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Digital images for colorimetric determination of copper in dietary supplements

Imagens digitais para a determinação colorimétrica de cobre em suplementos

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This study proposes a digital image analysis-based procedure for the determining copper content in dietary supplements. The method was established by measuring the color of the copper(II)-salicylic acid complex. Digital images obtained during analysis were captured using a smartphone equipped with the ColorMeter® application, which provided corresponding RGB (Red, Green, Blue) color values. The experimental chemical conditions were optimized using multivariate experimental design, and the optimal parameters were determined. The proposed method was validated, and the limits of detection (LD) and quantification (LQ), and precision for the RGB channels were established. A comparison with ultraviolet-visible (UV/Vis) spectroscopy results revealed no statistically significant differences at a 95% confidence level. The proposed method was applied to ten dietary supplement samples, which exhibited copper content ranging from 278 ± 13 to 3530 ± 75 µg (R channel) and 268 ± 14 to 2706 ± 275 µg (G channel). The mean values obtained showed excellent agreement with the values declared by manufacturers on product labels. These results demonstrate that the proposed method is suitable for copper determination in dietary supplements using digital images.
Keywords: RGB color system, factorial design, colorimetry.

Este estudo propõe um procedimento baseado em análise de imagem digital para determinação do teor de cobre em suplementos nutricionais. O método foi estabelecido medindo a cor do complexo de cobre(II)-ácido salicílico. As imagens digitais obtidas durante as análises foram capturadas utilizando um smartphone equipado com o aplicativo ColorMeter®, que forneceu os valores de cor RGB (Vermelho, Verde, Azul) correspondentes. As condições químicas experimentais foram otimizadas utilizando planejamento experimental multivariado, e os parâmetros ótimos foram determinados. O método proposto foi validado, e os limites de detecção (LD) e quantificação (LQ), e precisão para os canais RGB foram estabelecidos. Em comparação com os resultados da espectroscopia ultravioleta-visível (UV/Vis) não foram observadas diferenças estatisticamente significativas em um nível de confiança de 95%. O método proposto foi aplicado em dez amostras de suplementos, que apresentaram teor de cobre variando de 278 ± 13 a 3530 ± 75 µg (canal R) e 268 ± 14 a 2706 ± 275 µg (canal G). Os valores médios obtidos apresentaram excelente concordância com os valores declarados pelos fabricantes nos rótulos dos produtos. Esses resultados demonstram que o método proposto é adequado para determinação de cobre em suplementos nutricionais utilizando imagens digitais.
Palavras-chave: sistema de cores RGB, planejamento fatorial, colorimetria.

1. INTRODUCTION

Dietary supplements are products taken orally to supplement a person's healthy diet. They are also used to supplement the diets of individuals at greater risk of nutritional deficiency or those with medical conditions that limit nutrient bioavailability. They are sold without regulation and have a strong advertising appeal, claiming various benefits. They are widely available in establishments such as supermarkets and pharmacies and come in forms like tablets, capsules, gummies, powders, drinks, and bars [1, 2]. Over the last 20 years, the use of supplements has experienced significant growth, with the global market projected to reach U$20 billion by 2028. As this market expands and profits surge, the importance of ensuring product quality increases, along with the challenges associated with this task. Consequently, global quality standards must

be upheld, and greater attention should be devoted to addressing regulatory challenges surrounding nutritional supplements. Adulterated or mislabeled products can evade existing regulations, posing serious health risks due to contaminants or harmful substances. These risks can lead to severe side effects, some of which can be fatal, often due to adulterants present in the final product rather than the nutritional supplement ingredients themselves [3].

In Brazil, the National Health Surveillance Agency (ANVISA), through RDC No. 243 of July 26, 2018, sets the minimum health requirements for supplements, establishing rules for product designation, labeling, composition, and limits [4]. According to RDC No. 360 of December 23, 2003, a tolerance of ±20% is permitted regarding the declared nutrient values on product labels [5]. Pursuant to Normative Instruction No. 28 of July 26, 2018, maximum limits are established for nutrients, bioactive substances, enzymes, and probiotics in dietary supplements. Specifically, for individuals over 19 years old, the legislation permits up to 8,975.32 µg of copper per day. Copper in food supplements is available in various forms, including copper aspartate, copper bisglycinate, copper (II)-D-gluconate, copper oxide, copper sulphate, and copper sulphate pentahydrate [6].

