EJPMR, 2026, 13(9), 372-376 **Research Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

IDENTIFICATION AND ESTIMATION OF ALLURA RED IN RED VELVET CAKE BY UV SPECTROSCOPY

Narmatha K.[1]*, Afreen A.[2], Dhinakaran C.[3], Siva A.[4], Sivaramakrishnan K.[5], Swetha S.[6]

[1]Associate Professor, Department of Pharmaceutical Analysis, Kamalakshi Pandurangan College of Pharmacy, Ayyampalayam, Tiruvannamalai-606603, Tamil Nadu, India.
[2,3,4,5,6]Students, 8th Semester, B Pharmacy, Kamalakshi Pandurangan College of Pharmacy, Ayyampalayam, Tiruvannamalai-606603, Tamil Nadu, India.

***Corresponding Author: Narmatha K.**

Associate Professor, Department of Pharmaceutical Analysis, Kamalakshi Pandurangan College of Pharmacy, Ayyampalayam, Tiruvannamalai-606603, Tamil Nadu, India. **Email ID:** narmatha444@gmail.com
DOI: https://doi.org/10.5281/zenodo.22269642

How to cite this Article: Narmatha K.[1]*, Afreen A.[2], Dhinakaran C.[3], Siva A.[4], Sivaramakrishnan K.[5], Swetha S.[6]. (2026). Identification And Estimation of Allura Red In Red Velvet Cake By Uv Spectroscopy. European Journal of Pharmaceutical and Medical Research, 13(9), 372–376.

Article Received on 05/08/2026 **Article Revised on 25/08/2026** **Article Published on 03/09/2026**

ABSTRACT

Synthetic food colors are extensively used in the food industry to enhance visual appearance and product consistency. Allura Red, also known as FD&C Red No. 40 or E129, is a widely used azo dye with the chemical formula $C_{18}H_{14}N_2Na_2O_8S_2$ and molar mass 496.42 $g \cdot mol^{-1}$. It appears as a red, water-soluble powder with high thermal stability with melting point >300 °C. Due to increasing concerns over excessive consumption and potential health effects such as allergic reactions, hyperactivity in children, and long-term organ toxicity, accurate estimation of allura red in food products is essential. According to FASSI food color should be added within the permissible limit. This article focuses on identification and estimation of allura red present in red velvet cake by UV-Visible spectrophotometry. Sample preparation involves extraction, filtration, and clean-up based on food matrix. The estimation results obtained by plotting calibration curve for different concentrations of standard allura red dye. The study highlights the need for strict regulation, proper labelling, and reliable analytical methods to ensure safe use of allura red in food products.

KEYWORDS: Allura Red AC, Food dye estimation, UV-Visible spectrophotometry, FSSAI.

INTRODUCTION

Synthetic food colours are artificial dyes that are chemically manufactured and added to foods and beverages to enhance or restore their appearance. They are widely used in candies, soft drinks, bakery products, ice creams, snacks, desserts, and packaged foods to make products more attractive and consistent. Most synthetic colours are water-soluble and provide bright, stable shades that withstand heat, light, and pH changes better than natural colourants.[1]

They are classified into azo, triarylmethane, xanthene, and indigoid dyes, and are available as water-soluble, oil-soluble, or lake forms. Commonly approved colours include Tartrazine, Sunset Yellow, Carmoisine, Ponceau 4R, Brilliant Blue, Indigo Carmine, and Erythrosine. The use of synthetic colours is strictly regulated by FSSAI in India and by FDA, EFSA, and Codex globally, which set ADI values, purity standards, and labelling requirements. Their advantages include low cost, high stability, intense colour, longer shelf life, and uniform quality.[2]

However, excessive consumption raises health concerns. These include allergic reactions, hyperactivity in children, digestive disorders, nervous system effects like irritability and poor concentration, potential long-term effects on liver and kidneys, and nutritional problems as they are mostly used in processed foods high in sugar and fat. Therefore, while synthetic colours are important for the food industry, their safe use depends on regulation, proper labelling, and consumer awareness, with future trends moving toward a balance of synthetic and natural alternatives.[3]

