ANALYTICAL AND SAFETY STUDIES ON ALLURA RED FOOD DYE USING UV-VISIBLE SPECTROSCOPY AND PAPER CHROMATOGRAPHY

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ABSTRACT

Synthetic food colorants like Allura Red are widely used to enhance the appearance of food and beverages, but concerns about their potential health risks necessitate monitoring their concentrations. This study aimed to evaluate the concentration of Allura Red in selected food samples and assess their compliance with regulatory safety limits. Using UV-visible spectrophotometry and paper chromatography, the dye was quantified and identified in various commercially available products. A calibration curve was generated using standard Allura Red solutions ranging from 20 to 200 µg/mL, enabling the determination of unknown sample concentrations based on their absorbance values. Paper chromatography was employed to calculate Rf values for preliminary dye identification. Results showed that all tested samples had Allura Red concentrations between 31.2 µg/mL and 127.06 µg/mL, falling within the permissible range (up to 200 µg/mL) set by the Food Safety and Standards Authority of India. Chromatographic analysis confirmed the presence of Allura Red in specific samples, with Rf values matching known standards. The study demonstrated the reliability of UV-visible spectroscopy for precise quantification and the utility of chromatography for qualitative detection. In conclusion, the tested food products complied with safety regulations and did not pose significant health risks, underscoring the importance of routine monitoring and analytical methods to ensure food safety.

Keywords: Allura Red, Spectrophotometry, Food Safety, Regulatory

INTRODUCTION

The use of synthetic food dyes has become increasingly prevalent in the food and beverage industry due to their vivid colors, cost-effectiveness, and stability during processing.[1,2] Among these, Allura Red (also known as Red 40 or E129), an azo dye, is one of the most widely used colorants in products such as soft drinks, candies, and baked goods. While synthetic dyes offer desirable visual appeal, multiple studies over the years have raised health concerns associated with their excessive consumption.[3,4] Reported effects include allergic reactions, behavioral changes in children, and potential carcinogenicity. Consequently, regulatory bodies such as the Food Safety and Standards Authority of India (FSSAI), the U.S. FDA, and the European Food Safety Authority (EFSA) have established strict permissible limits for synthetic dyes in consumables.[5]

In the past, various analytical methods, including high-performance liquid chromatography (HPLC), capillary electrophoresis, and spectrophotometry, have been employed to identify and quantify food dyes in commercial products.[6,7] Among these, UV-visible spectrophotometry remains a reliable and cost-effective technique, particularly in routine food safety testing. Similarly, paper chromatography offers a simple yet efficient method for the qualitative detection of synthetic dyes.[8]

Despite regulatory efforts and technological advances, the presence and concentration levels of synthetic dyes in many food items remain a subject of public concern.[9,10] Therefore, there is a continued need for accessible, accurate, and rapid testing methods to ensure consumer safety and regulatory compliance.[11,12]

The purpose of this study is to determine the concentration of Allura Red in selected food products using UV-visible spectrophotometry and paper chromatography, and to evaluate whether these levels fall within the permissible limits set by FSSAI.[13]

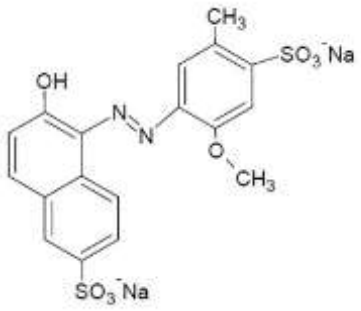

Fig. 1 Molecular structure of Allura Red AC (E 129).

