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Evaluation of tartrazine (E-102) content in orange-flavored soft drinks marketed in Guayaquil using UV–Visible spectrophotometry.

Evaluación del contenido de tartrazina (E-102) en bebidas refrescantes sabor naranja comercializadas en Guayaquil mediante espectrofotometría UV-Visible.

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Abstract.
Tartrazine (E-102) is a synthetic colorant widely used in flavored beverages to improve their appearance. Its use must be controlled due to potential adverse effects associated with excessive consumption. The objective was to quantify the presence of tartrazine. (E-102) in orange-flavored non-carbonated soft drinks marketed in supermarkets in Guayaquil and to evaluate compliance with internationally established permissible limits. The applied experimental study, with a quantitative approach. Twelve samples of orange-flavored non-carbonated soft drinks from three commercial brands were collected through stratified sampling in supermarkets in Guayaquil. Tartrazine quantification was performed using UV–Visible spectrophotometry at 427 nm. A calibration curve was prepared using standard solutions, and the method was validated by quantifying its precision, accuracy, repeatability, and linearity. The average tartrazine concentrations obtained were 134.85 mg/L A, 99.45 mg/L B, and 54.61 mg/L C, by beverage brand, respectively. The results showed that samples corresponding to brand A exceeded the maximum limit of 100 mg/L established by international organizations. In brand B, values close to the regulatory limit were analized, whereas brand C showed concentrations considerably lower than the permitted value. After subjecting the findings to the Shapiro–Wilk test, data obtained from the statistical analysis, revealing variability in tartrazine content among the analyzed brands. One of the analyzed brands exceeded the permissible limits of tartrazine, highlighting the need to strengthen the control of additives in beverages.

Keywords.
Tartrazine, UV-Visible Spectrophotometry, Flavored Beverages, Food Colors

Resumen.
La tartrazina (E-102) es un colorante sintético utilizado en bebidas saborizadas para mejorar apariencia. Su uso debe ser controlado debido a posibles efectos adversos asociados al consumo excesivo. El objetivo fue cuantificar la presencia de tartrazina (E-102) en refrescos de naranja sin gas comercializadas en expendios de Guayaquil evaluando su cumplimiento con límites permisibles establecidos internacionalmente. El estudio experimental aplicado, con enfoque cuantitativo. Se recolectaron doce muestras de bebidas refrescantes no carbonatadas sabor naranja pertenecientes a tres marcas comerciales, mediante muestreo estratificado en supermercados de Guayaquil. La cuantificación de tartrazina empleando espectrofotometría UV-Visible a 427 nm. La curva de calibración se elaboró con patrones de referencia y se evaluó el desempeño analítico del método mediante parámetros de linealidad, exactitud, precisión y repetibilidad. Las concentraciones promedio de tartrazina obtenidas fueron 134.85 mg/L para la marca A, 99.45 mg/L para la marca B y 54.61 mg/L para la marca C. Los resultados evidenciaron que las muestras correspondientes a la marca A superaron el umbral máximo de 100 mg/L establecido por organismos internacionales. En la marca B se observaron valores cercanos al límite regulatorio, mientras que la marca C presentó concentraciones considerablemente inferiores al valor permitido. Tras someter los hallazgos al test de Shapiro-Wilk, los resultados del procesamiento estadístico revelaron que la muestra no seguía una distribución normal lo que evidenció variabilidad en el contenido de tartrazina entre las marcas analizadas. Una de las marcas analizadas superó los límites permisibles de tartrazina, evidenciando la necesidad de fortalecer el control de aditivos en bebidas.

Palabras clave.
Tartrazina, Espectrofotometría UV-Visible, Bebidas saborizadas, Colorantes alimentarios

1. Introduction

The growing consumer demand for visibly more attractive products, synthetic food colorants have been developed for use in food production, in order to improve certain aspects of the product. To achieve consumer attention, therefore, every food product must mention on the label that it contains artificial substances such as Tartrazine, because there are studies where this additive can cause complications in a low number of people who suffer from diseases such as asthma and attention deficit disorders, the latter more likely in children. Many countries do not allow the use of this dye and others control it more rigorously. Tartrazine is one of the dyes that raise serious consumer safety concerns at low doses relevant to real-life human exposure. The scientific literature continues to grow following the re-evaluation by the European Food Safety Authority (EFSA) in 2009 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2016. [1] [2]

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[3]Food additives, indispensable elements in modern diets, are widely used in restaurants to align with the demands of the consumer market and improve their gastronomic experience. However, the potential risks associated with excessive or prolonged consumption cannot be overlooked. [4]The Codex Alimentarius defines food additives as a substance that is deliberately incorporated into foodstuffs for the purpose of fulfilling specific technical functions (including organoleptic functions) in the manufacture, processing, treatment, packaging, storage or transport of that food. However, they stressed that the definition given by the Codex Alimentarius does not include the term contaminants or substances added to foods to maintain or enhance nutritional qualities. According to some studies, there were several food additives that had been used in the past, but are no longer authorized due to the evidence found of their side effects, a high frequency of possible health incidents, as well as medium and long-term toxicity. It is also important to note that, apart from synthetic food colourings, certain commercial additives of plant or animal origin have also been discontinued. The classification of naturally occurring dyes can be very complex due to the wide variety of intrinsic properties of the coloring substances. These can come from various natural sources and therefore have a wide range of chemical compositions that affect their properties, solubility and stability differently, and can have different origins, whether plant or animal. [5][6][7]

