Indian Journal of Chemical Technology
Vol. 32, May 2025, pp. 334-342
DOI: 10.56042/ijct.v32i3.15056

Spectrophotometric analysis of food colourants as synthetic dyes in different types of food products

Abhijit Kargupta[1], Kakoli Dutta[1]* & Subhasis Rana[2]

[1]Department of Basic Science and Humanities, Institute of Engineering & Management, Sector V, Salt Lake Electronics Complex, Kolkata – 700091, India

[2]Department of Basic Science and Humanities, University of Engineering & Management, New Town Action Area-III, West Bengal – 700160, India

*E-mail: kakoli.dutta@iem.edu.in

Received 13 November 2024; accepted 11 January 2025

The present objective is to detect three food colourants (FCs) viz. Sunset Yellow, Tartrazine and Allura Red in the commonly consumed beverages and foods with the help of spectrophotometric analysis. Moreover, water and ethanol reference indicated that yellow coloured soft drink containing Tartrazine and orange coloured energy drink containing Sunset Yellow have similar absorbance (290 and 479 nm). Pinkish red coloured energy drink and fruit juice containing Allura Red (510 and 505 nm) have similar absorbance (300 nm) for both solvent, same absorbance for deep yellow coloured food as powder form containing Tartrazine (300 nm), grey coloured and light brown dry food containing Allura red (310 and 300 nm), yellow and light-yellow coloured soft drinks containing Tartrazine has similar absorbance (440 and 470 nm) for both solvents. The mixture of synthetic dye, deep red coloured containing Tartrazine, Sunset Yellow and Allura red shows absorbance at 480 nm. In conclusion, the concentration of these three FCs is easily detected through time-of-flight mass spectrometric analysis, which could be suitable measurement to detect the presence of dyes in the food products prior to quantitative assessment for food industry.

Keywords: Beverages, Food colourants, Spectrophotometric analysis, Synthetic dyes

Introduction

To date, major attentions have been concerned to evaluate the food colourants (FCs) as synthetic dyes in foods, beverages and drugs[1-3]. It is necessary to control the concentration of FCs due to health hazards to children and adults as this pose toxicity[1,3,4]. Almost all of them are not harmless and have varying degrees of toxicity associated with allergic, carcinogenic, and mutagenic effects[4,5]. According to Lehto *et al.*[6], the maximum accepted levels of food dyes are progressively decreased and now have reached 0.01 g of a dye per 100 g of a food products. Therefore, it is mandatory to monitor the dye content in food products as per strict regulation.

As per earlier studies, several methods such as UV-visible spectrophotometry[7-10], high performance liquid chromatography or HPLC[11], mass-spectrometry[12] and digital image analysis[13] have been established for the detection of food dyes. Time-of-flight mass spectrometry analysis for dyes in foods and beverages are common to know the illegal mixing of synthetic dyes as colouring agent[14]. Al Tamim *et al.*[15] evaluated 18 types of FCs in different food samples by using liquid chromatography/tandem mass spectrometry (LC-MS/MS) technique.

It was mentioned that there are some advantages of these methods for directly detecting coloured dyes in the samples of food products, but these techniques are considered laborious, expensive, and time-consuming[7]. Moreover, few studies have reported multicomponent analysis of FCs suitable by using spectrophotometry where the majority of results explored on binary mixture analysis and did not encompass the partial least squares (PLS) approach nor structured experimental designs[16-18]. It was reported that there exists some problems related to analysis of samples containing a mixture of dyes, especially of Sunset Yellow and Tartrazine (TAR, food additive E102) in which absorption maxima at 427 and 484 nm, respectively, recorded[10].

Generally, beverages contain a mixture of two dyes such as Sunset Yellow and Tartrazine[19]. Tartrazine is a synthetic lemon-yellow azo dye used as a food colourant (E102), which is called as “Cl food yellow

4"[20]. The food colouring Sunset Yellow FCF (E110), called as "orange-yellow *S"* or "Cl food yellow Cl 3" (European Commission, 2013). Allura red AC, called as "CL Food Red 17", is a synthetic red azo food colourant dye (E129)[20].

Generally, spectrometric analysis could be faster, less chemical and reagent usage as well as higher solubility in water. Moreover, it is easy to analyse all the FCs in foods and beverages prior to be consumed by children and adults because beyond the permissible limit may pose toxicity after consumption[1,3,4,21]. According to Robert *et al*.[22], the mean recovery ranged between 93.8- 115.2% and the precision of the analysis was also found satisfactory for all test compounds in both within-laboratory repeatability (0.8-7.7%) and within-laboratory reproducibility (1.6-7.7%) studies for liquid chromatography-tandem quadrupole mass spectrometry.

In the present study, we attempted to detect these three FCs in the commonly consumed beverages and foods with the help of spectrophotometric and time-of-flight mass spectrometry analysis.

Experimental Section

Materials and methods

Food colouring dyes viz. Tartrazine, Sunset Yellow and Allura Red as pure compounds were purchased from Sigma-Aldrich, USA. The food samples like beverages and foods were purchased from local market. The ethanol was purchased as analytical grade.

UV-visible spectrophotometer (Model: Labtronics LT 2201) and Quadrupoletime-of-flight mass spectrometry (Model: Waters XEVO-G2 Xs-QT) were used to perform spectrophotometric analyses in the wavelength range of 300-550 nm.