Copper is the third most abundant trace element in the human body, playing a vital role in various physiological processes. These include metabolism, enzyme function, antioxidant defense, hemoglobin production, melanin synthesis and collagen production. Copper is distributed throughout the body, with significant stores found in liver, brain, heart, kidneys and muscles. However, excessive copper intake can lead to various adverse health effects, including epigastric pain, anemia, kidneys and liver failure, and other complications. Furthermore, copper supplements are contraindicated in patients with hepatolenticular degeneration, also known as Wilson's disease, due to the risk of exacerbating the condition [7, 8]. Typically, copper is quantified using conventional analytical techniques, including flame atomic absorption spectrometry (FAAS), inductively coupled plasma optical emission spectrometry (ICP OES), inductively coupled plasma mass spectrometry (ICP-MS) [8], high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GFAAS) [9], energy dispersive X-ray fluorescence spectrometry (EDXRF) [10], and others. However, some of these techniques are relatively expensive and, sometimes, needs a complex and rigorous pretreatment of the sample.

Recent advancements in analytical technology have introduced innovative approaches, including digital image-based chemical analysis. This technique utilizes digital images captured from various devices, such as digital cameras, webcams, scanners, tablets, or smartphones. Offering benefits like rapid analysis, easy operation and portability of devices. The rapid advancement of smartphone technology has enabled high storage capacities, expanded internal memory, enhanced camera resolution, and seamless connectivity to external devices. These features facilitate colorimetric analysis, transforming smartphones into portable and versatile analytical tools for various applications, including environmental monitoring, food quality assessment, biomedical diagnostics, and field-based research. The most common filters used in smartphones to obtain image pixels are CMYK (cyan, magenta, yellow, and black) and RGB (red, green, and blue), which establish relationships between analyte concentrations and color components. The RGB system, used in this study, assigns values ranging from 0 to 255 to colors. For example, the color violet has RGB values of R = 238, G = 130, and B = 238 [11, 12].

The literature reports the use of digital images for colorimetric determination of copper. Pessoa et al. (2017) [13] evaluated copper content in sugarcane spirits using cuprizone, a bidentate organic reagent that forms a blue complex in slightly alkaline media. Digital images for RGB value extraction were captured using a digital camera. Similarly, Fernandes et al. (2020) [14] determined copper in sugarcane spirits based on the colorimetric reaction between Cu(II) and diethyldithiocarbamate (DDTC), resulting in a yellow-brownish complex. Digital images were captured using an ordinary flatbed scanner, and parameters derived from the RGB color space were utilized for quantification. Franco et al. (2021) [15] analyzed copper in distilled beverages based on the complexation reaction between Cu(II) and thiocarbazone, forming a yellow-colored complex. The RGB values were extracted using a smartphone. Al-Nidawi and Alshana (2021) [16] determined copper in edible oil samples based on the complexation reaction between copper and N,N-diethyl-N'-benzoylthiourea, which forms a green-colored complex. The RGB values were extracted using a smartphone. Maia et al. (2022) [17] evaluated copper in sugarcane spirits

using a complexation reaction between Cu(II) and carbon dots functionalized with cuprizone. The RGB values were extracted using a smartphone. Experimental design techniques, such as two-level and Doehlert designs, can be utilized to determine the optimal conditions for colorimetric reactions [18-20].

For the first time, this study introduces a digital image-based analytical methodology for determining copper in dietary supplements. Salicylic acid was chosen for its advantages, such as stable complex formation, simplicity, low cost, speed, and effectiveness. The most common complexing agents for copper are cuprizone, DDTC, neocuproine, and others. Compared to salicylic acid, cuprazone has greater sensitivity but less robustness. DDTC is significantly affected by interference, and neocuproine requires the reduction of Cu(II) to Cu(I) [13, 14]. This procedure exploits the colorimetric reaction between Cu^{2+} and salicylic acid, leveraging the advantages of smartphone-based analytical measurement.