The reasons why this colorant is quantified are due to the high per capita consumption of flavored beverages in Ecuador, occupying the third place behind carbonated beverages and bottled water, likewise, within sugary drinks with 83% in the commerce of stores and supermarkets in the city of Guayaquil, in addition to the little control that exists of the national entities in the use of the colorant, since in the category of this beverage there is no maximum limit of the concentration of the colorant stipulated by the INEN regulations, who is the national regulation in controlling the use of additives in food. . [8]

Due to concerns about these potential health effects, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) have established internationally recognized standards in the Codex Alimentarius on the maximum allowable levels of this colorant depending on the type of food or beverage. [9]In addition, from a regulatory point of view, there is a growing attention and interest in the risk assessment of these dyes used in food products (i.e. azo dyes). In the case of azo dyes, a limiting factor for their use is their carcinogenicity potential, which occurs after their reduction to carcinogenic metabolites in the intestine. [10]

In recent research, there is consensus that synthetic food dyes have diverse impacts on consumers, especially the pediatric population, due to their influence on sensory appeal, which can encourage a preference for certain foods. The results revealed that these color additives are directly linked to a number of health problems, with a greater impact on children. [11]

In some studies, the identification and quantification of dyes in different beverages of mass consumption is carried out, using methods such as thin layer chromatography and other visible spectrophotometry in a reliable way to determine that they comply with permissible limits established in the regulations. A variety of matrix-dependent methods have been developed and applied to determine food dyes, employing different analytical techniques along with appropriate sample preparation protocols. The main techniques used for its determination are chromatography with spectrophotometric detectors and spectrophotometry, while sample preparation procedures depend largely on the food matrix. In food technology, food colourings of different origins are chemical substances that are incorporated into food matrices to maintain or enhance the organoleptic characteristics of the product, which may be affected or lost during processing or storage, and to preserve the desired colour. The most common and widely used classification is based on the distinction between soluble and insoluble coloring additives (dyes or pigments), which in turn can be categorized as natural or synthetic.[12][13] [14]

In recent years, research on the detection and quantification of synthetic food dyes has evolved towards the treatment of sensitive, analytical, selective and sustainable techniques. Recent studies in high-impact journals have reported the use of advanced methods such as electrochemical sensors based on nanomaterials, spectroscopy coupled to intelligent systems, and high-performance liquid chromatography with improved detectors, which allow the detection of tartrazine at trace levels with high accuracy and shorter analysis time. For example, recent research has shown that electrochemical sensors modified with nanoparticles have significantly lower detection limits compared to conventional methods, while techniques such as UHPLC coupled to spectrophotometric detectors continue to be the standard for analytical validation in complex matrices. [15] [16]Likewise, there has been a growing interest in more sustainable and low-cost methodologies, such as optimized UV-Visible spectrophotometry, which continues to be widely used in contexts where accessibility is required without compromising analytical reliability. These trends reflect the need to balance accuracy, cost, and applicability in the monitoring of food additives in consumer products.

It is evident that the analysis of trace food dyes is essential with the application of the appropriate analytical techniques, with high specificity and selectivity. Several studies have reported that there is a growing interest in monitoring the concentration of synthetic food dyes in various products. Therefore, this research aims to

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determine the concentration of Tartrazine (E-102) or also called Yellow No. 5 in non-carbonated orange flavored beverages refreshed by UV-VIS. UV/VIS spectrophotometry. [17]

1.1. Food additives

These additives are incorporated to improve the appearance and preservation of the product. Although some provide nutrients, their main function is technological and sensory, anticipating the consumer's experience of nutritional value.[18]

Its inclusion in processing such as production and transportation or storage seeks to stabilize the product. These additives reinforce conservation against environmental or microbial factors, preventing or delaying spoilage and guaranteeing food safety. [19]

1.2. Dyes

From 400 B.C. When the Egyptians colored wine, the visual aspect has conditioned food decisions. Chemically, this occurs thanks to two groups: the chromophore, responsible for the tone, and the auxochrome, which allows it to be fixed. Until the nineteenth century, people relied solely on natural sources to stimulate that sense of desire and freshness that color brings to consumers. [20]

1.3. Designation "E" in Dyes

To supply the food trade with additives, the European Union implemented a system of codes (letter E followed by 3 or 4 digits). These numbers allow the chemical name and function of each substance to be equated, and it is mandatory to detail them on the labelling to ensure transparency and differentiate each ingredient unequivocally. For example: E1- colorant, E4- emulsifiers, stabilizers, thickeners and gelling agents, E3- antioxidants, E2- preservatives [21]

1.4. Classification of dyes according to their nature

1.4.1. Natural Colors

From animal, vegetable or mineral sources, these dyes stand out for their safety. As they lack sanitary restrictions and do not require certifications, they are freely included in the production of cosmetics, pharmaceuticals and food, consolidating themselves as a safe and versatile option in various industrial sectors compared to synthetic options.. [22]

During product production processes, the instability of natural dyes could degrade in the presence of high temperatures, inadequate storage, this leads to changes in water activity and pH and even to be photoensible. [23]

1.4.2. Synthetic Dyes

Known as artificial, which are obtained from chemical synthesis. These dyes were widely used at the beginning of the nineteenth century, starting in 1826. By decomposing indigo, the German researcher Unverdorben isolated aniline compounds for the first time, marking a milestone in the study of this dye. This is how it made room for the manufacture of many artificial colorants. Due to the low cost and greater dyeing and glossing power in the products, synthetic dyes stand out, however, in some studies harmful effects have been reported in infants affecting their behavior and generating attention deficit and hyperactivity. Therefore, in some European countries, the use of artificial colors has been restricted, giving priority to the use of colorants of natural origin, while in others it has been regularized by determining permissible limits established by the FDA [24] [25]

