Hindawi
Journal of Chemistry
Volume 2018, Article ID 6486250, 6 pages
https://doi.org/10.1155/2018/6486250

Research Article

Simultaneous Determination of Sunset Yellow FCF, Allura Red AC, Quinoline Yellow WS, and Tartrazine in Food Samples by RP-HPLC

Hakan Alp,[1] **Davut Başkan**,[1] **Ahmet Yaşar**,[2] **Nurettin Yaylı**,[2] **Ümmühan Ocak**,[1] **and Miraç Ocak**[1]

[1]*Department of Chemistry, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey*
[2]*Faculty of Pharmaceutical Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey*

Correspondence should be addressed to Hakan Alp; hakanalp@ktu.edu.tr

Received 11 July 2018; Accepted 18 September 2018; Published 14 October 2018

Academic Editor: Teodorico C. Ramalho

An efficient method was developed for the simultaneous determination of Sunset Yellow FCF (E110), Allura Red AC (E129), Quinoline Yellow WS (E104), and Tartrazine (E102) in food samples by RP-HPLC. The mentioned food dyes were analyzed at room temperature for 23 min with gradient elution. Three mobile phases were used for the elution, and mobile phase A was an acetate buffer (pH = 7.5, 1%), mobile phase B was acetonitrile, and mobile phase C was methanol. The flow rate was 1.0 mL min^{-1}, and the injection volume was 20 μL. The linear ranges were 0.72–50 mg L^{-1}, 0.24–50 mg L^{-1}, 0.75–10 mg L^{-1}, and 0.69–50 mg L^{-1} for Tartrazine, Quinoline Yellow WS, Sunset Yellow FCF, and Allura Red AC, respectively. R^2 values were 0.999 for all dyes. Limits of detection were 0.24 mg L^{-1}, 0.08 mg L^{-1}, 0.25 mg L^{-1}, and 0.23 mg L^{-1} for Tartrazine, Quinoline Yellow WS, Sunset Yellow FCF, and Allura Red AC, respectively. The relative standard deviation (RSD) of the measurements for all of the four dyes was between 0.56 and 1.65% intraday measurements. This method was successfully applied in the determination of the mentioned dyes in ice pops, gummy bears, chewing gum, and sweets candy samples.

1. Introduction

Synthetic food dyes are legally used in order to increase the attractiveness and color of the food [1]. Other than natural dyes, synthetic dyes are also used as additives in foodstuffs within legal boundaries. Sunset Yellow FCF, Allura Red AC, Quinoline Yellow WS, and Tartrazine are synthetic food dyes that are widely used in ice cream, popsicles, confectionery, gummy bears, juices, bakery, meat, sweets candy, desserts, snacks, drinks, and sauces [2, 3]. It has been thought that these food dyes cause some adverse effects, especially on the nervous system [4–6]. The health problems related to the synthetic food dyes depending on the reactions of the aromatic azo compounds in the structure are attention deficit hyperactivity disorder, allergies, food intolerance, and asthma [6–8]. The toxicity of the food dyes on the living organisms has been investigated [9–11]. Many countries have banned and strictly controlled the uses of these dyes in foodstuff and drinks [12].

Some spectrophotometric methods have been proposed for the analysis of food dyes [2, 3, 13–15]. However, mostly high-performance liquid chromatography methods for determining synthetic food dyes have been used. In these methods, the chromatographic conditions in which many dyes can be simultaneously determined have been investigated [16–20]. In particular, it is desirable that the determination time is short, and the operations before the determination are simple and not time-consuming. It is preferred that the mobile phase contains less amount of organic solvent in terms of environment. For this reason, there is still a need to develop a simple, fast, and environmentally friendly new methods.

Spectrophotometric methods are simple but usually require enrichment procedures before measurements [3].

HPLC methods are the most widely used methods due to their high sensitivity. However, the extraction of the dye and the chromatographic process can be time-consuming. In the present study, the development of a RP-HPLC method for the simultaneous determination of Sunset Yellow FCF, Allura Red AC, Quinoline Yellow WS, and Tartrazine in many types of foods has been aimed. Therefore, a faster and more environmentally friendly method of determination is proposed for the simultaneous determination of four food dyes using less chemical than those of literature methods. The four synthetic food dyes are efficiently separated using an optimized gradient elution in a single run within 15.1 min. The proposed method is validated in different food matrices, such as ice pops, gummy bears, chewing gum, and sweets candy, which is successfully used in the analysis of commercial samples.

2. Experimental

2.1. Chemicals and Samples. Ammonium acetate (GPR Rectapur) was from VWR Chemicals, and sodium hydroxide was from Merck Company (Darmstadt, Germany). The HPLC grade methanol (JT Baker) and acetonitrile (Merck) were used. Water was purified using an ultrapure water purification system (Sartorius Stedim Arium Pro UV).

Sunset Yellow FCF, Allura Red AC, Quinoline Yellow WS, and Tartrazine were purchased from Sigma-Aldrich (Steinheim, Germany). The structures, names, and C.I. numbers of the used dyes in this study are given in Scheme 1. The stock dye solutions (1000 mg L^{-1}) were prepared in water. Working solutions (50 mg L^{-1}) were prepared freshly from the stock solutions by dilution with water.

The mobile phase A consists of an aqueous ammonium acetate solution 1% (m/v), brought to pH 7.5 by drop-wise addition of a sodium hydroxide solution 10% (m/v) (m/v: mass/volume). Acetonitrile and methanol were used as mobile phases B and C, respectively.

Ice pops, gummy bears, chewing gum, and sweets candy were purchased from local supermarkets in Trabzon, Turkey.