2. MATERIAL AND METHODS

2.1 Reagents and samples

All solutions were prepared using high-purity water (resistivity: 18.2 MΩ cm) from a Gehaka OS 20 LXE water purification system (São Paulo, SP, Brazil). The following reagents were used: copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), acetone (C_3H_6O), salicylic acid ($C_7H_6O_3$), and sodium hydroxide (NaOH) from Sigma-Aldrich; and nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) from Merck (Darmstadt, Germany). Stock solutions were prepared as follows: Cu^{2+} (100 mg L^{-1}): 0.396 g of $CuSO_4.5H_2O$ dissolved in 1 L of ultra-pure water; salicylic acid (4% w v^{-1}): 4 g dissolved in 100 mL of acetone; NaOH (0.1 mol L^{-1}): 4 g dissolved in 1 L of ultra-pure water. Ten representative dietary supplement samples were purchased from local drugstores in Aracaju and Simão Dias, Sergipe, Brazil, as well as from a compound pharmacy in Aracaju. Copper determination in dietary supplement samples was carried out using Ultraviolet/Visible (UV/Vis) spectroscopy on an Edutec EEQ-9023 spectrophotometer, with an analytical wavelength of 622 nm.

2.2 Sample preparation

One tablet or capsule of dietary supplement was placed in a Teflon pump, and 2 mL of HNO_3 (70% w v^{-1}), 1 mL of H_2O_2 (30% v v^{-1}) and 7 mL of ultra-pure water were added. The mixture was then allowed to stand for 40 minutes for pre-digestion. The mixture was subsequently digested in a digester block at 170 °C for 2 hours. Following digestion, the samples were removed from the block, cooled to room temperature, and transferred to centrifuge tubes, where they were stored at room temperature until analysis, adapted from Santos et al. (2021) [21]. All experiments were performed in triplicate.

2.3 Formation of the Cu/Salicylic acid complex

The choice of salicylic acid as complexing agent was based on the literature [22, 23], which reports its use for the complexation of copper with salicylated ligands, being within the requirement for using the digital imaging method, which is the formation of coloration. The complex between copper and salicylic acid was formed in a 25 mL volumetric flask, the complex was formed by adding 4 mL of 4% (w v^{-1}) salicylic acid solution. The sample volume was adjusted to fit the calibration curve. The pH was adjusted to 11 using 0.1 mol L^{-1} NaOH, and the final volume was completed with ultrapure water while monitoring the pH. Consistent with Rojas et al. (2016) [24], who investigated the interaction between copper and salicylic acid at pH 1-8, our study confirms the formation of the mixed complex $[Cu(OH)Sal_2]^{3-}$ at pH values above 8.

2.4 Optimization of analytical method experimental conditions

The colorimetric reaction was optimized by evaluating the effects at coded (real) levels of: pH [+1 (11) and -1 (9)]; time [+1 (20) and -1 (10) min] and salicylic acid volume (4% w v^{-1}) [+1 (6) and -1 (2) mL], using a full two-level factorial design, with a total of 8 experiments and three central points. Subsequently, a Doehlert design was employed to refine the optimization of the significant variables, pH [-1 (8), -0.5 (9), 0 (10), +0.5 (11) and +1 (12)] and time [-0.866 (20), 0 (25) and +0.866 (30) min]. The experimental domains for copper determination were established based on previous studies carried out in the laboratory and the literature [24]. The experimental error was estimated by triplicating the central points. A single sample of dietary supplements was utilized for the experimental designs. Statistica® 8.0 software was used to construct and analyze the factorial designs.

2.5 RGB data acquisition and evaluation

A closed system, adapted from Neto et al. (2019) [19], was designed for digital image capture. The system consisted of a wooden box (34 x 28 x 22 cm) with a side aperture (2.5 x 3.5 cm) and an internal light control compartment to ensure reproducible image acquisition (Figure 1). The utilization of a LED lamp obviated the need for flash photography. The sample support was positioned 10 cm from the side opening, as studies by Porto et al. (2019) [25] demonstrated no significant differences in image quality at distances of 5, 10, and 15 cm. Digital images of the colorimetric systems were captured using a Motorola Moto G10 smartphone, featuring a 48-megapixel camera (4000x3000 pixels resolution). The ColorMeter® app was utilized to extract R (red), G (green), and B (blue) color parameters.