1.5. Dyes as food additives

The Food and Drug Administration (FDA) defines dyes and pigments as those compounds obtained through artificial synthesis or extracted directly from mineral, vegetable, or animal sources. The essential feature of these substances is their intrinsic ability to transfer color to cosmetic products, drugs, or foods, regardless of whether their original chemical structure underwent modifications during the process. [26]

1.6. Synthetic dye Tartrazine E-102

Tartrazine is a synthetic azo dye with a lemon-yellow coloration used as a food pigment (E102), also called "Food Yellow Cl 4". Azo food dyes are synthetic and come from aromatic amines, which contain an azo group of two nitrogen atoms bonded together (-N = N-) and unified to aromatic rings. [27] [28]
The average daily intake of tartrazine was established at 7.5 mg/kg [28]

1.7. Chemistry of the dye Tartrazine

The N5 yellow with mass is 534.4 g/mol and its chemical formula C16H9N4Na3O9S2, and structurally it has an azo group (–N=N–), joined by aromatic rings, tartrazine is therefore recognized as belonging to the azo dye family. [29]

1.8. Toxicology of the dye Tartrazine

E-102 or yellow E-5, is a very controversial dye since other studies showed that they could cause, respiratory conditions, allergic reactions even hyperactivity in children, since then, the maximum permissible limits and the daily intake which does not affect people's health, were investigated in laboratory rats and thus maintain control in the use of this food additive and that it is reported in the formulation and it is demonstrated on the label that contains tartrazine. [30]

1.9. Non-carbonated beverages

That which does not contain carbon dioxide in its composition, produced from drinking water that complies with the safety parameters established in Ecuadorian standards, and may contain sugar such as sweeteners, synthetic flavorings or of natural origin. [31]

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1.10. Regulations for non-carbonated beverages of the colorant Tartrazine E-102

In Ecuador, the concentration of the colorant Tartrazine in non-carbonated beverages or according to the INEN 192 Standard "Flavored water-based beverages without gas, including fruit punches and lemonades and similar beverages", is not established in the regulation, Only NTE INEN 2304 of "Soft drinks or non-carbonated beverages. Requirements", there is no description of the concentration of tartrazine, it only indicates that it must not exceed the food additives established by the INEN 192 standard, which does not establish the maximum permissible limits for this colorant. In other similar beverages, in the NTE INEN 1101 version 2016 "Carbonated or carbonated beverages. Requirements", if it establishes a maximum concentration of 200mg/L of Tartrazine in its composition. The insufficient control of national regulations in this type of beverage leads to an unrestricted problem when using additives, causing a possible risk to the health of users. [32]

2. Materials and methods.

The research used as main materials orange-flavored non-carbonated soft drinks of three commercial brands, acquired in supermarkets in different sectors of the city of Guayaquil. Analytical reagents were used for the preparation of standard tartrazine (E-102) solutions, as well as distilled water and conventional laboratory equipment, including volumetric flasks, volumetric pipettes, and quartz cells. The instrumental analysis was used using a UV-Visible spectrophotometer, operated at 427 nm wavelength, specifically chosen because it coincided with the peak of maximum absorbance in the detection of the dye.

The methodology was experimental with a quantitative approach. A calibration curve was constructed from standard solutions of tartrazine, allowing the relationship between concentration and absorbance to be established. The samples were previously prepared and analyzed under controlled conditions, recording their absorbance values to determine their concentration by interpolation in the curve. Additionally, the performance of the analytical method was evaluated through parameters of linearity, accuracy, precision and repeatability. The data obtained were subjected to statistical analysis to verify their behavior and variability. The analysis and management of the experimental data were carried out with Microsoft Excel software**,** using integrated statistical tools for the calculation of parameters such as standard deviation, mean, coefficient of variation and linear regression analysis. The reproducibility of the method was evaluated from repeated measurements under controlled conditions, verifying the consistency of the results by analyzing the intra-assay variability. Although Excel is a general-purpose tool, its application in this study was adequate due to the characteristics of the data obtained and the type of analysis required, allowing reliable results to be obtained and comparable to the criteria established in the validation of analytical methods.

2.1. Raw material

The raw material analyzed was made up of orange-flavored non-carbonated soft drinks, corresponding to three commercial brands available in commercial premises in the city of Guayaquil. The samples were selected considering their high availability and consumption in the local market, guaranteeing representativeness. Units were acquired in different establishments located in different geographical areas, in order to minimize biases associated with a single point of sale. The products were in original sealed containers, within their expiration date and stored under normal commercial conditions.

The selected beverages presented similar physicochemical characteristics in terms of coloration, transparency and type of formulation, being homogeneous liquid matrices suitable for direct spectrophotometric analysis. The composition declared on the labeling included the presence of tartrazine (E-102) as an artificial color. Prior to the analysis, the samples were conditioned at room temperature and gently homogenized to ensure the uniformity of the system. No complex preparation treatments were performed, given the liquid nature of the matrix, which allowed adequate reproducibility in the analytical measurements.

2.2. Elaboration of the Mother Solution

a. The standard containing 99.0% to 100% purity was adjusted.
b. The standard of 10mg = 10.10mg can be adjusted to 100%. Purity
c. 10.10 mg standard weight of tartrazine dye on the analytical balance, and dissolved in a volume of 100ml, with distilled water it is taken to the gauging line.
d. Stays at room temperature and protected from light

2.3. Construction of the calibration curve

a. Starting from the stock solution, 5 dilutions with different concentrations of 6, 12, 18, 24, 30 mg/L were prepared in 10ml volumetric flasks by volume with distilled water.
b. The spectrophotometer is calibrated to 427nm, which corresponds to the maximum absorption of the dye at that wavelength. The concentrations of solutions were read in an ascending manner, using distilled water as a target.
c. By means of a scatter plot, absorbance vs. concentration was related
d. The equation of the curve line was determined.