Analytical procedure

UV-Vis spectrophotometric analysis

All the food and beverages samples were dissolved in distilled water, which was also used as a blank for the analyses of prepared mixtures and blank in the first set of samples. Moreover, all the food and beverages samples were dissolved in ethanol, which was also used as water blank for the analyses of prepared mixtures and ethanol blank in the second set of samples. The standard curve was prepared for each food colourant separately. The sample was prepared after taking 1 mL of liquid sample or 1 g of solid sample and the volume was made up of 10 mL using distilled water/ethanol. For measurements, the prepared samples were diluted 40 times with the respective solvents, which were taken as references.

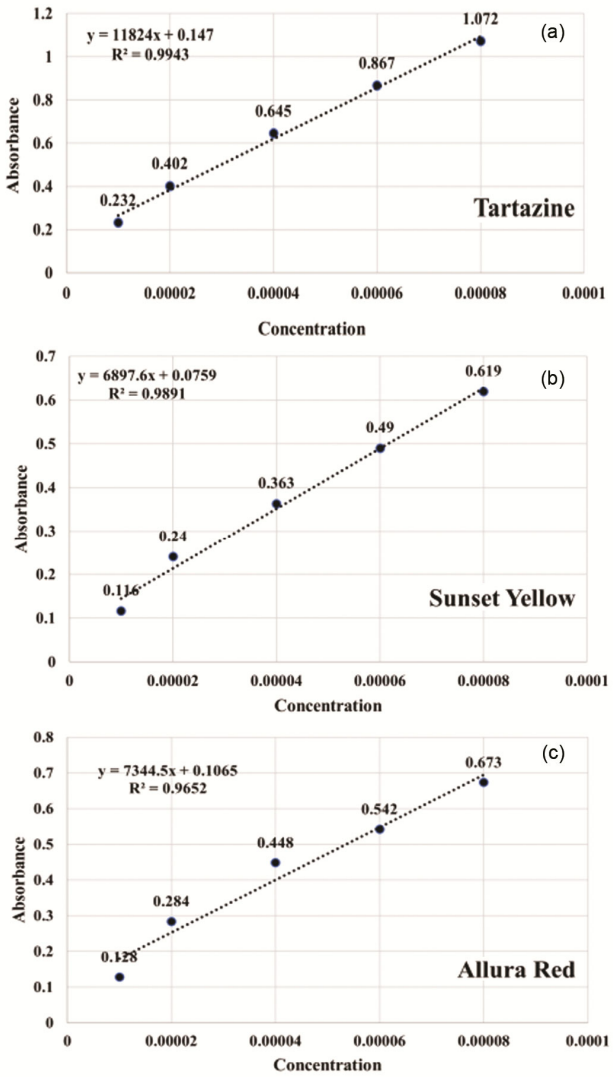

Fig. 1 — Standard curve for (a) Tartrazine, (b) Sunset Yellow and (c) Allura Red

Time-of-flight mass spectrometric analysis

All the food and beverages samples were dissolved in distilled water (10 mL was prepared after dissolving 1 g of powder and 1 mL of liquid was used to made 10 mL of solution). Each solution was analysed by using above-mentioned instrument and the results were expressed in m/z unit.

Results and Discussion

Absorption Spectrometry for dyes in foods and beverages

The absorbance was estimated as per the standard curve of each synthetic dye viz. Tartrazine (Fig. 1a),

Sunset Yellow (Fig. 1b) and Allura Red (Fig. 1c) in which the R2 was found to be 99.0%, 99.0% and 96%, respectively.

Table 1 — Spectrophotometric analysis of dyes in food products as per water reference

Sample No	Food products	Colour	Peak observed (nm)
1	Beverage (soft drink)	Yellow	290
2	Beverage (energy drink)	Orange	479
3	Beverage (energy drink)	Red	510
4	Beverage (fruit juice)	Pinkish red	300
5	Beverage (food)	Deep yellow	420
6	Food (dry food)	Grey	310
7	Food (dry food)	Light brown	300
8	Mixture of SY, T, AR	Deep red	480
9	Beverage (soft drink)	Yellow	440
10	Beverage (soft drink)	Light yellow	470

Table 1 evaluates spectrophotometric analysis of dyes in food products as per water reference. In sample 1 yellow coloured beverage (soft drink) containing Tartrazine, the peak was observed at 290 nm (Fig. 2). In sample 2 as beverage (energy drink), orange coloured containing Sunset yellow, the peak was observed at 479 nm (Fig. 3). In sample 3 as beverage (energy drink), red coloured containing Allura Red, the peak was observed at 510 nm (Fig. 4a). In sample 4 as beverage (fruit juice), pinkish red coloured containing Allura Red, the peak was observed at 300 nm (Fig. 4b). In sample 5 as food (in powder form), deep yellow coloured containing Tartrazine, the peak was observed at 300 nm (Fig. 5). In sample 6 as food (dry food), grey coloured containing Allura red, the peak was observed at