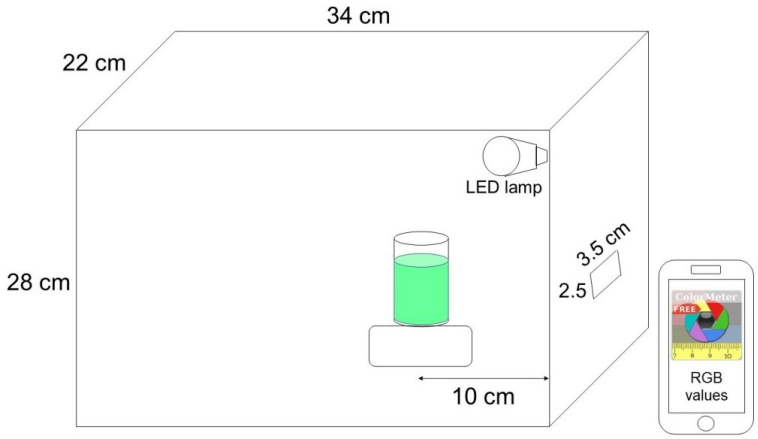

Figure 1: Digital image analysis systems.

3. RESULTS AND DISCUSSION

3.1 Stability evaluation of the formed complexes

To assess the stability of the complexes formed in the samples (2.3 section), three samples were randomly selected, and the tests were conducted using a UV/Vis spectrophotometer. Evaluating the stability of the complex, we observe that two tablet samples exhibit stabilization between 60-420 minutes, with absorbance reaching minimum values at 24 h. In contrast, capsule 05 displays an increase in absorbance from 60-420 min, followed by a decline to minimum values after 24 hours. This initial increase can be attributed to sample oxidation, which beginning around 60 minutes, as shown in Figure 2.

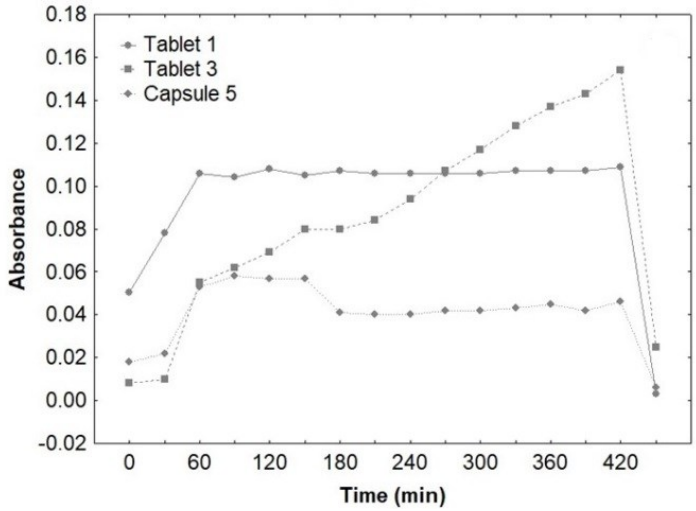

Figure 2: Stability evaluation of copper complexes formed in samples: tablet 1, tablet 3, and capsule 5, analyzed by UV/Vis.

3.2 Optimization of the procedure for copper analysis in dietary samples

The optimization of experimental conditions for copper(II) determination was conducted in two stages. Firstly, a two-level full factorial design was employed to establish optimal conditions for determining the copper(II)-salicylic acid complex. The factors investigated were pH, time, and volume of 4% (w v^{-1}) salicylic acid. Table 1 presents the experimental domains of the factors, including coded and real values, along with the corresponding RGB values for each experiment.

Following evaluation of the design data using Statistica 8.0 software, the Pareto chart (Figure 3) revealed that pH and time variables were statistically significant (p-values < 0.05). Notably, both variables exhibited positive effects, indicating that increased levels of pH and time resulted in enhanced responses in the experiments. The interaction between pH and time was also assessed. Notably, when one variable was at its highest level, while the other was not, an increase in response was observed (experiments 3 and 6). However, when both pH and time were at their highest levels (experiments 1 and 2), the responses were not optimal, as presented in Table 1. Since the volume of salicylic acid variable was not significant in the two-level full factorial design, it was maintained at its center point value of 4 mL.