Food colours are commonly added to cakes to improve their appearance and make them more attractive. Both

synthetic and natural food colours are used depending on the type of cake and consumer preference.[4] Allura Red AC, also called FD&C Red No. 40 or E129, is a synthetic azo food dye with the chemical formula $C_{18}H_{14}N_2Na_2O_8S_2$ and a molar mass of 496.42 g·mol^{-1}. It appears as a red powder that is highly water-soluble and has a melting point >300 °C, showing good thermal stability.[5] It is widely used in beverages, candies, bakery items, desserts, and packaged foods to provide bright red-orange colour. The dye is regulated by FSSAI, FDA, and EFSA with set ADI limits. While approved for use, excessive intake may cause allergic reactions, hyperactivity in children, and other health concerns, so proper labelling and controlled use are essential.[6] The literature survey revealed that many research works were carried out to estimate the synthetic food colours in various food stuffs. For the present study, the red velvet cake was selected to estimate the concentration of Allura Red due to its insufficient investigation.

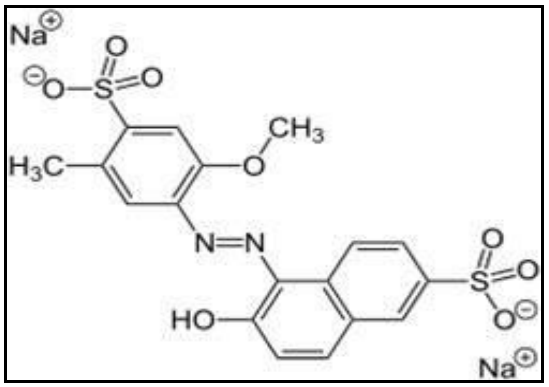

Figure 1: Structure of Allura Red.

MATERIALS METHODS AND APPROACHES

For the estimation of Allura Red food colour, the main materials include Allura Red standard, which is procured online, and the red velvet cake was brought from local bakery. Ethanol, ammonia, glacial acetic acid, sulphuric acid, sodium hydroxide, Prussian blue was analytical grade. The methods include UV-Visible spectrophotometry at 504 nm after aqueous extraction.[7]

SAMPLE PREPARATION

The confectionery sample type used was **Red Velvet Cake. Preliminary treatment**

10 g of sample was weighed and blended it into uniform paste then 50 ml of 2% ammonia and 70% alcohol was added to oil-removed sample, to break the food matrix bond and release the anionic dye. The above mixture was warmed in water bath for about 2-3 mins for the starch to settle down. The resulting colourful liquid was centrifuged at 30,000 rpm for 15 minutes. Supernatant liquid layer was collected and evaporated using a water bath.[8]

Figure 2.

Dye extraction

3 ml of 5% glacial acetic acid was added to the pretreated sample to slightly acidify it and then add 2 ml of distilled water. To concentrate the colour intensity, boil it on a water bath for a few minutes before diluting it with water.[9]

Identification of extracted colour

Allura Red AC is a synthetic monoazo dye containing an **–N=N–** (azo) group and sulfonate (-SO_3^-) groups. Its identification is commonly based on its colour reactions, reduction of the azo bond, reaction with acids and bases and its solubility in water.[10]

1. Reduction Test (Azo Dye Identification)

The reduction reaction involving Prussian blue and Allura Red serves as a colorimetric sensor mechanism used to detect and quantify the synthetic food dye Allura Red (E129).[11]

Allura Red + Prussian Blue reagent → Intense blue colour

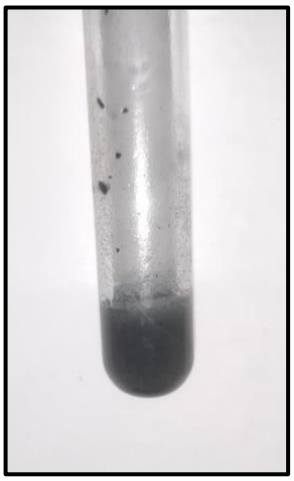

Figure 3.

2. Acid–Base Colour Stability Test

Unlike many natural dyes, Allura Red is relatively stable in acidic and alkaline media.

Reaction in Acid

When a few drops of concentrated sulphuric acid is added to a solution containing Allura Red, it produces crimson to dark red colour. The red colour remains almost

unchanged because the molecular structure of Allura Red is resistant to acidic conditions.

