	50.0	0.38	1.20	1.38	0.87	0.60	0.53	0.82
	200.0	1.52	1.97	1.44	1.12	0.87	1.34	1.37
	6.2	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Fe^{3+}	50.0	1.20	0.58	0.47	0.49	2.33	0.54	0.94
	200.0	2.05	0.77	1.02	0.91	3.10	0.92	1.46

C: Combination of reagent/solvent given in table 1; RSD: relative standard deviation (%); n = 31, 38.

Percentage of iron ions chelating (PIC%)

The results obtained can be converted into a percentage of iron ions chelating (PIC%), promoted by the EDTA, using Equation 6. The values varied from 1 to 45% for Fe^{2+}, and from 3 to 47% for Fe^{3+}, showing great chelation of these iron ions (Table 6). EDTA is an excellent sequestering agent (Oviedo & Rodríguez, 2003), however, the purpose of this validation is to use this method in the future to evaluate the potential of natural substances that sequester iron ions to replace EDTA.

Table 6. Percentage of iron ions chelating with EDTA.

Iron	EDTA (μg mL^{-1})	Absorbance (570 nm)	PIC%
	6.2	0.68 ± 0.001	1.16 ± 0.21^{b}
Fe^{2+}	50.0	0.61 ± 0.004	10.61 ± 0.57^{b}
	200.0	0.37 ± 0.004	45.89 ± 0.56^{b}
	6.2	0.72 ± 0.004	3.21 ± 0.47^{a}
Fe^{3+}	50.0	0.65 ± 0.005	12.72 ± 0.62^{a}
	200.0	0.38 ± 0.002	47.91 ± 0.20^{a}

PIC%: Percentage of iron ions chelating; SD: Standard deviation; n = 6. Different letters indicate statistically significant differences by Tukey's test ($p \leqslant 0.05$).

The validation of a method must demonstrate that the analytical method produces reliable results and is suitable for the purpose for which it is intended, in a documented manner and according to objective criteria (Brasil, 2017).

In this study, gallic acid proved to be a great substitute for a technique that uses ferrozine. Gallic acid is a more favorable option as it is cheaper, easy to commercially acquire, and has applicability to other laboratory techniques, thereby being of greater cost/benefit to laboratories that have low budgets. Given its wide occurrence in natural products, gallic acid and its derivatives are seen as having potential in the food and pharmaceutical industries (Choubey et al., 2018).

The analysis of iron ions chelating using ferrozine was developed in the 70's, because deferoxamine mesylate did not affect the determination of iron at levels of 0.10 mg mL^{-1} in blood serum (Carter, 1971). However, currently, most iron chelation analyzes are to determine the concentration of iron ions in other non-human matrices, such as in water or food samples. This highlights the need for new methods to be properly validated in order to be applied to samples of different origins. The limited number of validation

studies with ferrozine makes it impossible to say that the method demonstrated here with gallic acid is better, however, it offers a viable alternative for the evaluation of iron ion chelators.

Conclusion

All the validation parameters for the developed method were studied as per ANVISA and ICH guidelines. The reported UV-vis spectrophotometric method was found to be accurate, simple, specific, selective, precise, and reproducible, and therefore was successfully validated as a suitable approach for the determination of iron ions chelating. The advantages of this method include its simplicity, speed, low cost, and the fact that it produces very low levels of dangerous residues thereby having little effect on the environment. Hence, the method is an alternative that can be used in the routine laboratory analysis of iron ion chelation in food samples.

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