Azo colors are synthesized in a two-step chemical process, initially forming a di-azo compound through the reaction of an aromatic amine. This involves the creation of nitrosamine and di-azo-hydroxide. In the subsequent step, the di-azo compound couples with various reactive aromatic systems.[14] The resulting molecules possess a conjugated system due to the π -π-

electrons spanning the two aromatic moieties and the azo groups. This conjugation enables the absorption of specific wavelengths of visible light, giving the compounds their distinct colors. The azo group's destruction results in cleavage into two aromatic amines, leading to the loss of color.[15]

The color of these dyes is modulated by selecting specific substituents and determining the number of azo groups. Yellow hues are achieved using acetoacetic acid arylides and heterocyclic compounds, while red shades result from the reaction of an aniline derivative as the di-azo component with a naphthol derivative. A blue shade is attained by substituting the aniline derivative with a benzidine derivative.[16]

Although a variety of azo dyes are available, food manufacturers predominantly use yellow and red synthetic dyes rather than blue or brown ones.[17] According to the European Food Safety Authority (EFSA), azo dyes account for about 70% of all dyes used in food production.[18]

Notably, there have been historical concerns about adverse reactions to the synthetic food color Tartrazine, dating back over 50 years.[19] Calls for discontinuing its use in food arose, but many studies on the subject were deemed inadequate. The EFSA's recent statement concludes that studies on Tartrazine do not provide evidence of reproductive or developmental effects. There is only minor evidence of hypersensitivity reactions in a very small segment of the population. The overall impact of Tartrazine on health remains uncertain, and the EFSA advocates for further research to substantiate any potential adverse effects.[20]

Table 1. Permitted Levels of Allura Red AC in beverages and foodstuffs (European Food Safety Authority [EFSA], 2009).

Product Class	**Types of Food**	**Permitted Level (mg L^{-1})**
Soft drinks and other non-alcoholic beverages	Ready-to-drink cordials, vending machine concentrates, instant teas, bitter soda, bitter vino, liquid food supplements/dietary integrators	10–100
Alcoholic beverages	Beers, ciders, fortified and aromatized wines, spirituous beverages, aromatized wine-product cocktails, fruit wines, cider and perry	Up to 200
Confectionery	Boiled sweets, toffees, caramels, gums, jellies, pastilles, licorice, chewing gum	50–300

Fine bakery wares	Biscuits, wafers, cakes, baking ingredients, preserves of red fruits, extruded or expanded savory snack products	Up to 200
Soups	Complete formulae for weight control and nutritional supplements	Up to 50
Meat products	Breakfast sausage (min. 6% cereal), luncheon meat, burger meat	20–25
Meat and fish analogs	Based on vegetable proteins	Up to 100
Fish products	Surimi	500
Cheese	Edible cheese rind	No maximum level specified
Special dietary foods	Liquid food supplements	Up to 100
Desserts	Blancmanges, custards, mousses, dry mixes, sauces	Up to 150
Snack foods	Other savory products and nuts	Up to 100
Sauces	Pickles, relishes, chutney, curry powder, tandoori	Up to 500

MATERIALS AND METHOD

Instrument

A Double Beam UV-Visible Spectrophotometer (Model: LABINDIA UV-3000) equipped with UV Win Software was used to record the absorbance and wavelength of Allura Red. Quartz cuvettes with a path length of 1 cm were used for all measurements. A digital analytical balance (WENSAR) was employed for accurate weighing of samples.

Chemicals and Reagents

- Allura Red AC (Brand: SUN) was used as the analytical standard.

- Distilled water served as the primary solvent due to the high solubility of Allura Red.
- All glassware used (beakers, pipettes, volumetric flasks, funnels) was made of borosilicate glass and rinsed with distilled water before use.
- All reagents were of analytical grade.

Selection of Solvent

Distilled water was selected as the solvent for the analysis of Allura Red due to its high solubility profile, chemical inertness, and compatibility with UV-visible spectrophotometric measurements. The dye exhibited complete dissolution in water, forming a stable and homogeneous solution, which is essential for accurate absorbance readings. Water also ensures minimal background interference in the UV region, supporting reliable baseline correction. Alternative solvents such as ethanol and methanol were evaluated but showed limited solubility, while non-polar solvents were deemed unsuitable due to complete insolubility of the analyte. Thus, distilled water was deemed the most appropriate and analytically robust solvent for this study.