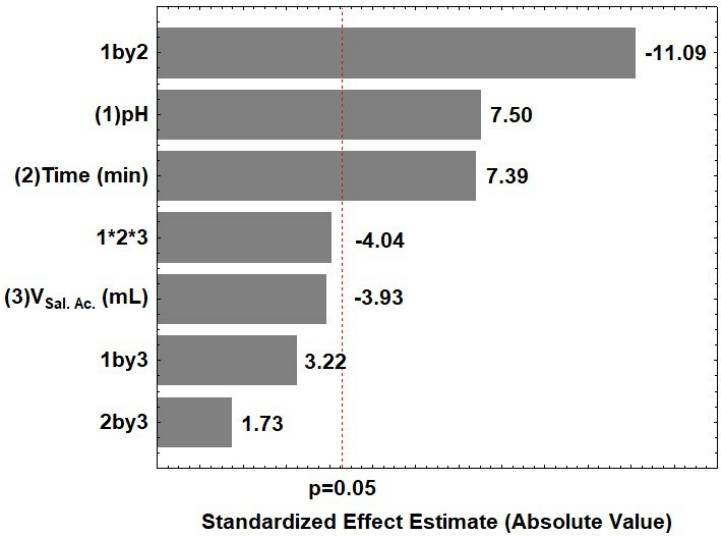

Figure 3: Pareto chart for two-level full factorial design (2^3): effects of pH, waiting time, and salicylic acid volume.

Table 1: Two-level full factorial design (2^3) for the variables pH, waiting time, and volume of 4% (w v^{-1}) salicylic acid, with R, G, and B values as responses.

Experiment	pH	Time (min)	V (mL)	R	G	B	Normalized sum
1	+1 (11)	+1 (20)	+1 (6)	146	150	157	2.83
2	+1 (11)	+1 (20)	-1 (2)	151	156	159	2.91
3	+1 (11)	-1 (10)	+1 (6)	155	160	164	2.99
4	+1 (11)	-1 (10)	-1 (2)	153	158	161	2.95
5	-1 (9)	+1 (20)	+1 (6)	153	158	161	2.95
6	-1 (9)	+1 (20)	-1 (2)	154	161	163	2.99
7	-1 (9)	-1 (10)	+1 (6)	120	122	125	2.29
8	-1 (9)	-1 (10)	-1 (2)	137	140	146	2.64
9(CP)	0 (10)	0 (15)	0 (4)	148	153	156	2.86
10(CP)	0 (10)	0 (15)	0 (4)	144	149	152	2.78
11(CP)	0 (10)	0 (15)	0 (4)	144	151	154	2.81

CP: central point; coded value (real value); V: 4% salicylic acid volume.

To investigate the interaction between the two significant variables, a Doehlert design (Table 2) was employed. This design enables a more comprehensive visualization of the experimental region through response surface methodology. The pH and time variables were investigated, with the most significant factor (pH) evaluated at five levels and the least significant factor (time) evaluated at three levels in the experimental design. The response variable was calculated as the sum of R, G, and B values for each experiment. To minimize systematic errors, the experiments were conducted in a random order.

Table 2: Doehlert design for pH and time variables, with RGB sum as response variable.

Experiment	pH	Time (min)	R	G	B	Normalized sum
1	0 (10)	0 (25)	113	110	105	2.62
2	+1 (12)	0 (25)	110	106	103	2.55
3	+0.5 (11)	+0.866 (30)	128	125	122	3.00
4	-1 (8)	0 (25)	115	107	105	2.62
5	-0.5 (9)	-0.866 (20)	106	100	97	2.42
6	+0.5 (11)	-0.866 (20)	109	104	101	2.51
7	-0.5 (9)	+0.866 (30)	108	103	101	2.50
8(CP)	0 (10)	0 (25)	125	124	120	2.95
9(CP)	0 (10)	0 (25)	109	103	100	2.50

CP: central point; coded value (real value).

Utilizing Statistica 8.0 software, the data from the Doehlert design were analyzed, yielding a Pareto chart that revealed neither pH nor time significantly impacted the design. This indicates that any point within the experimental region can be considered optimal for further experimentation. However, visual analysis of the response surface revealed that experiment 3, with pH 11 and 30 min reaction time, yielded the optimal response, closely approaching the ideal value, as shown in Figures 4(a) and (b).