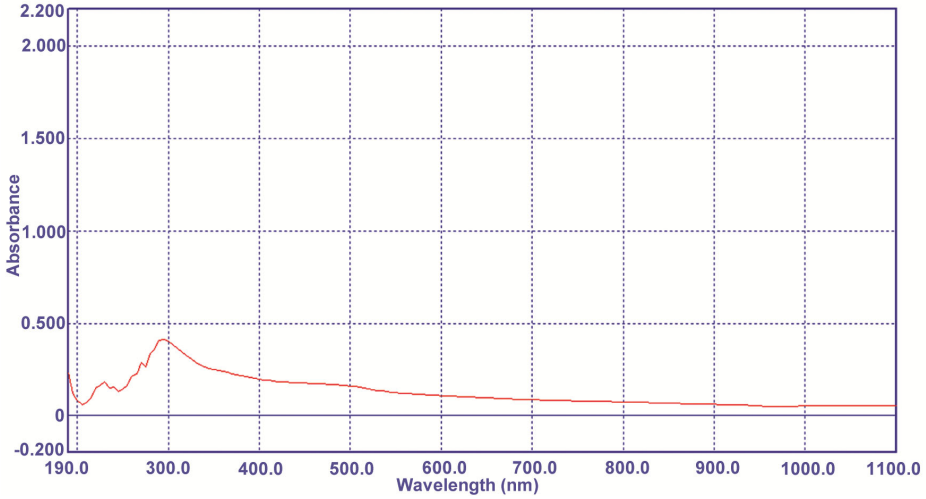

Fig. 2 — Peak observed in sample 1 as beverage (soft drink) presence of Tartrazine

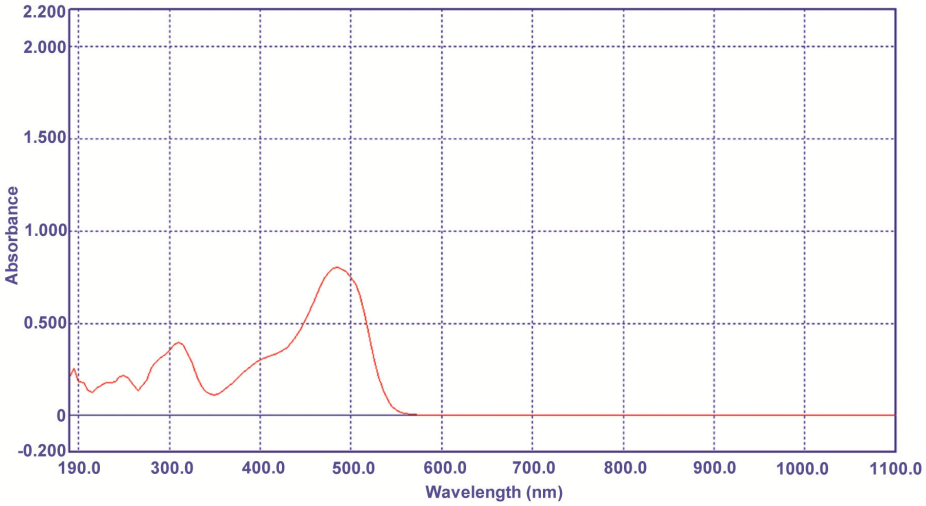

Fig. 3 — Peak observed in sample 2 as beverage (energy drink) presence of Sunset Yellow