Preparation of Standard Stock Solution

A stock solution (Solution A) was prepared by accurately weighing 200 mg of Allura Red AC and dissolving it in a small volume of distilled water in a 100 mL volumetric flask. The solution was shaken and diluted up to the mark with distilled water to achieve a concentration of 2000 µg/mL.

From this, Solution B was prepared by pipetting 1 mL of Solution A into a new 100 mL volumetric flask and diluting to volume with distilled water, resulting in a working stock of 20 µg/mL. Serial dilutions were then made from Solution B to prepare concentrations ranging from 20 to 200 µg/mL.

Selection of Wavelength (λmax Determination)

To determine the wavelength of maximum absorbance (λmax) for Allura Red, a 20 µg/mL solution was scanned in the UV-visible range from 200–800 nm. The dye exhibited maximum absorbance at 504 nm, which was selected for all subsequent quantitative analysis.

Sample Collection and Preparation

Collection of Food Samples

Representative food samples containing synthetic dye additives were procured from local markets, ensuring they were within expiration dates and free from visible contamination. Both solid and liquid food products were included to assess the presence and concentration of Allura Red across different formulations.

Identification and Classification

Each sample was visually inspected under natural or standardized white light to identify its apparent color. The shade (e.g., red, brown, yellow) was recorded for reference. The physical nature of the samples was categorized as solid, liquid, or semi-solid. Solids were further assessed for texture (e.g., powdery, granular, fibrous), and liquids were evaluated for viscosity (e.g., watery, thick).

Dissolution Procedure

An accurately weighed 1-gram portion of each food sample was transferred to a clean 100 mL beaker. Subsequently, 50 mL of distilled water was added gradually with continuous stirring. The degree of solubility was noted whether the sample was fully soluble, partially soluble, or insoluble.

Filtration

Samples that were not completely soluble underwent filtration using Whatman filter paper and a glass funnel. The resulting filtrate was collected in a clean container and visually examined for clarity to ensure suitability for spectrophotometric analysis.

Sample Analysis

The prepared aqueous extracts were analyzed using UV-visible spectrophotometry. Absorbance values were recorded at the predetermined wavelength (504 nm) for quantification of Allura Red content.

RESULT:

Chromatographic Profiling of Food Samples for Allura Red Content:

Table 2. Rf value of different food samples

SAMPLE	DISTANCE TRAVELLED BY SOLVENT	DISTANCE TRAVELED BY THE SUBSTANCE	RF VALUE	SPOT COLOUR CHARACTERIZATION
Sample 1	7 cm	1.9	0.27	Red (not E129)
Sample 2	**7 cm**	**2.5**	**0.35**	**Red (E129)**
Sample 3	**7 cm**	**2.5**	**0.34**	**Red (E129)**
Sample 4	**7 cm**	**2.4**	**0.35**	**Red (E129)**

Sample 5	7 cm	5	0.7	Red (not E129)
Sample 6	**7 cm**	**2.4**	**0.34**	**Red (E129)**
Sample 7	7 cm	1.1	0.15	Red (not E129)
Sample 8	7 cm	1.8	0.25	Red (not E129)
Sample 9	7 cm	1	0.14	Red (not E129)
Sample 10	**7 cm**	**2.4**	**0.34**	**Red (E129)**
Sample 11	7 cm	1.8	0.25	Red (not E129)
Sample 12	7 cm	5.1	0.71	Red (not E129)

Paper chromatography was employed to qualitatively confirm the presence of Allura Red in the food samples based on their retention factor (Rf) values. All tested samples displayed red-colored spots, with varying migration distances. The calculated Rf values ranged from 0.14 to 0.70, indicating the presence of colorants with different polarities.