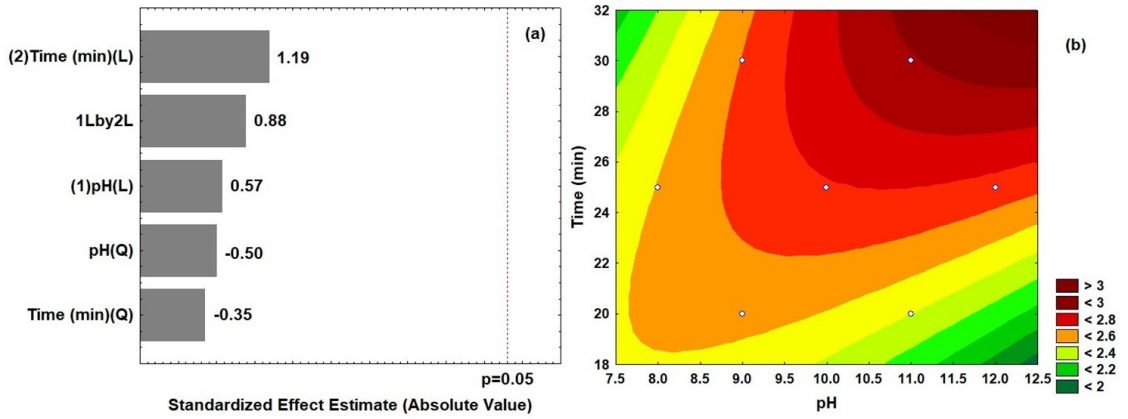

Figure 4: (a) Pareto chart and (b) contour surface chart, obtained for the Doehlert design, with pH and time variables.

The Doehlert design results in a quadratic model, which was validated through analysis of variance (ANOVA). The lack-of-fit test revealed a non-significant p-value (0.374) > 0.05, indicating that the model adequately explains the experimental data. Additionally, ANOVA revealed that pH, time, and their interactions were non-significant (p-values > 0.05). Given the absence of significant variables, experiment 3 was selected, consistent with the contour surface chart findings. Consequently, pH (11) and 30 min waiting time were established as the optimal conditions for subsequent experiments.

3.3 Evaluation of analytical method performance

Calibration curves for copper determination were constructed using a 100 µg mL^{-1} copper standard solution (prepared from copper sulfate) over a concentration range of 1.0-10 µg mL^{-1}. The data was processed, results in RGB channel and UV/Vis values, with the corresponding figures of merit summarized in Table 3. Calibration curve equations were obtained using linear regression analysis, for both digital images and UV/Vis spectroscopy, results in correlation coefficients (r) exceeding 0.99, indicating excellent linearity and correlation.

Table 3: Figures of merit for digital image and UV/Vis methods.

	R channel	G channel	B channel	UV/Vis
Equation	y = 10.846x + 66.851	y = 14.562x + 61.590	y = 10.662x + 72.224	y = 0.0055x + 0.0078
r	0.994	0.996	0.997	0.990
RSD %	2.0	2.7	3.4	1.4
LD (µg mL^{-1})	0.08	0.11	0.12	0.22
LQ (µg mL^{-1})	0.25	0.36	0.39	0.74

LD: limit of detection; LQ: limit of quantification; r: correlation coefficient; RSD: relative standard deviation.

The precision, expressed as relative standard deviation (RSD), was evaluated for each color channel: 2.0% (R), 2.7% (G), and 3.4% (B). LD and LQ were calculated according to IUPAC guidelines [26], using the following expressions: LD = 3σ/s and LQ = 10σ/s, where σ represents the standard deviation of ten analytical blank measurements, and s is the slope of the calibration curve.

The LDs obtained in this study are consistent with literature values reported for similar techniques. Pessoa et al. (2017) [13] founded an LD of 0.078 µg mL^{-1} for copper determination in sugarcane samples using complexation with cuprizone and colorimetric analysis by digital imaging (CID), where the R channel was selected for quantification. Fernandes et al. (2020) [14] also analyzed copper in sugarcane, employing complexation with diethyldithiocarbamate

(DDTC) and CID detection. Notably, they achieved a LD of 1.6 µg mL^{-1}, utilizing the B channel for quantification. Franco et al. (2021) [15] analyzed distilled beverage samples and determined copper via complexation with thiocarbazone, utilizing CID detection. The B channel was selected for analysis, results in a LD of 0.18 µg mL^{-1}. Al-Nidawi and Alshana (2021) [16] reported a LD of 0.3 µg mL^{-1} for copper determination in edible oils using complexation with N,N-(diethyl)-N'-benzoylthiourea (DEBT) and CID, with channel B selected for analysis. The coefficients of determination (R^2) values were found in this work were in agreement with the literature, in which the values were obtained were greater than 0.90, what indicated that the channels R, G and B linearly respond to these studied concentration range [13].