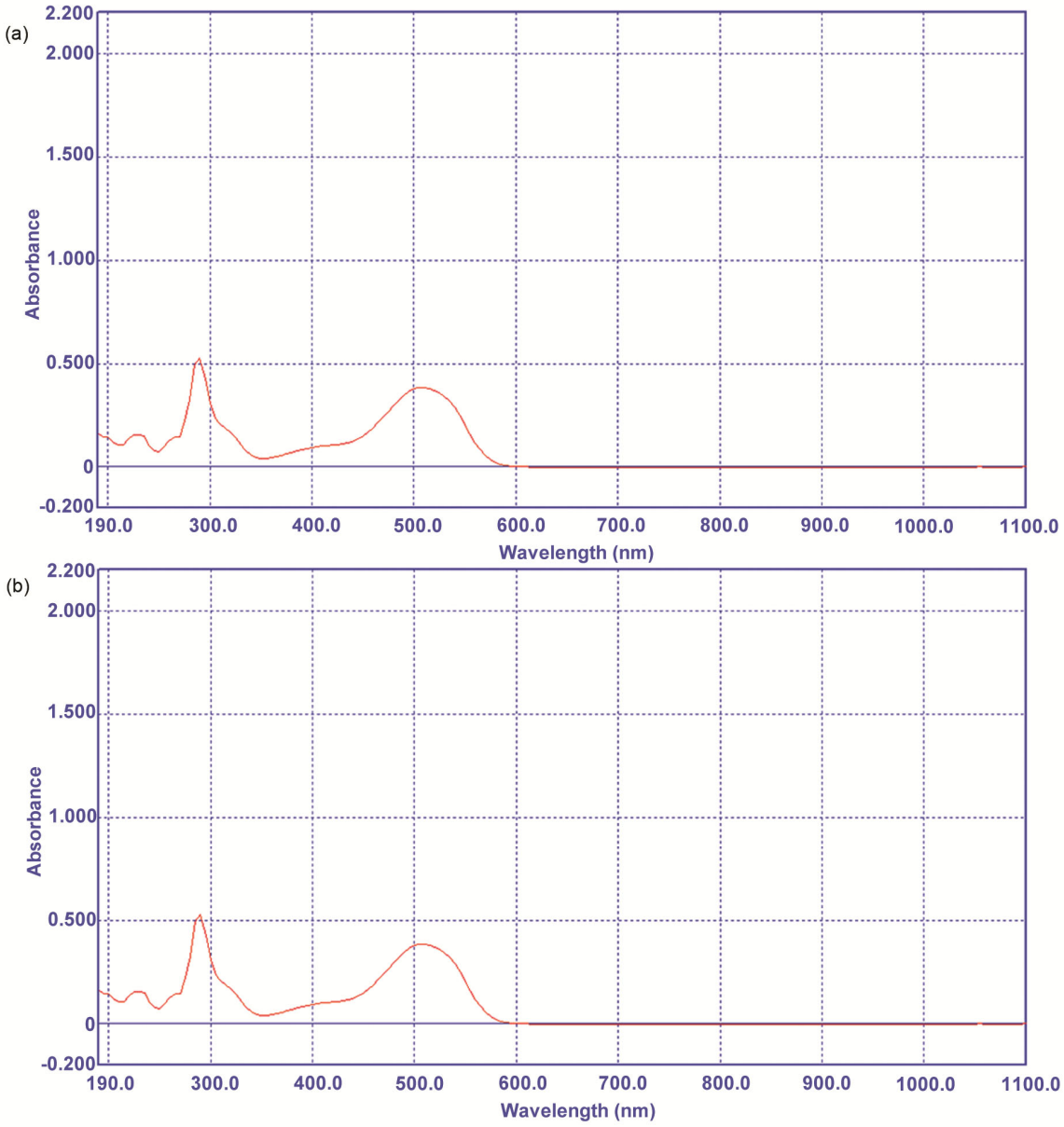

Fig. 4 — Peak observed in (a) sample 3 as beverage (energy drink) and (b) sample 4 as beverage (fruit juice) presence of Allura Red

310 nm (Fig. 6). In sample 7 as food (dry food), light brown coloured containing Allura red, the peak was observed at 300 nm (Fig. 7). In sample 8 as mixture of synthetic dye, deep red coloured containing Tartrazine, Sunset Yellow and Allura red, the peak was observed at 480 nm (Fig. 8). In sample 9 (Fig. 9a) and 10 (Fig. 9b) as beverage (soft drink), yellow and light-yellow coloured containing Tartrazine, the peaks were observed at 440 nm and 470 nm, respectively.

Table 2 evaluates spectrophotometric analysis of dyes in food products as per ethanol reference. In sample 1 as beverage (soft drink), yellow coloured containing Tartrazine in which the peak was observed at 290 nm. In sample 2 as orange coloured beverage (energy drink) containing Sunset Yellow, the peak was observed at 479 nm. In sample 3 as beverage (energy drink), red coloured containing Allura Red in which the peak was observed at 505 nm. In sample 4 as beverage (fruit juice), pinkish red coloured containing Allura Red, the peak was observed at 300 nm. In sample 5 as food (in powder form), deep yellow coloured containing Tartrazine, the peak was observed at 480 nm. In sample 6 as food (dry food), grey coloured containing Allura red in which the peak

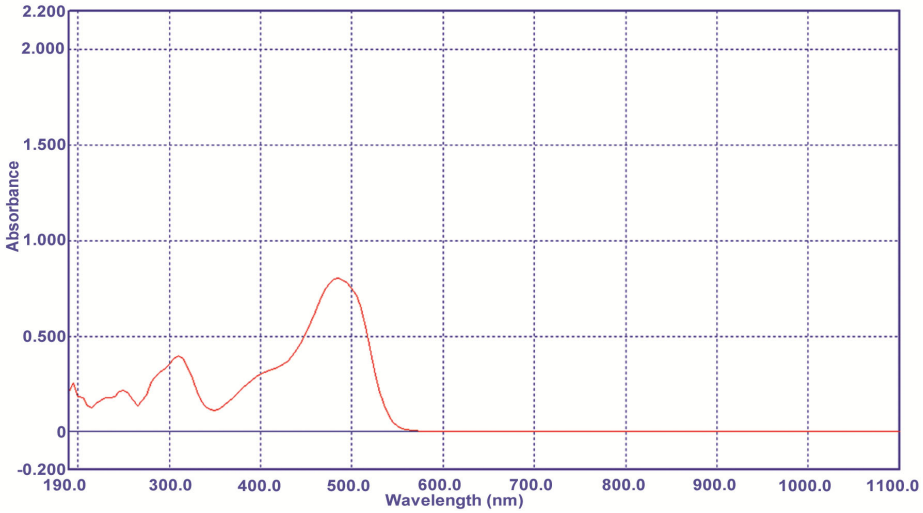

Fig. 5 — Peak observed in sample 5 as food (in powder form) presence of Tartrazine