Samples identified as containing Allura Red (E129) exhibited consistent Rf values between 0.34 and 0.35, aligning with the reference standard. In contrast, samples not labeled as E129 showed lower Rf values, ranging from 0.14 to 0.27, suggesting either a lower concentration of Allura Red or the presence of other red dyes. These results support the spectrophotometric findings and confirm the qualitative identity of Allura Red in selected samples.

Standard Absorbance Values

The standard absorbance values for Allura Red at varying concentrations were recorded and are presented in Table 6.2, establishing a clear relationship between concentration (µg/mL) and absorbance. The corresponding calibration curve (Figure 6.2) demonstrated a linear correlation across the tested range of 20–200 µg/mL, with absorbance values ranging from 0.046 to 0.626. This linearity confirms the suitability of the method for quantitative analysis and supports the accurate determination of Allura Red concentrations in food samples using UV-visible spectrophotometry.

Table 3. Relationship between concentration and absorbance.

CONCENTRATION (µg / mL)	**ABSORBANCE**	**CONCENTRATION (µg / mL)**	**ABSORBANCE**
20	0.046	120	0.377
40	0.101	140	0.422
60	0.170	160	0.471

80	0.241	180	0.521
100	0.288	200	0.626

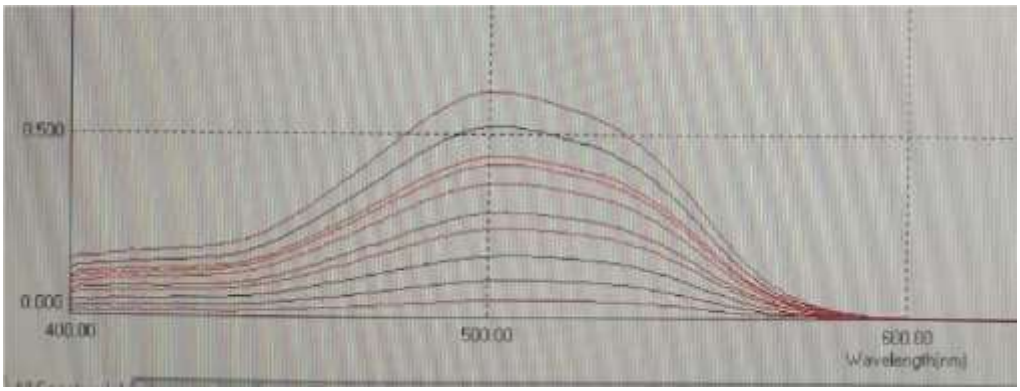

Fig 2. Different concentration wavelengths vs absorbance

Calibration Curve

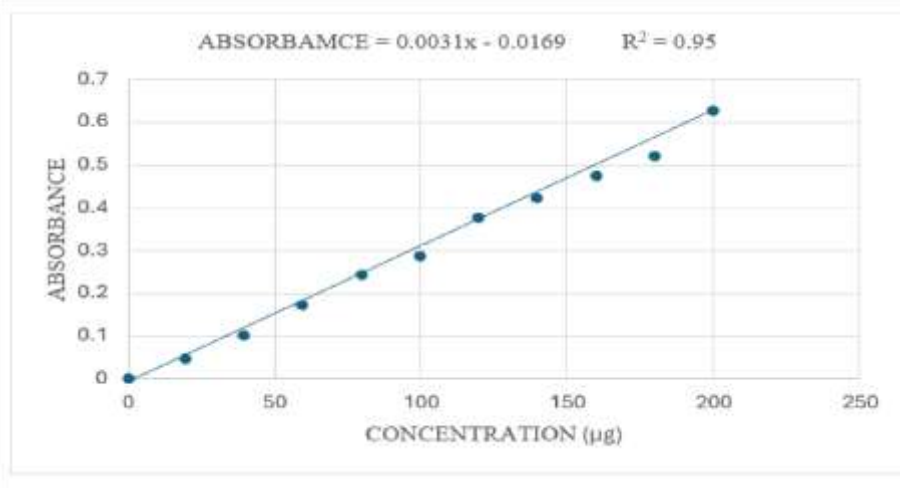

Fig 3. Concentration vs absorbance

This study relies on the calibration curve, a key tool in analytical chemistry used to determine the relationship between a substance's concentration in a solution and its absorbance. To develop the calibration curve, known concentrations of Allura Red and their corresponding absorbance values were measured, as shown in Table 6.3.