Allura red + H2SO4 → Crimson to dark red colour

Reaction in Alkali

Adding sodium hydroxide (1M) to aqueous solution of the dye shifts the colour to slightly yellowish red.[12]

Allura Red +NaOH → Slight yellowish red colour

Figure 4

Figure 5

Solubility test

Allura red dissolves readily in water to produce a bright red solution. Shows limited solubility in ethanol and is insoluble in acetone and chloroform.[13]

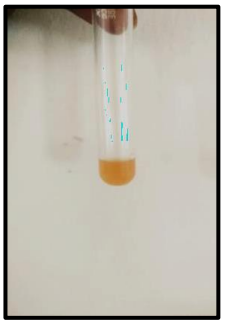

Figure 6.

ESTIMATION OF ALLURA RED BY UV SPECTROSCOPY METHOD

1. Preparation of Standard Solution

Stock Solution: Accurately weigh 0.100g of pure allura red and dissolve it in 100ml of distilled water in a volumetric flask, to create a 1000mg/L stock.

Working Standard: Prepare at least 5 serial dilutions spanning your expected concentration range (eg. 10mg/L, 20mg/L, 30mg/L, 40mg/L, 50mg/L).

Figure: 7.

2. Sample Preparation

As given in above procedure.

3. Spectroscopic Analysis

Set the spectrophotometer to a wavelength λmax = 504nm. Use distilled water as a blank to set zero. Measure the absorbance of the blank, working standards, and food sample.

4. CALCULATION

Plot a calibration curve using the absorbance values of standard solution on the y-axis and their concentrations on the x-axis. Use the equation of the line (y = mx + c) to calculate the concentration of allura red in prepared sample. Apply dilution factors to find the concentration in the original product (i.e. in cake).[14]

RESULTAND DISCUSSION

The UV–Visible spectrophotometric method was successfully employed for the estimation of **Allura Red AC (E129)** extracted from red velvet cake. The standard solution of Allura Red exhibited a maximum absorbance (λmax) at **504 nm**, which was selected as the analytical wavelength for quantitative estimation. The procedure described in the methodology enabled efficient extraction of the dye from the cake matrix using ammonia–ethanol solution followed by acidification with glacial acetic acid.

Linearity data of standard Allura Red food colour

The calibration curve was prepared using standard Allura Red solutions in the concentration range of 10–50mg/L. The absorbance increased proportionally with concentration, indicating compliance with Beer–Lambert's law within the selected range.

Table 2: Linearity data of standard Red food colour.

S.No.	Concentration [mg/L]	Absorbance (AU)
1.	10	0.460
2.	20	0.936
3.	30	1.403
4.	40	1.880
5.	50	2.364
6.	Sample	2.192

Calibration Curve Of Allura Red

Using the calibration curve method, a graph was created by plotting absorbance on the y-axis and concentration on the x-axis. The resulting straight line shows linearity, meaning that absorbance rises linearly as the solution's concentration rises. The standard graph can be extrapolated to determine the sample's concentration.

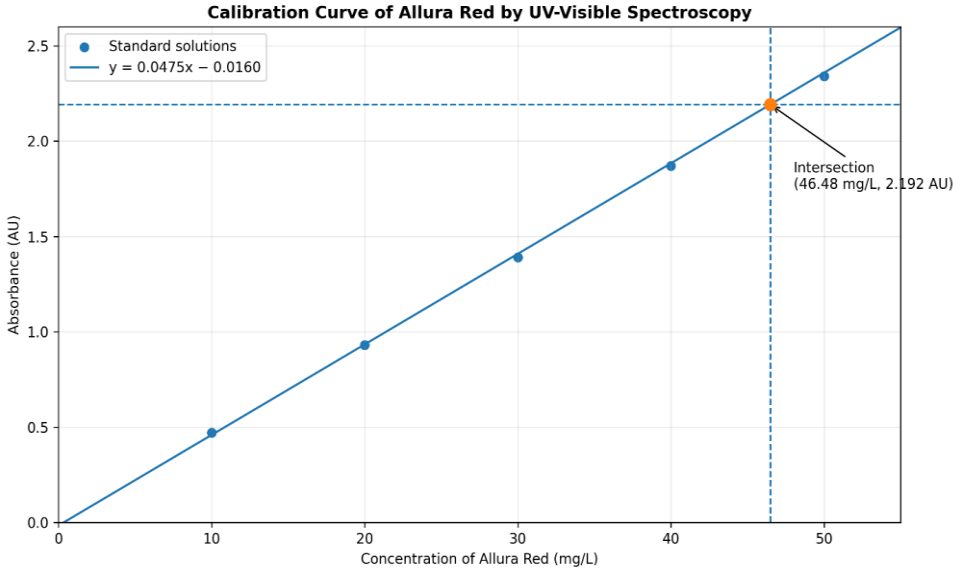

Figure 7: Calibration Curve of Allura Red Food Colour (Graph 1).