3.4 Analytical application

The proposed method (CID) was applied for analyzing copper in ten dietary supplement samples. Copper concentrations ranged from 278 ± 13 µg to 3530 ± 75 µg (R channel), 268 ± 14 µg to 2706 ± 275 µg (G channel), and 243 ± 2.2 µg to 2656 ± 147 µg (B channel). These values showed agreement with the reported values on product labels, with variations of 91 ± 10% to 118 ± 2.5% (R channel), 85 ± 4.2% to 109 ± 2.8% (G channel), and 62 ± 4.4% to 133 ± 7.4% (B channel). Notably, the Brazilian regulatory agency allows a ±20% tolerance for nutrient values on labels [5]. However, the B channel proved to be less suitable for analysis, since it presented considerable disagreements with the reported values by the manufacturers, which did not occur with channels R and G, as demonstrated in Table 4.

The results obtained using the CID method were compared to those from the UV/Vis method. Statistical analysis using paired t-tests (n=9, 95% confidence level) yielded calculated t-values of -1.73 (R), 1.44 (G), and 2.01 (B), all below the critical t-value (2.26), this does not necessarily imply absolute equivalence between the methods, only that, within the sample size (n=10) and observed variability, no statistically significant difference between the CID and UV/Vis methods was detected. These findings are illustrated in Figures 5(a), (b), and (c).

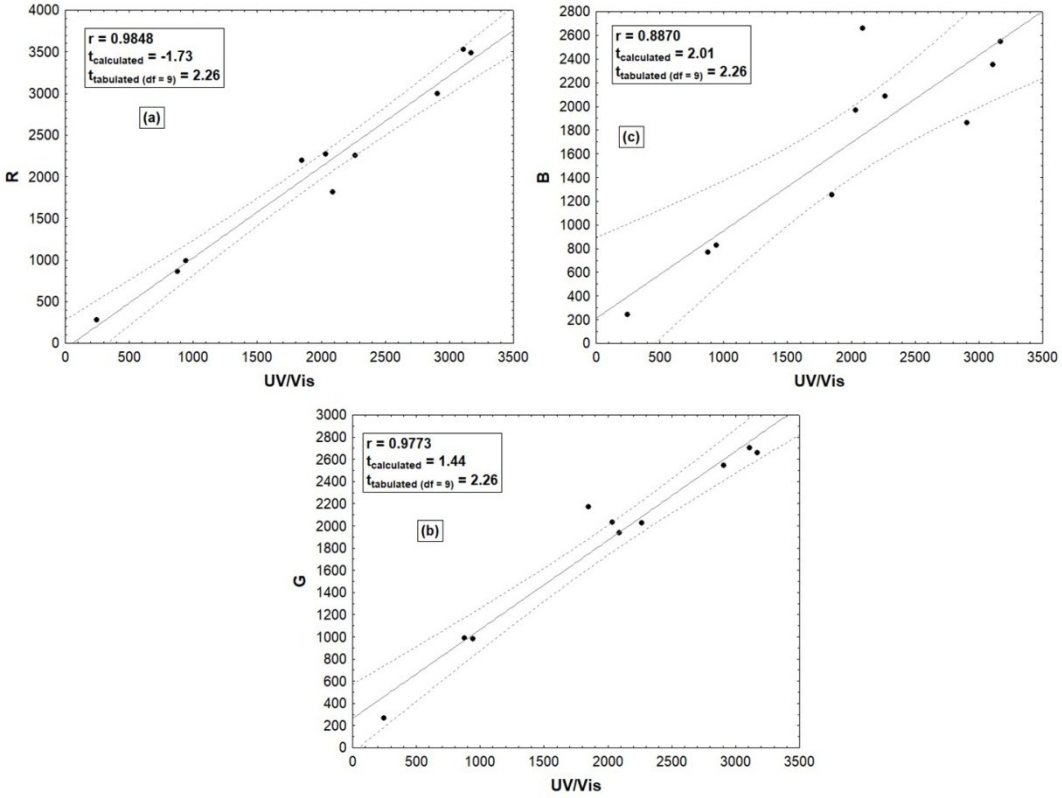

Figure 5: Comparison of UV/Vis and digital image methods for copper analysis in dietary supplements: correlation between R(a), G(b) and B(c) channels

Table 4: Agreement between manufacturer-reported concentrations and those obtained using the digital image method (R, G and B channels).