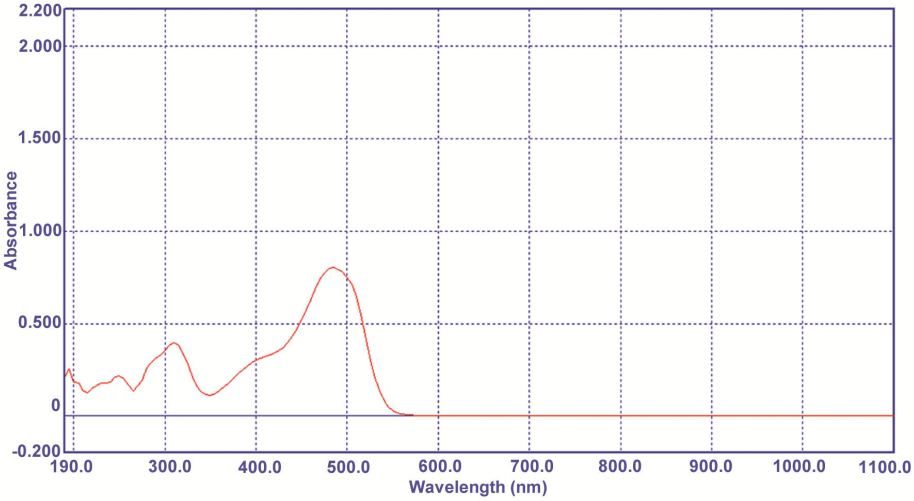

Fig. 6 — Peak observed in sample 6 as food presence of Allura Red

was observed at 310 nm. In sample 7 as food (dry food), light brown coloured containing Allura red in which the peak was observed at 300 nm. In sample 8 as mixture of synthetic dye, deep red coloured containing Tartrazine, Sunset Yellow and Allura red in which the peak was observed at 480 nm. In sample 9 and 10 as beverage (soft drink), yellow and light-yellow coloured containing Tartrazine in which the peaks were observed at 440 nm and 470 nm. Similar peaks were observed as like water reference for common dye.

Time-of-flight Mass spectrometry for Dyes in Food and Beverages

Fig. 10a exhibits Tartrazine (E102) at m/z 475.3106 in a mixture as a standard sample. In specific sample of beverage (energy drink), the similar value (m/z 475.3195) was obtained and confirmed the presence of Tartrazine (Fig. 10b).

Fig. 11a exhibits Sunset Yellow (E110) at m/z 535.3192 in a mixture as a standard sample. In specific sample of beverage (soft drink), the similar value (m/z 535.2009) was obtained and confirmed the presence of Sunset Yellow (Fig. 11b).

Fig. 12a exhibits Allura Red (E129) at m/z 495.3571 in a mixture as a standard sample. In specific sample of beverage (energy drink), the similar value (m/z 495.4071) was obtained and confirmed the presence of Allura Red (Fig. 12b).

In this study, it is a suitable measurement by using spectrophotometer to detect the concentration of synthetic dyes in the beverages and foods. It is

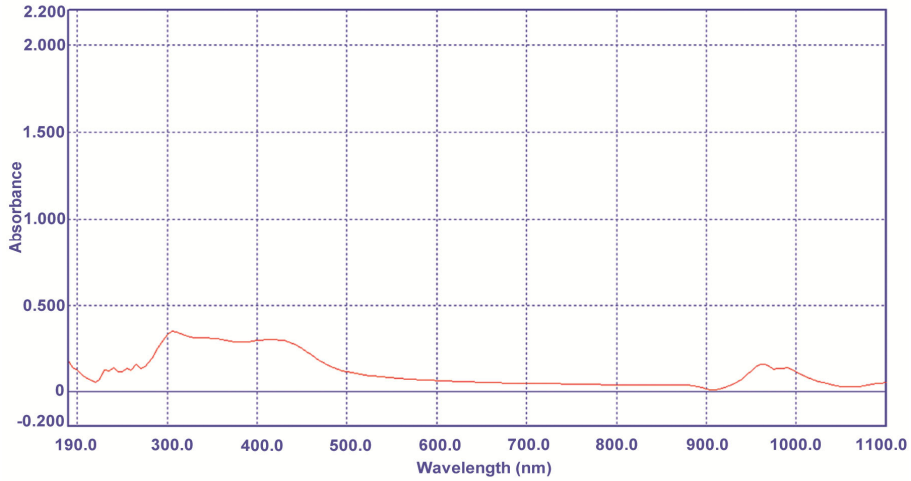

Fig 7 — Peak observed in sample 7 as food presence of Allura Red

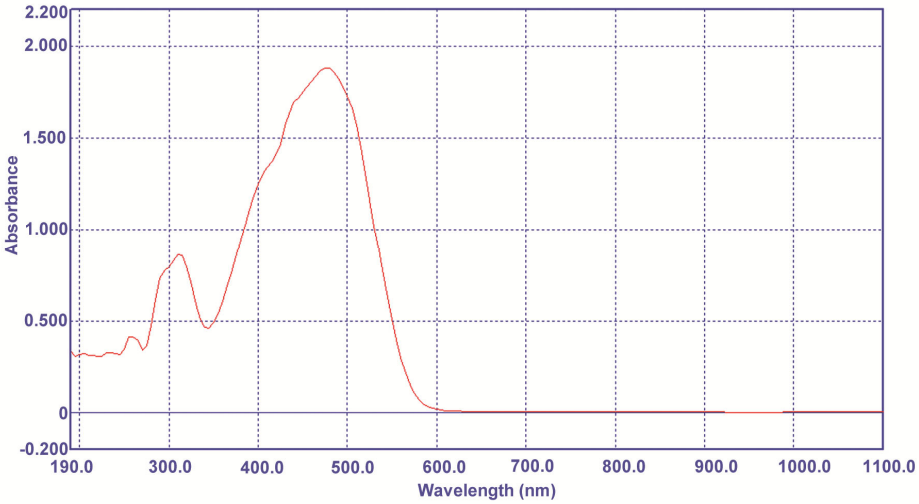

Fig. 8 — Peak observed in sample 8 as mixture of Tartrazine, Sunset Yellow and Allura Red using water/alcohol as reference

well-known fact that higher absorbance poses higher concentration of chemicals in the soluble sample as per Beer's Law. These three FCs are utilized in the beverages and food samples detected through UV-visible spectrophotometer easily as per the concept of previous studies[23,24].