The concentration range varied from 20 µg/mL to 200 µg/mL, providing a broad scope for accurate calibration. The calibration curve was plotted with absorbance on the y-axis and concentration on the x-axis, demonstrating a linear relationship that validated the Beer-Lambert Law. The calculated slope of the curve was 0.0031, with an intercept of -0.0169. These values were essential in formulating the equation used to determine the concentration of Allura Red in food samples. $x = \underline{y} + 0.0031 \underline{0.0169}$

Where x represents the concentration in µg/mL, y is the absorbance value of the sample, 0.0031 is the slope of the calibration curve, and −0.0169 is the intercept. The concentrations of Allura Red in each sample were determined by substituting the absorbance values of the food samples into this equation.

Food Sample Absorbance:

Table 4. Presence of sample and absorbance.

SELECTED FOOD SAMPLE	ABSORBANCE
Food Sample 2	0.377
Food Sample 3	0.106
Food Sample 4	0.080
Food Sample 6	0.108
Food Sample 10	0.185

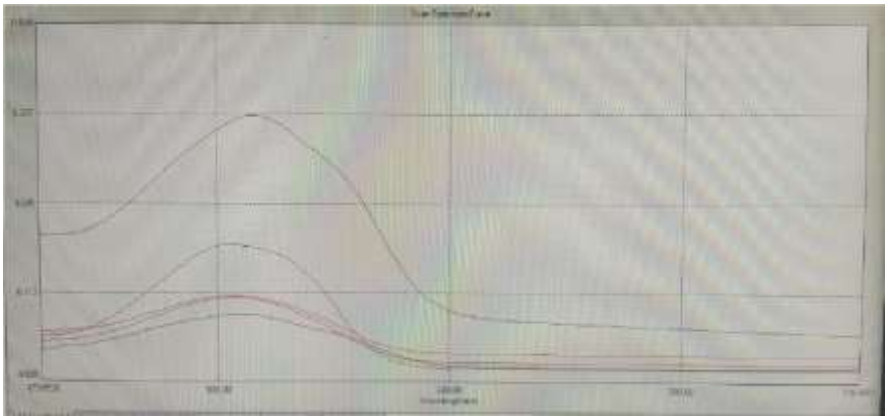

Fig 4. Different food sample wavelengths vs absorbance

Quantification of Allura Red in Food Samples

The concentration of Allura Red in food samples was determined using the calibration curve (Figure 6.2), which had a slope of 0.0031 and an intercept of -0.0169. The concentrations were calculated using the equation:

$$x = (y + 0.0169) / 0.0031,$$

where *y* is the absorbance value.

Among the tested samples:

- **Food Sample 2** showed an absorbance of 0.377, corresponding to **127.06 µg/mL**.
- **Food Sample 3** had an absorbance of 0.106, giving **39.6 µg/mL**.

- **Food Sample 4** exhibited 0.080 absorbance, equal to **31.2 µg/mL**.
- **Food Sample 6** (absorbance 0.108) had **40.2 µg/mL**.
- **Food Sample 10** (absorbance 0.185) was found to contain **65.1 µg/mL**.

All concentrations were well within the FSSAI permissible limit of 0–200 µg/mL, indicating regulatory compliance and consumer safety.

Table 5. shows the samples, their concentrations (µg /mL), the FSSAI limit in (µg /mL), and the resulting inferences.