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2.4. Verification of the analytical method by UV-VISIBLE spectrophotometry

2.4.1. Selected Wavelength

For the colorant Tartrazine in beverages, the maximum wavelength was established in previous studies, which established 427nm as the maximum wavelength. [33]

2.4.2. Specificity

Matrix Effect Study. The method used in addition to the standard. Choosing a non-carbonated beverage that encompasses the possible interferents of the matrix and that does not contain the coloring that Tartrazine.

A 1:10 dilution in a 100ml volumetric flask from the selected drink.
The same aliquots of the tartrazine stock solution were taken, which was used to construct the calibration curve, and diluted at various concentrations of 6, 12, 18, 24 and 30 mg/L, in the volume filling with the 1:10 dilution of the chosen beverage.
The spectrophotometer is calibrated and read at 427nm wavelength. The solutions were read in ascending concentration, using the 1:10 dilution as the target of the selected drink.
The equation of the curve line was determined. [34]
Statistically, the effect curve was compared with the calibration curve matrix of the standard by means of the T-student test with the Microsoft Excel Data Analysis tool for related samples.

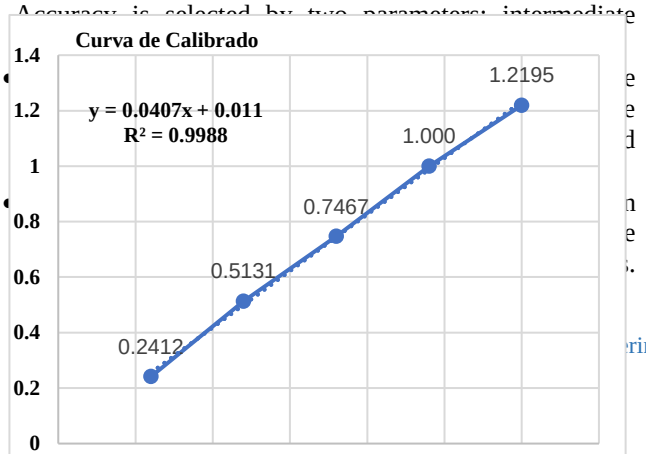
2.4.3. Accuracy.

According to the following steps:
With a flask without the analyte of interest, it is fortified with the standard tartrazine dye at three concentration levels. Being tall. medium and low in the order of 6, 18 and 30 mg/L respectively.
With the data collected from the calibration curve, the percentage of recovery carried out with the fortified matrix was calculated.
The reliability of the method was verified by the percentage of recovery, evaluated under the international AOAC criteria. For this calculation, the mathematical relationship was applied that allows the amount of analyte recovered to be contrasted with the real value added, thus guaranteeing the accuracy of the analysis:

$$\%\text{Recuperación} = \frac{\text{Valor obtenido } (\bar{x})}{\text{Valor real}} \times 100 \qquad (1)$$

2.4.4. Precision.

Accuracy is selected by two parameters: intermediate



- **Calculate** from the data obtained from intermediate accuracy and repeatability with the statistical parameters below:

$$\%\ \text{CV} = \frac{\text{S}}{\text{X}} \times 100\% \qquad (2)$$

X: Medium
S: Standard deviation.
% CV: coefficient of variation

The method is more accurate the lower the %CV, so according to the acceptance limit of this parameter it must be less than 2% [35]

2.4.5. Sample reading using UV/VIS spectrophotometer.

1. The samples were labeled according to the sector obtained.
2. 1:10 dilutions were prepared in 25ml volumetric flasks, flushed to volume with distilled water for each drink
3. It was performed in triplicate, the readings were timated, at 427nm wavelength.
4. The concentration of tartrazine from the equation of the line was determined in the samples, and multiplied by the dilution factor 1:10.

2.4.6. Statistical analysis of the samples.

1. SPPS Statistics was applied
2. Shapiro-Wilk test for each sample was applied
3. For each sample per beverage brand, the Shapiro-Wilk test was applied
4. Linear regression and correlation analysis were determined for each sample by beverage brand.

3. Results.

3.1. Construction of the Calibration Curve

The calibration curve was based on other studies with modification of the last point of the curve.

Table 1. Absorbance vs Tartrazine Concentration

CONCENTRATION OF TARTRAZINE mg/L	ABSORBANCE (ABS)
6	0.2412
12	0.5131
18	0.7467
24	1.000
30	1.2195

Source: Bonilla, et al, 2026

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Figure 1. Calibration curve for the tartrazine standard.

3.2. Testing an analytical method using a UV-VISIBLE spectrophotometer

3.2.1. Specificity

Matrix Effect Study. He calculated the specificity of the method, adding the Standard to a blank matrix.