CALCULATION

The concentration of the sample was calculated using the regression line equation by substituting the sample absorbance value in place of y.

The regression line equation was found to be

$2.192 = 0.048x - 0.032$

$X = \frac{2.192 + 0.032}{0.048}$

$X = 46.33$ mg/L

The concentration of sample was found to be **46.33mg/L.**

COMPLIANCE

Table 3: Comparison of sample concentration with permissible limit.

Colour	Sample	Observed Concentration in (mg)	Permissible Limit Set By Food Safety thorities in (mg)
Allura Red	Red velvet Cake(10g)	46.33 mg	1 mg

CONCLUSION

The study showed that the concentration of Allura Red in the analysed red velvet cake sample was found to be 46.33mg in 10g of sample. This value was compared with the maximum permissible limit of 1mg in 10g of sample prescribed by food safety authorities. Since the observed concentration is higher than the permissible limit, **the sample does not comply with the recommended food safety standards.** Therefore, the analysed red velvet cake is unsafe with respect to its Allura Red content, indicating that the manufacturer has used the synthetic food colour more than the regulatory limits. The study was intended to create consumer awareness. This result demonstrates that the UV–Visible spectrophotometric method is effective for monitoring Allura Red in food products and can be applied for routine quality control and regulatory compliance testing.

ACKNOWLEDGEMENT

The authors are grateful to the management of **Kamalakshi Pandurangan College of Pharmacy, Ayyampalayam** for their encouragement and providing facilities for this work.

REFERENCES

1. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2018). *Principles of Instrumental Analysis* (7th ed.). Cengage Learning.

2. Food Safety and Standards authority of India. *Food Safety and Standards (Food Products Standards and Food Additives) Regulations*, 2011; *2011*. New Delhi: FSSAI.
3. Christian, G. D., Dasgupta, P. K., & Schug, K. A. (2014). *Analytical Chemistry* (7th ed.). Wiley.
4. Sharma, B. K. (2014). *Instrumental Methods of Chemical Analysis* (25th ed.). Goel Publishing House.
5. Chatwal, G. R., & Anand, S. K. (2014). *Instrumental Methods of Chemical Analysis*. Himalaya Publishing House.
6. Kirk, R. S., & Sawyer, R. (1991). *Pearson's Composition and Analysis of Foods* (9th ed.). Longman.
7. Scotter, M. J. Methods for the determination of permitted synthetic food colours in foods. *Food Additives & Contaminants*, 2015; 32(10): 1565–1594.
8. Minioti, K. S., Sakellariou, C. F., & Thomaidis, N. S. Determination of synthetic food colourants by HPLC. *Analytica Chimica Acta*, 2007; *583*(1): 103–110.
9. Marmion, D. M. (1991). *Handbook of U.S. Colourants: Foods, Drugs, Cosmetics and Medical Devices* (3rd ed.). Wiley.
10. Clydesdale, F. M. Colour as a factor in food choice. *Critical Reviews in Food Science and Nutrition*, 1993; *33*(1): 83–101.
11. International Organization for Standardization. (2018). *ISO/IEC 17025: General requirements for the competence of testing and calibration laboratories*. Geneva: ISO.
12. Khopkar, S. M. (2018). *Basic Concepts of Analytical Chemistry* (4th ed.). New Age International.
13. Kumar, A., & Sharma, V. UV–Visible spectrophotometric determination of synthetic food colours in beverages and confectionery products. *Journal of Food Science and Technology*, 2019; *56*(8): 3870–3878.
14. U.S. Food and Drug Administration. (2024). Colour Additives Permitted for Use in Food. Silver Spring, MD: FDA.