According to Hashem *et al.*[24], the two reactions were monitored spectrophotometrically at maximum absorbance 350 and 610 nm, which is within the range of present study of Sunset Yellow. Thani[25] reported the absorption spectra of Allura red at a range of 400-800 nm, which was comparatively higher absorption in food products. Gräb & Geidel[26] estimated these three FCs in which absorption maxima at 428 nm, 482 nm and 502 nm for Tartrazine, Sunset Yellow and Allura Red were obtained. Rukosueva *et al.*[10] reported that the Sunset Yellow and Tartrazine (TAR, food additive

Table 2 — Spectrophotometric analysis of dyes in food products as per ethanol reference

Sample No	Food products	Colour	Peak observed (nm)
1	Beverage (energy drink)	Yellow	290
2	Beverage (soft drink)	Orange	485
3	Beverage (energy drink)	Red	505
4	Beverage (fruit juice)	Pinkish red	300
5	Beverage (food)	Deep yellow	420
6	Food (dry food)	Grey	310
7	Food (dry food)	Light brown	300
8	Mixture of SY, T, AR	Deep red	480
9	Beverage (soft drink)	Yellow	440
10	Beverage (soft drink)	Light yellow	470

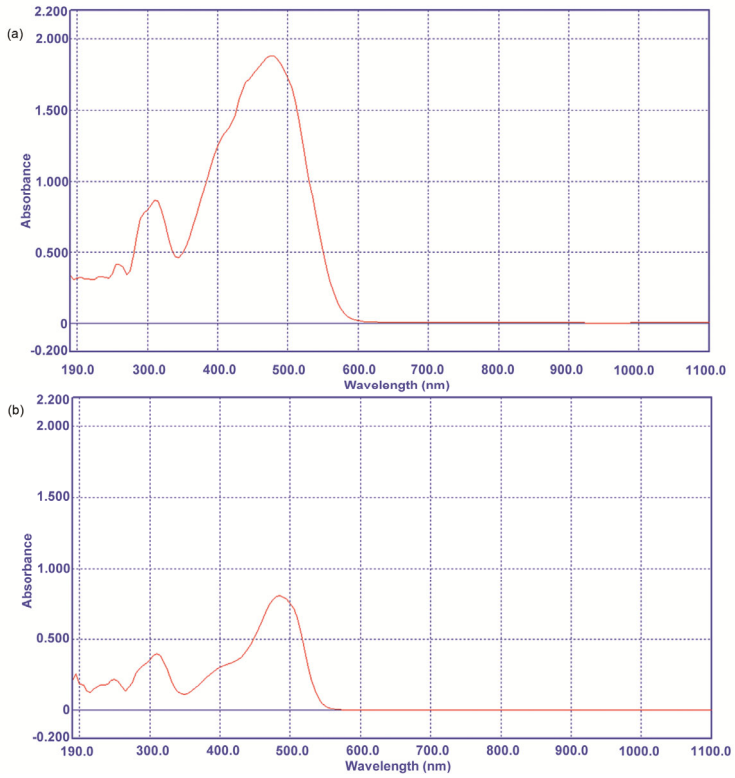

Fig. 9 — Peak observed in (a) sample 9 and (b) sample 10 as beverage (soft drink) presence of Tartrazine

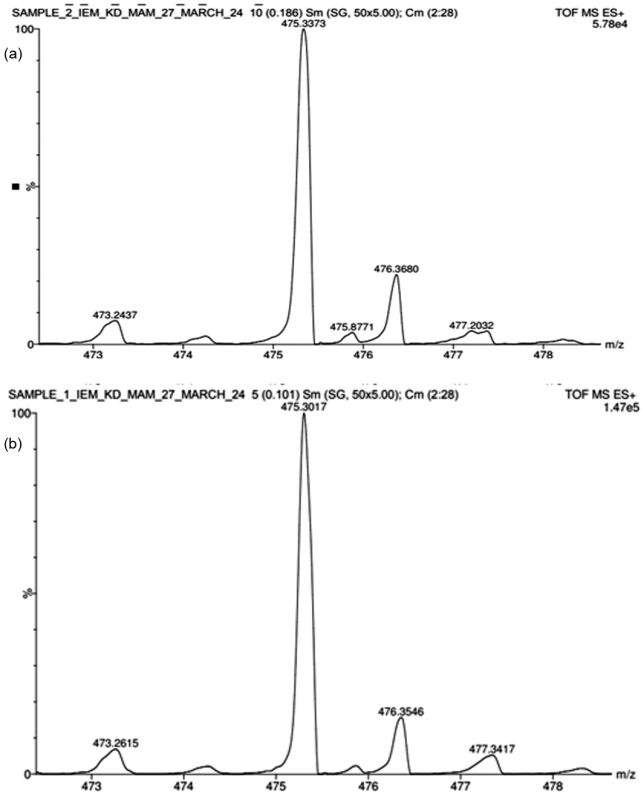

Fig. 10 — Quadrapole-time of flight analysis of Tartrazine (E102) in (a) pure compound and (b) beverage