		Concentration (μg)					Agreement with informed values (%)		
Sample	**Copper source**	**Informed value**	**Real value (R)**	**Real value (G)**	**Real value (B)**	**UV/Vis**	**R**	**G**	**B**
Tablet 1	Anhydrous copper sulfate	3000	2995 ± 123	2542 ± 126	1860 ± 133	2909 ± 138	100 ± 4.1	85 ± 4.2	62 ± 4.4
Tablet 2	Copper oxide	900	861 ± 17	985 ± 25	768 ± 64	879 ± 58	96 ± 1.9	109 ± 2.8	85 ± 7.1
Tablet 3	Copper oxide	900	987 ±36	980 ± 33	827 ± 54	945 ± 91	110 ± 4.0	109 ± 3.6	92 ± 6.0
Capsule 1	Anhydrous copper sulfate	2000	2196 ± 141	2173 ± 48	1253 ± 495	1849 ± 13	110 ± 7.0	109 ± 2.4	63 ± 25
Capsule 2	Anhydrous copper sulfate	3000	3530 ± 75	2706 ± 275	2350 ± 483	3109 ± 273	118 ± 2.5	90 ± 9.2	78 ± 16
Capsule 3	Anhydrous copper sulfate	2000	2250 ± 140	2027 ± 70	2088 ± 65	2267 ± 168	112 ± 7.0	103 ± 1.6	104 ± 3.3
Capsule 4	Anhydrous copper sulfate	2000	1812 ± 196	1935 ± 116	2656 ± 147	2093 ± 93	91 ± 10	97 ± 5.8	133 ± 7.4
Capsule 5	Copper quelate	2000	2270 ± 27	2034 ± 35	1968 ± 174	2036 ± 182	113 ± 1.3	102 ± 1.8	98 ± 8.7
Capsule 6	Copper quelate	3000	3487 ± 187	2657 ± 192	2549 ± 717	3170 ± 139	116 ± 6.2	89 ± 6.4	85 ± 24
Capsule 7	Copper quelate	250	278 ± 13	268 ± 14	243 ± 2.2	247 ± 4.8	111 ± 5.2	107 ± 5.7	97 ± 0.9

This may indicate linearity issues at low concentrations or interference in color reading. Low statistical power (due to a small n) can lead to false conclusions of non-significance. An equivalence test or an increase in sample size would be recommended to confirm robustness. Strong correlations were observed, with correlation coefficients of 0.9848 (R), 0.9773 (G), and 0.8870 (B), demonstrating high agreement between the two methods, except for B channel. Channel B is close to the significance threshold, suggesting that under different conditions (more samples or greater variability), and differences could emerge. The R and G channels enabled more reliable determination of copper concentrations. The accuracy range (R: 90–119%; G: 84–118%) shows that, although there is no clear systematic bias, there is considerable variation, especially in the G channel (84% at the lower limit). It would be important to evaluate whether these variations are consistent across different concentration ranges (e.g., low vs. high copper concentrations), as colorimetric methods may lose sensitivity at extremes.

4. CONCLUSION

For the first time, this work validates an efficient analytical approach for copper quantification in dietary supplements through complexation with salicylic acid. Factorial design optimization enabled the identification of favorable conditions for forming a stable, green-colored complex (420 min). Notably, the analyzed dietary supplement concentrations aligned with the manufacturers' reported values. Combining colorimetric reactions with smartphone-based detection enables a fast, precise, accurate, and low-cost analytical solution. In this perspective, new research can be developed to improve further and expand the use of complexing agents and digital images as an analytical tool for determinations of metals in various matrices. For a more critical analysis, it is necessary to increase the sample size, evaluate different concentration ranges, as well as residual analysis and alternative regression models.

5. ACKNOWLEDGEMENT

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