S.NO	SELECTED FOOD SAMPLE	CONCENTRATION	FSSAI LIMIT (µg/mL)	LIMIT
1	Food Sample 2	127.06	Upto 200 µg/mL	PASS
2	Food Sample 3	39.6		PASS
3	Food Sample 4	31.2		PASS
4	Food Sample 6	40.2		PASS
5	Food Sample 10	65.1		PASS

All five food samples analyzed in this study contained Allura Red concentrations well below the maximum limit of 200 µg/mL set by FSSAI, ranging from 31.2 µg/mL to 127.06 µg/mL. These findings confirm that the samples comply with regulatory standards and are considered safe for consumption. The relatively low levels of Allura Red also indicate that the use of this dye in the tested products is within acceptable limits and does not pose a significant health risk.

DISCUSSION

Synthetic food dyes, such as Allura Red (E129), are widely used in the food industry to improve the aesthetic appeal of products. Despite their functional benefits, their safety has been questioned due to reported health concerns, including allergic reactions and behavioral effects, particularly in children. Regulatory agencies like the Food Safety and Standards Authority of India (FSSAI) have therefore established permissible limits for their use.

In the present study, the concentration of Allura Red in five commercially available food samples was determined using UV-visible spectrophotometry and supported by paper chromatography. The calibration curve established for the spectrophotometric analysis demonstrated a strong linear relationship between concentration and absorbance, consistent with the Beer-Lambert Law. The calculated dye concentrations ranged from 31.2 µg/mL to

127.06 µg/mL, all within the FSSAI's permissible limit of 0–200 µg/mL (Figure 3). These results indicate that the tested products comply with current food safety regulations and are unlikely to pose health risks to consumers.

Chromatographic analysis further confirmed the presence of Allura Red in selected samples. Those identified as E129 exhibited Rf values between 0.34 and 0.35, which align with standard references for Allura Red, whereas samples with lower Rf values (0.14–0.27) likely contained other red dyes (Figure 2). This supports the reliability of paper chromatography as a complementary technique for preliminary dye identification.

Compared to earlier studies that have reported either excessive dye concentrations or lack of labeling compliance, our findings reflect a relatively safer use of synthetic dyes in the sampled products. However, the study is limited by its small sample size and geographic scope. Only five food products were analyzed, and results may not represent broader market trends. Additionally, spectrophotometric analysis, though efficient and cost-effective, lacks the specificity of more advanced methods like HPLC or LC-MS.

Future studies should expand the sample size, include a broader range of food matrices, and employ multi-method validation for improved accuracy. Further exploration into the long-term health impacts of synthetic dye consumption, especially in vulnerable populations such as children, remains essential. Continuous monitoring and stricter enforcement of labeling standards will also support public health initiatives and consumer trust.

CONCLUSION

The present study successfully determined the concentration of Allura Red in selected food samples using UV-visible spectrophotometry, supported by qualitative analysis through paper chromatography. The major finding reveals that all tested samples contained Allura Red within the permissible limit of 0–200 µg/mL set by FSSAI, indicating compliance with food safety regulations. The established calibration curve confirmed the method's reliability for quantitative analysis, while chromatographic data further validated dye identification. These findings affirm the initial hypothesis that Allura Red is present in food products at safe concentrations and that spectrophotometry serves as an effective and accessible tool for routine food colorant analysis. The study underscores the value of continuous monitoring of synthetic dyes in foods to ensure consumer safety and regulatory adherence.

REFERENCES

1. Kizil N, Basaran E, Erbilgin D, Yola ML, Uzcan F, Soylak M. Deep eutectic solvent (DES) based dispersive liquid-phase microextraction of Sunset Yellow FCF in food and pharmaceutical products. Microchem J. 2022;181:107734.

2. McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, et al. Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children: a randomised, double-blinded, placebo-controlled trial. Lancet. 2007;370(9598):1560-7.

3. Hashem MM, Atta AH, Arbid MS, Nada SA, Asaad GF. Immunological studies on Amaranth, Sunset Yellow and Curcumin as food colouring agents in albino rats. Food Chem Toxicol. 2010;48(6):1581-6.