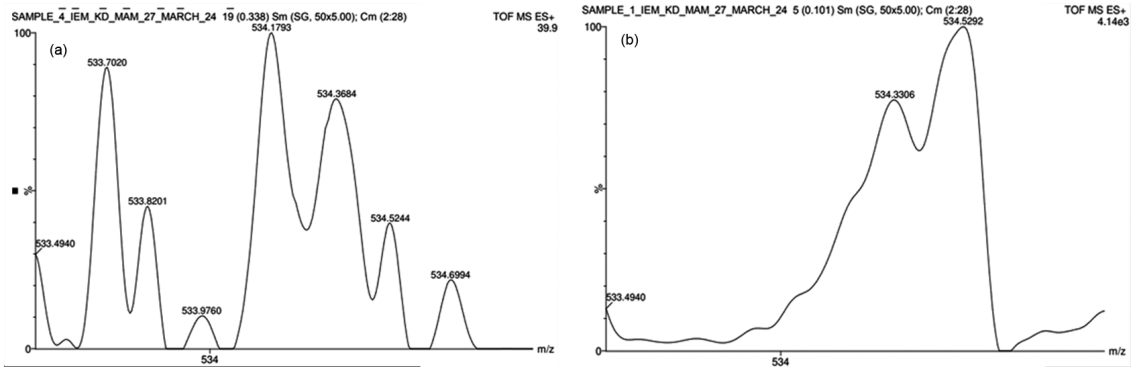

Fig. 11 — Quadrapole-time of flight analysis of Sunset Yellow (E110) in (a) pure compound and (b) beverage

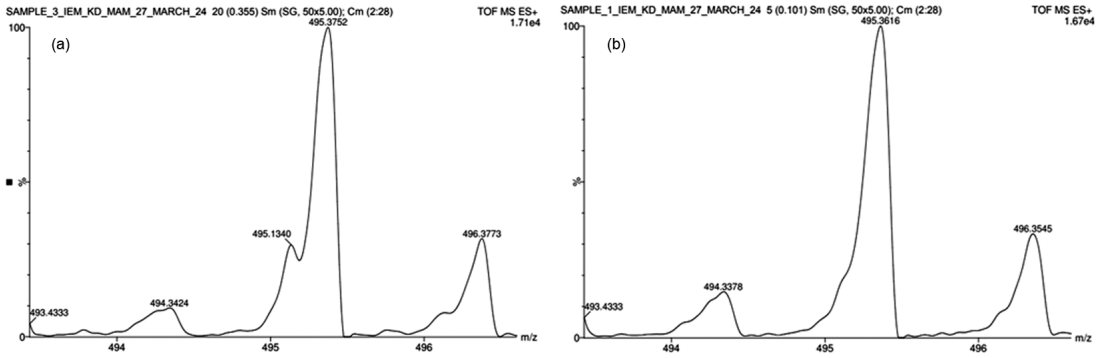

Fig. 12 — Quadrapole-time of flight analysis of Allura Red (E129) present in (a) pure compound and (b) beverage

E102) recorded the absorption maxima at 427 and 484 nm, respectively.

Regarding Quadrapole-time of flight analysis, Nagappan *et al*.[27] revealed that the ESI-Q-TOF spectrum obtained for Tartrazine at a molecular mass of 534 Da in which the molecule provided distinct signals corresponding to three negative pseudo molecular ions at m/z 511, m/z 489 and m/z 467, respectively. Moreover, Salivo[28] stated that negative mode detection of sulfonated azo colourants in sweets/candies in which the negative ion molecular mass for Tartrazine (m/z 467.017), Sunset Yellow (m/z 407.071) and Allura Red (m/z 451.008), respectively.

Conclusion

It is concluded that the higher absorbance confirmed the higher concentration of dyes in the food products (beverages and foods) as per spectrophotometric analysis. Moreover, an accurate molecular mass was obtained easily as per Quadrapole-time of flight analysis in the beverages for each FC, which could be suitable measurement to detect the presence of dyes in the food products prior to quantitative assessment for food industry.

Acknowledgments

Authors are indebted to the Chemistry laboratory of Department of Basic Science and Humanities, Institute of Engineering & Management and Central facilities of Bose Institute.

References

1 Amchova P, Kotolova H & Ruda-Kucerova J, Health safety issues of synthetic food colorants, *Regul Toxicol Pharmacol*, 73 (2015) 914.

2 Yamjala K, Nainar M S & Ramisetti N R, Methods for the analysis of azo dyes employed in food industry-A review, *Food Chem*, 192 (2016) 813.

3 Christ-Ribeiro A, Maciel J V, Bier E M, Pinto J S & Dias D, Application of electrochemical sensors in the determination of synthetic dyes in foods or beverages and their toxicological effects on human health: A review, *Food Anal Methods*, 15 (2022) 2394.

4 Kargupta A, Dutta K, Roychoudhury D, Rana S & Talapatra S N, Toxicity, genotoxicity and ecotoxicity of food colourants especially synthetic dyes and its metabolites: An in silico approach, *Asian J Chem*, 34 (2022) 2955.

5 Silva M M, Reboredo F H & Lidon F C, Food colour additives: A synoptical overview on their chemical properties, applications in food products, and health side effects, *Foods*, 11 (2022) 379.