Table 2. Concentration of tartrazine in fortified matrix vs Absorbance
Source: Bonilla, et al, 2026

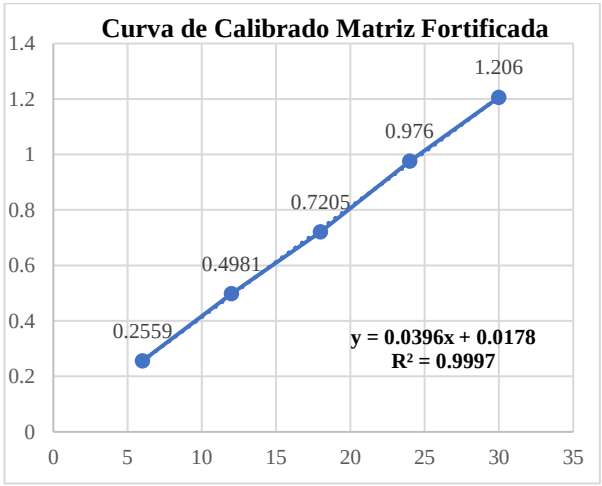

Figure 2. Fortified Matrix Calibration Curve

The evaluation of the specificity of the method by studying the matrix effect was carried out through a calibration curve in a fortified matrix, obtaining the linear relationship between the concentration of tartrazine. And absorbance. The equation of the line (y = 0.0396x + 0.0178) presented a high coefficient of determination (R^2 = 0.9997), this evidences the excellent linear correlation in the range of concentrations analyzed. This behavior indicates that the concentration of the analyte is directly proportional to the response of the method even in the presence of components of the matrix itself.

Likewise, the low dispersion of the experimental points with respect to the regression line suggests that there is no significant interference of the matrix in the determination of tartrazine. The near-zero intercept value (0.0178) confirms the minimal contribution of background signals or possible interfering compounds. Together, these results demonstrate that the method is specific for the quantification of tartrazine in the evaluated matrix, ensuring that the analytical signal obtained corresponds specifically to the analyte of interest.

The selectivity of the method was assessed by comparing the absorbances of a pure calibration curve and a matrix fortified with the same doses of tartrazine. By means of a unilateral student T-test, it was examined whether the matrix induced a significant increase in the signal, thus guaranteeing that the results obtained depended exclusively on the dye and not on external interferers. The hypotheses were evaluated:

Hi: If there is a significant difference

Ho: There is no significant difference

Decision: If $t_{critical} > t_r$ Ho is admitted, since there is no significant difference.

Table 3. Unilateral T-Student Test of Related Samples

Concentration of tartrazine mg/L	Absorbance (ABS)
6	0.2559
12	0.4981
18	0.7205
24	0.9760
30	1.2060

Prueba t-student	Abs. Standard curve	Abs. Fortified curve
Media	0.7441	0.7314
Variance	0.1495	0.1415
Observations	5	5
Pearson's correlation coefficient	0.9995	
Hypothetical difference of the means	0	
Degrees of freedom	4	
Tr Statistic	1.7528	
P-value a tail	0.0773	
Critical value of tcritic (one queue)	2.1318	

Source: Bonilla, et al, 2026

The value of t_r is 1.7528 and the critical value t is 2.1318, since critical t is greater than t_r, because the null hypothesis (Ho) was admitted. Therefore, no significant difference is observed in the absorbance means, and the matrix does not influence the readings in the absorbance spectrophotometer considered a specific method.

3.2.2. Accuracy.

The accuracy of the method was demonstrated by the percentage of recovery of the fortified matrices. The concentrations analyzed were made in triplicate at three concentration levels: high, medium and low of the fortified matrices.

Table 4. Percentage of recovery of fortified matrices.

Conc. Mg/l	Absorbance			Prom.	Conc. Obtained	% Recovery
6	0.2432	0.2423	0.2423	0.2426	5.6768	94.6128
18	0.7405	0.7408	0.7414	0.7409	18.2601	101.4450

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30	1.2018	1.2035	1.2024	1.2026	29.9184	99.7278

Source: Bonilla, et al, 2026

The recovery study showed an adequate performance of the analytical method in the evaluated matrix. For the concentration levels of 6, 18 and 30 mg/L, recovery percentages of 94.61%, 101.45% and 99.73%, respectively, were obtained, values that are within the acceptable range for spectrophotometric methods. Likewise, the concentrations obtained (5.68, 18.26 and 29.92 mg/L) showed a high agreement with the theoretical concentrations, which indicates good accuracy of the method. The low variability between absorbance measurements reflects adequate accuracy. Overall, these results demonstrate that the method is reliable for the quantification of tartrazine in the analyzed matrix, without evidencing significant interferences that affect its recovery.

3.2.3. Precision.

Quantification was assessed using the Intermediate precision and repeatability method. The precision of the method was established by Standard Deviation (SD) and the criterion was admitted with the percentage of the Coefficient of variation (CV%).

3.2.3.1. Repeatability.

The readings were made in triplicate from three concentration levels of the standard's control solutions, with the same laboratory, equipment and analyst in a short period of time.

Table 5. Repeatability at 3 concentration levels.

Conc. Mg/l	Absorbance			Prom.	Ds	Cv%
6	0.2422	0.2369	0.2447	0.2413	0.0040	1.6508
18	0.7335	0.7458	0.7491	0.7428	0.0082	1.1068
30	1.2162	1.2262	1.2110	1.2178	0.0077	0.6344

Source: Bonilla, et al, 2026

The percentage of the coefficient of variation is located below the acceptance criterion in the 3 levels (low, medium and high), which must be <2%, for the first level it is 1.6508%, for the second level it is 1.1068% and finally the third level is 0.6344%.

3.2.3.2. Intermediate precision.

It was determined starting from the highest level of concentration, in three days the readings in triplicate, by the same laboratory, equipment and different analysts.