4. Tanaka T. Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. Food Chem Toxicol. 2006;44(2):179-87.

5. Rajamanickam D, Shanthi M. Photocatalytic degradation of an azo dye Sunset Yellow under UV-A light using TiO_2/CAC composite catalysts. Spectrochim Acta A Mol Biomol Spectrosc. 2014;128:100-8.

6. El-Shahawi MS, Hamza A, Al-Sibaai AA, Bashammakh AS, Al-Saidi HM. A new method for analysis of Sunset Yellow in food samples based on cloud point extraction prior to spectrophotometric determination. J Ind Eng Chem. 2013;19(2):529-35.

7. Miri Z, Elhami S, Zare-Shahabadi V, Jahromi HJ. Fe_3O_4@PDA@PANI core–shell nanocomposites as a new adsorbent for simultaneous preconcentration of Tartrazine and Sunset Yellow by ultrasonic-assisted dispersive micro solid-phase extraction. Spectrochim Acta A Mol Biomol Spectrosc. 2021;262:120130.

8. Yoosuf NA, Joseph JT, Shah JM. Mutagenicity assessment of Sunset Yellow on chromosomal aberrations and whole genome DNA strand breaks in Allium cepa. J Cytol Genet. 2020;21:121-30.

9. Carocho M, Barreiro MF, Morales P, Ferreira IC. Adding molecules to food, pros and cons: a review on synthetic and natural food additives. Compr Rev Food Sci Food Saf. 2014;13(4):377-99.

10. Rovina K, Siddiquee S, Shaarani SM. Extraction, analytical and advanced methods for detection of Allura Red AC (E129) in food and beverage products. Front Microbiol. 2016;7:798.

11. Kucharska M, Grabka J. A review of chromatographic methods for determination of synthetic food dyes. Talanta. 2010;80(3):1045-51.

12. Hamzeh S, Mahmoudi-Moghaddam H, Zinatloo-Ajabshir S, Amiri M, Nasab SA. Eco-friendly synthesis of mesoporous praseodymium oxide nanoparticles for highly efficient electrochemical sensing of Carmoisine in food samples. Food Chem. 2024;433:137363.

13. Xing Y, Meng M, Xue H, Zhang T, Yin Y, Xi R. Development of a polyclonal antibody-based enzyme-linked immunosorbent assay (ELISA) for detection of Sunset Yellow FCF in food samples. Talanta. 2012;99:125-31.

14. Medeiros RA, Lourencao BC, Rocha-Filho RC, Fatibello-Filho O. Simultaneous voltammetric determination of synthetic colorants in food using a cathodically pretreated boron-doped diamond electrode. Talanta. 2012;97:291-7.

15. Lawal A, Abdulkarim S. Toxicological study and quantitative determination of Allura Red (E129) dye additive in some food beverages consumed in Katsina Metropolis, Nigeria. FUDMA J Sci. 2024;8(3):228-32.

16. Hofseth LJ, Hebert JR, Murphy EA, Trauner E, Vikas A, Harris Q, et al. Allura Red AC is a xenobiotic. Is it also a carcinogen?. Carcinogenesis. 2024;45(10):711-20.

17. Siddiquee S, Shafwanah AM. Toxicology and analytical methods for the analysis of Allura Red (E129) in food and beverage products: a current perspective. In: Safety issues in beverage production. Cambridge: Academic Press; 2020. p. 335-57.

18. Scotter MJ, editor. Colour additives for foods and beverages. Amsterdam: Elsevier; 2015.

19. König J. Food colour additives of synthetic origin. In: Scotter MJ, editor. Colour additives for foods and beverages. Cambridge: Woodhead Publishing; 2015. p. 35-60.

20. Coleman RE. The truth about Red Dye 40. Nutrient Fact Sheets [Internet]. 2024 Aug 13 [cited 2025 Jun 24]. Available from: http://data.europa.eu/eli/reg/2012/231/2024-04-23.