6 Lehto S, Buchweitz M, Klimm A, Straßburger R, Bechtold C & Ulberth F, Comparison of food colour regulations in the EU and the US: A review of current provisions, *Food Addit Contam: Part A*, 34 (2017) 335.

7 Arroz E, Jordan M & Dumancas G G, Development of a direct spectrophotometric and chemometric method for determining food dye concentrations, *Appl Spectrosc*, 71 (2017) 1633.

8 Benvidi A, Abbasi S, Gharaghani S, Dehghan T M & Masoum S, Spectrophotometric determination of synthetic colorants using PSO-GA-ANN, *Food Chem*, 220 (2017) 377.

9 Dudkina A A, Volgina T N, Saranchina N V, Gavrilenko N A & Gavrilenko M A, Colorimetric determination of food colourants using solid phase extraction into polymethacrylate matrix, *Talanta*, 202 (2019) 186.

10 Rukosueva E A, Aliyarova G R, Tikhomirova T I, Apyari V V & Nesterenko P N, Simultaneous determination of synthetic food dyes using a single cartridge for preconcentration and separation followed by photometric detection, *Int J Anal Chem*, 2020 (2020) 2409075.

11 Loriano L, Ribeiro L C, Saibt N, Bandeira N M G, Prestes O D & Zanella R, Determination of six synthetic dyes in sports drinks by dispersive solid-phase extraction and HPLC-UV-Vis, *J Braz Chem Soc*, 29 (2017) 602.

12 Chao Y Y, Chen Y L, Chen W C, Chen B H & Huang Y L, Rapid detection of illegal colorants on traditional Chinese pastries through mass spectrometry with an interchangeable thermal desorption electrospray ionization source, *Food Chem*, 252 (2018) 189.

13 Vidal M, Garcia-Arrona R, Bordagaray A, Ostra M & Albizu G, Simultaneous determination of color additives tartrazine and allura red in food products by digital image analysis, *Talanta*, 184 (2018) 58.

14 Brazeau J, Identification and quantitation of water-soluble synthetic colors in foods by liquid chromatography/ ultraviolet–visible method development and validation, *ACS Omega*, 3 (2018) 6577.

15 Al-Tamim A, Al-Rabeh M, Al-Tamimi A, Al-Ajlan A & Alowaifeer A, Fast and simple method for the detection and quantification of 15 synthetic dyes in sauce, cotton candy, and pickle by liquid chromatography/tandem mass spectrometry, *Arab J Chem*, 13 (2020) 3882.

16 Vidotti E C, Cancino J C, Oliveira C C & Rollemberg M, Simultaneous determination of food dyes by first derivative spectrophotometry with sorption onto polyurethane foam, *Anal Sci: Int J Japan Soc Anal Chem*, 21 (2005) 149.

17 Turak F & Özgür M U, Validated spectrophotometric methods for simultaneous determination of food colorants and sweeteners, *J Chem*, 2013 (2013) 127847.

18 Turak F, Dinç M, Dülger O & Ozgür M U, Four derivative spectrophotometric methods for the simultaneous determination of Carmoisine and Ponceau 4R in drinks and comparison with high performance liquid chromatography, *Int J Anal Chem*, 2014 (2014) 650465.

19 USEPA (United States Environmental Protection Agency), Estimation ProgramInterface (EPI) Suite, US Environmental Protection Agency, Washington, DC, USA, (2004).

20 European Commission, Commission Regulation (EU) No. 1274/2013 of the European Parliament and of the Council of 6 December 2013 Amending and Correcting Annexes II and III to Regulation (EC) No. 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No. 231/2012 as Regards Certain Food Additives, *J Eur Union*, L328 (2013) 79.

21 Barciela P, Perez-Vazquez A & Prieto M A, Azo dyes in the food industry: Features, classification, toxicity, alternatives, and regulation, *Food Chem Toxicol*, 178 (2023) 113935.

22 Robert C, Hulle M V, Hird S, Brasseur P Y & Gillard N, The analysis of sudan and other azo dyes in spices using liquid chromatography-tandem quadrupole mass spectrometry, *Waters*, (2021).

23 El-Sheikh A H & Al-Degs Y S, Spectrophotometric determination of food dyes in soft drinks by second order multivariate calibration of the absorbance spectra-pH data matrices, *Dyes Pigm*, 97 (2013) 330.

24 Hashem E I, Saleh M S, Al-Salahi N O A & Youssef A K, Advanced spectrophotometric analysis of sunset yellow dye E110 in commercial food samples, *Food Anal Methods*, 10 (2017) 865.

25 Thani M Z, A Simple spectrophotometric evaluation method of Allura red (E129) in several food samples using cloud point extraction, *Al-Mustansiriyah J Sci*, 28 (2017) 134.

26 Gräb P & Geidel E, Spectroscopic studies of food colourings, *World J Chem Edu*, 7 (2019) 136.

27 Nagappan K, Yamjala K, Sathyaseelan M & Byran G, Stability evaluation of tartrazine by liquid chromatography-diode array detector and high-resolution electron spray ionization quadrupole time-of-flight mass spectrometry/mass spectrometry analysis, *Asian J Pharm Clin Res*, 10 (2017) 295.

28 Salivo S, Negative mode detection of sulfonated azo colourants in sweets/candies using the MALDI8030 dual polarity benchtop MALDI-TOF mass spectrometer, Application News. Shimadzu Corporation, 1st Edn, (2022).