Table 6. Assessment of Intermediate Accuracy at the highest concentration level

Conc. mg/L	Absorbance			Prom.	Ds	Cv%
	Day 1	Day 2	Day 3			
30	1.2392	1.239	1.2162	1.2277	0.0118	0.9603
	1.2193	1.2199	1.2262			
	1.2434	1.2355	1.211			

Source: Bonilla, et al, 2026

At the highest concentration level, the standard deviation is 0.0118 and the coefficient of variation is 0.9603%, which is accepted as it must be <2%. Complying with the two parameters, it is evident that the method is Precise.

3.3. UV-VISIBLE spectrophotometric determination of Tartrazine in non-carbonated orange-flavored soft drinks.

Each sample was labeled following the collection parameters, which are assigned the codes for the respective marks, being A, B and C followed by the first letter of the selected sector, being N (North), S (South), O (WEST) and E (EAST), finally, numbered from 1 to 4 to distinguish from the chosen lot. To determine the concentration of Tartrazine in orange-flavored soft soft drinks, using the equation of the line arranged by the calibration curve and the response was multiplied by the Dilution Factor (FD), as follows:

$$y = mx + b \quad (3)$$

Example: In the sample with the code "AN1" it was analyzed as follows:

$$0.5712 = 0.0407x + 0.0110 \quad (4)$$

$$x = \frac{0.5712 - 0.0110}{0.0407} * 10 \quad (5)$$

$$x = 137.6413\ mg/L \quad (6)$$

Table 7. Concentrations of Tartrazine in samples of non-carbonated soft drinks.

Code	Tartrazina mg/L	Code	Tartrazine mg/L	Code	Tartrazine mg/L
AN1	137.6413	BN1	96.6585	CN1	55.6020
AN2	133.8329	BN2	96.5111	CN2	55.0614
AN3	136.6830	BN3	93.8329	CN3	55.2334
AS1	138.8206	BS1	99.8034	CS1	57.9361
AS2	139.2875	BS2	99.6069	CS2	58.4275
AS3	138.9926	BS3	99.5577	CS3	57.6904
AE1	133.0958	BE1	100.0491	CE1	52.2359
AE2	132.7273	BE2	99.4103	CE2	51.0319
AE3	132.3833	BE3	98.9189	CE3	50.8600
AO1	132.5799	BO1	103.5872	CO1	54.1523
AO2	131.9410	BO2	103.2678	CO2	53.6118
AO3	130.1794	BO3	102.1450	CO3	53.4398
Prom.	134.8471	**Prom.**	99.4457	**Prom**	54.6069
DS	3.2178	**DS**	2.8242	**DS**	2.5545
CV%	2.2938 %	**CV%**	2.8399 %	**CV%**	4.6779 %

Source: Bonilla, et al, 2026

The study demonstrated critical contrasts between the brands analyzed. The "A" mark systematically exceeds the

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limit of 100 mg/L stipulated by the FAO and the regulations of Colombia and Mexico, complying only with Salvadoran legislation, which is less restrictive. On the other hand, the "B" mark presents an irregular conformity, exceeding international limits only in its first three samples (BO1-BO3). In contrast, the "C" brand stood out as the only product that respects global standards in all of its samples, ensuring responsible use of tartrazine and compliance with food safety specifications.

3.4. Statistical Analysis of Orange-flavored non-carbonated soft drinks.

3.4.1. Shapiro - Wilk.

It was statistically analyzed with the SPSS Statistics to check if the variable (Tartrazine Concentration) fits a normal distribution, the acceptance criteria for the hypothesis are as follows:

Hi: The samples do not have normal distribution. (Non-parametric data)

Ho: Samples have normal distribution (Parametric Data)

Table 8. Shapiro-Wilk Normality Test

1.	**Hypothesis**	Ho: the variable "tartrazine" comes from a normal population Hi: the variable "tartrazine" does not come from a normal population
2.	**Significance**	a – 5% – 0.05
3.	**Calculated Value**	0.846
4.	**P - value**	p= <0.001
5.	**Decision**	If p > α I accept my Ho, otherwise Hi is accepted 0.001< 0.05, because the Ho
6.	**Conclusion**	The variable "Tartrazine" does not come from a normal population, so the data are non-parametric.

Source: Bonilla, et al, 2026

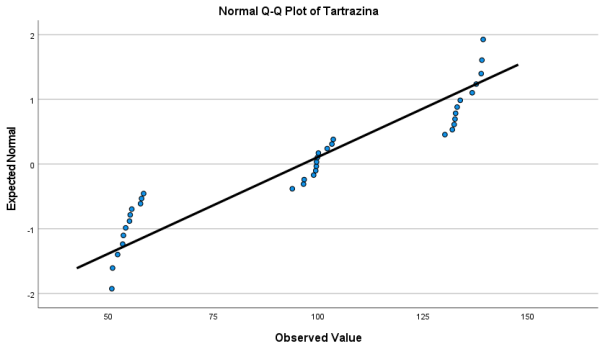

Figure 3. Normality of tartrazine

Figure 3 shows the normal Q-Q graph of tartrazine showing the comparison between the expected values and the values observed under a normal distribution. Almost all points are seen to line up close to the baseline, suggesting a general trend towards normality of the data. However, slight deviations are evident at the extremes, particularly in the lowest and highest values, indicating possible asymmetries or the presence of variability in the data. Overall, although the data show some adjustment to the normal distribution, these deviations justify that normality is not completely met, which is consistent with the data obtained by the Shapiro-Wilk test.

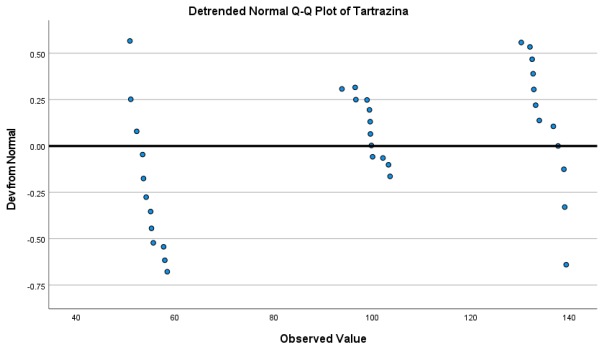

Figure 4. Normality without tartrazine tendency

Figure 4 shows the normal Q-Q graph with detrend complements the interpretation of Figure 3 Q-Q above by showing the deviations of the data from the normal distribution as a function of the baseline (zero). In this case, it can be seen that the points are not randomly distributed around the horizontal line, but present systematic patterns: negative values grouped at the lower end and positive values at the upper end. This behavior confirms the presence of deviations from normality, evidencing asymmetry in the distribution of the data. In accordance with the previous Q-Q graph, where slight separations of the line at the ends were already visible, the detrend graph reinforces that these deviations are not random but structural. Therefore, both graphs support the result of the Shapiro-Wilk test, indicating that the data do not have a normal distribution and present variability associated with the differences between concentrations of the samples analyzed.

3.5. Statistical analysis on each brand of non-carbonated orange flavored refreshing drink.

Using SPSS Statistics, the results obtained from each brand of beverages were statistically analyzed independently, by means of the Shapiro-Wilk test, it was determined if the data are non-parametric or parametric and to use an adequate analysis of Correlation between the two variables (Dependent: Absorbance and Independent: Tartrazine coloring), evidencing the degree of variance or relationship found between the samples, culminating with the linear regression analysis for Forecast the value of one variable from another.

3.5.1. Statistical analysis: Brand "A" soft drink

3.5.1.1. Shapiro-Wilk.

Criteria for the admission of the hypothesis:

Ho: The samples have a normal distribution. (Parametric Data)

Hi: The samples do not have a normal distribution. (Non-parametric data)

Table 9. "Shapiro-Wilk" normality test for "A" brand beverage

1.	**Hypothesis**	Ho: the variable "tartrazine" comes from a normal population Hi: the variable "tartrazine" does not come from a normal population

2.	**Significance**	a – 5% – 0.05
3.	**Calculated Value**	0.884
4.	**P - value**	p= .098
5.	**Decision**	If p > α I accept my Ho, otherwise Hi is accepted 0.098 > 0.05, so Ho
6.	**Conclusion**	The variable "Tartrazine" in Brand "A" of soft drink comes from a normal population, therefore, the data are Parametric.

Source: Bonilla, et al, 2026

3.5.1.2. Linear correlation.

The drink with brand "A" corresponds to parametric data (they adjust to a normality), so a Pearson Correlation analysis was done

Table 10. Correlation of beverages with "A" brand

Correlation			
		Abs	**Tartrazina**
Abs	Pearson Correlation	1	0.997**
	Sig. (1-tail)		<0.001
	N	12	12
Tartrazina	Pearson Correlation	0.997**	1
	Sig. (1-tail)	<0.001	
	N	12	12
**. The correlation is significant at the 0.01 (1-tail) level.			

Source: Bonilla, et al, 2026

A positive correlation between the two variables greater than 0 and close to 1 is observed, being 0.997, so a high degree of relationship between them is found.

3.5.2. Statistical analysis: Brand "B" soft drink

3.5.2.1. Shapiro-Wilk.

Criteria for acceptance of the hypothesis:
Ho: The samples follow a normal distribution. (Parametric Data)
Hi: Samples do not tend to a normal distribution (Non-parametric data)

Table 11. "Shapiro-Wilk" normality test for brand "B" soft drink

1.	**Hypothesis**	Ho: the variable "tartrazine" comes from a normal population Hi: the variable "tartrazine" does not come from a normal population
2.	**Significance**	a – 5% – 0.05
3.	**Calculated Value**	0.942
4.	**P - value**	p= .524
5.	**Decision**	If p > α I accept my Ho, otherwise Hi is accepted 0.524 > 0.05, therefore Ho is accepted
6.	**Conclusion**	The variable "Tartrazine" in the Marde ca "B" soft drink comes from a normal population, therefore, the data are Parametric.

Source: Bonilla, et al, 2026

3.5.2.2. Linear correlation.

The brand "B" soft drink is parametric data fits a normality), therefore, a Pearson Correlation analysis was performed.

Table 12. Correlation of "B" brand soft drinks

Correlation			
		Abs	**Tartrazina**
Abs	Pearson Correlation	1	0.996**
	Sig. (1-tail)		<0.001
	N	12	12
Tartrazina	Pearson Correlation	0.996**	1
	Sig. (1-tail)	<0.001	
	N	12	12
**. The correlation is significant at the 0.01 (1-tail) level.			

Source: Bonilla, et al, 2026

Between the two variables, a positive correlation close to 1 and greater than 0 is demonstrated, with a value of 0.996, since a high degree of relationship is established between the samples.

3.5.3. Statistical analysis: Refreshing drink Brand "C"

3.5.3.1. Shapiro-Wilk.

Criteria for acceptance of the hypothesis:
Ho: The samples tend to be distributed normally. (Parametric Data)
Hi: The samples do not follow a normal distribution. (Non-parametric data)

Table 13. "Shapiro-Wilk" normality test for the "C" brand soft drink

1.	**Hypothesis**	Ho: the variable "tartrazine" comes from a normal population Hi: the variable "tartrazine" does not come from a normal population
2.	**Significance**	a – 5% – 0.05
3.	**Calculated Value**	0.945
4.	**P - value**	p= .576
5.	**Decision**	If p > α admits my Ho, otherwise Hi is accepted 0.098 > 0.05, so the Ho
6.	**Conclusion**	The variable "Tartrazine" in the "C" Brand beverage comes from a normal population, therefore, the data are Parametric.

Source: Bonilla, et al, 2026

3.5.3.2. Linear correlation.

The "C" mark of beverage is parametric data (they follow a normality), so a Pearson Correlation analysis was performed.

Table 14. Correlation of beverages with "C" mark

Correlation			
		Abs	**Tartrazina**
Abs	Pearson Correlation	1	1,000**
	Sig. (1-tail)		<0.001
	N	12	12
Tartrazina	Pearson Correlation	1,000**	1
	Sig. (1-tail)	<0.001	
	N	12	12
**. The correlation is significant at the 0.01 (1-tail) level.			

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Source: Bonilla, et al, 2026

A perfect positive correlation is evidenced between the two variables, being 1,000, so a perfect degree of relationship between the samples is determined.

4. Discussion

The results achieved showed a significant variability in the content of tartrazine among the brands analyzed, highlighting that one of them exceeded the maximum permissible limit of 100 mg/L. This finding refers to possible deficiencies in quality control during industrial formulation, which represents a potential risk for the consumer. Recent studies have reported that azo dyes, such as tartrazine, are still widely used due to their stability and low cost, although their inadequate dosage can lead to regulatory non-compliance. [15]

From the analytical point of view, the UV-Visible spectrophotometric method used demonstrated high reliability, evidenced by excellent linearity ($R^2 = 0.9997$) and adequate recovery percentages. These results are consistent with recent research that emphasizes the usefulness of accessible and low-cost optical methods for the quantification of dyes in foods, which can achieve correlation coefficients greater than 0.99 and be comparable to more complex techniques such as HPL [36]

In relation to the statistical analysis, the non-normality of the data suggests a heterogeneous distribution of tartrazine content among commercial samples. This variability has been reported in recent studies, which indicate that differences in formulation, industrial processes and quality control directly influence the final concentration of dyes in feeds. In addition, these results reinforce the need to apply adequate statistical tools to interpret real data in food matrices.[16]

When compared with the current literature, it is observed that the presence of concentrations close to or above regulatory limits is not an isolated case. Several studies have raised concerns about the safety of tartrazine, including possible toxicological effects associated with prolonged use, which has led to recent scientific reviews focused on its biological effects and potential risks. [37][38]

Finally, the results of the present study are aligned with a growing trend in research: the development of more sensitive, sustainable and faster methods for the detection of food dyes, including fluorescent sensors and advanced electrochemical techniques. There is also a growing interest in replacing synthetic dyes with natural alternatives, driven by consumer demand and stricter regulations. In this context, the importance of strengthening monitoring and control systems to guarantee food safety is highlighted. [39]

5.- Conclusions

A sampling protocol was established in the city of Guayaquil, selecting one of the most popular commercial premises, in order to guarantee the representativeness of the non-carbonated, refreshing orange-flavored beverages available in the local market. A random stratified sampling was applied, which allowed obtaining representative samples of different commercial brands, optimizing the efficiency and validity of the data collection process according to the proposed objective.

The quantification of tartrazine (E-102) by UV-Visible spectrophotometry demonstrated that the analytical method used is suitable, reliable and reproducible for this type of matrix. Excellent linearity ($R^2 > 0.99$) was obtained, confirming the proportionality between absorbance and concentration. The specificity was verified by studying the matrix effect, evidencing the absence of significant interference from the components of the drink. Likewise, the accuracy presented recovery percentages between 94% and 101%, within the ranges accepted by the AOAC, and the precision, evaluated in terms of repeatability and intermediate precision, showed coefficients of variation less than 2%. However, the observed variability between brands, reflected in high standard deviations, suggests possible effects associated with the stability of the colorant, influenced by factors such as storage time, exposure to light and industrial processing conditions.

In relation to the objective of the study, it was found that one of the three brands analyzed (Brand A) presented concentrations of tartrazine higher than the maximum permissible limit of 100 mg/L stipulated by international regulations (FAO and regulations of countries such as Colombia and Mexico). This finding shows a potential non-compliance with food safety standards, which highlights the need to strengthen control and regulation methods in the Ecuadorian context, where there are currently no specific regulations for this additive in beverages. In this sense, the study provides relevant scientific evidence that contributes to the knowledge about the presence and variability of artificial colors in marketed beverages, and constitutes a starting point for other studies and the development of public policies aimed at consumer protection and the control of food additives.

6.- Author Contributions (Contributor Roles Taxonomy (CRediT))

1. Conceptualization: Carlos Valdiviezo.
2. Data curation: Stefanie Bonilla.
3. Formal analysis: Stefanie Bonilla.
4. Acquisition of funds: Not applicable.
5. Research: Stefanie Bonilla, Carlos Valdiviezo
6. Methodology: Luis Romero, Stefanie Bonilla, Carlos Valdiviezo, Michael Rendón.
7. Project management: Stefanie Bonilla

8. Resources: Carlos Valdiviezo, Michael Rendón.
9. Software: Not applicable.
10. Supervision: Carlos Valdiviezo
11. Validation: Stefanie Bonilla, Carlos Valdiviezo
12. Visualization: Luis Romero, Michael Rendón.
13. Writing - original draft: Luis Romero, Stefanie Bonilla
14. Writing - revision and editing: Stefanie Bonilla, Carlos Valdiviezo, Luis Romero, Michael Rendón.

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