2.2. Apparatus. Chromatographic analysis was carried out with an Agilent 1100 liquid chromatograph system integrated with a degasser Agilent 1260 and a UV detector. A pH meter (Eutech pH 510) equipped with a combined glass-calomel electrode was employed for pH measurements. An ultrasonic bath (Isolab Laborgerate GmbH) was used for the pretreatment of solid food samples.

2.3. Chromatographic Conditions. A Zorbax Eclipse^{R} XDB-C8 Analytical column (4.6 × 150 mm 5 micron, Agilent) was used as the column. The mobile phase A was filtered through a membrane filter with a pore diameter of 0.45 μm. For optimization, a number of gradient elution programs were tested by gradually decreasing the amount of the organic solvent. The optimum flow rate of eluent was 1.0 mL min^{-1}, and the injection volume was 20 μL. The optimized gradient program is shown in Table 1. All experiments were performed at room temperature.

The UV detector was used to detect the dyes at the specific wavelengths for each dye. These wavelengths were 427, 417, 482, and 507 nm for Tartrazine, Quinoline Yellow WS, Sunset Yellow FCF, and Allura Red AC, respectively.

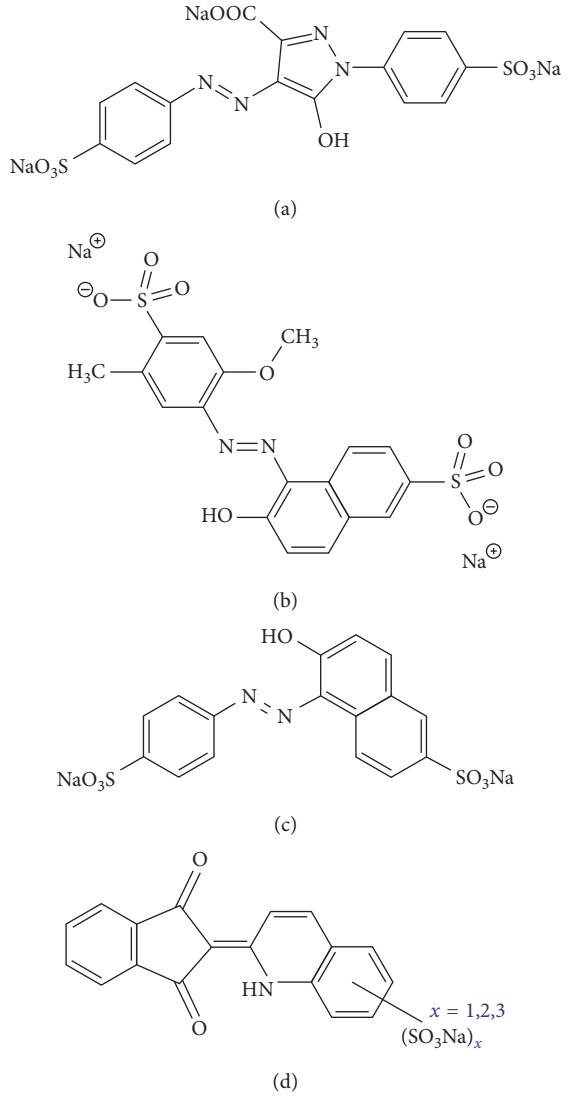
The chromatographic system was initially conditioned by passing the mobile phase A through the column for about 1 h to obtain a stable baseline signal.

2.4. Preparation of Dye Standards and Sample Solutions. Individual standard stock solutions (1000 mg L^{-1}) containing each dye were prepared by dissolving appropriate amount of dye in 25 mL distilled deionized water. The solutions were kept in dark flasks. The working standard solutions of each dye were prepared by appropriate dilution of stock solutions with water to give concentrations between 0.10 and 50 mg L^{-1}. The mixed standard solutions containing all dyes at concentrations between 1.0 and 50 mg L^{-1} were also prepared by mixing and dilution of appropriate aliquots from standard stock solution of each substance. All solutions were stored at 4°C in the dark and were stable at least for 2 months.

Gummy bear samples (10.0 g) in water (75 mL) were shaken to dissolve for 25 min in an ultrasonic bath, and the volume was made up to 100 mL in a volumetric flask. Sweets candy samples (20.0 g) were homogenized with a blender. The 4 g homogenized sample in water (75 mL) was shaken for 25 min in an ultrasonic bath at 25°C. The solution was filtered through a folded paper filter and then through 0.45 μm disposable syringe filter. The filtrate was collected in a volumetric flask of 100 mL. The sweets candy extract was filtered only through 0.45 μm disposable syringe filters. Chewing gum samples (20 g) in water (75 mL) were shaken in an ultrasonic bath for 15 min at room temperature. The solution was filtered through 0.45 μm disposable syringe filter. The filtrate was collected in the volumetric flask of 100 mL. Ice pop samples (10 g) were dissolved in water (75 mL). Then, they were filtered through 0.45 μm disposable syringe filters. The solution volumes were completed to 100 mL in the volumetric flask.

2.5. Validation of the Method. In order to determine the linear region of each dye, the measurements were carried out using different concentrations among 0.10–50 mg L^{-1} for all compounds, with three replicates per concentration. Calibration curves were also prepared with the mixed standard solutions at concentration levels of 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 mg L^{-1} to check the selectivity of the method. The calibration curve of each dye was used for the validation of experiments and determination.

The limit of detection (LOD) (3× Sd/m) and the limit of quantification (LOQ) (9× Sd/m) were determined by using the residual standard deviation of the regression line (Sd) and the slope of the calibration line (m) [21]. To verify the accuracy of the proposed method, analysis of the same food samples was carried out by using a standard method [22]. The Allura red amount in the gummy bear found using the standard method NMKL 130 was not statistically different from those of the proposed method. The results are given in Table 2. Statistical analysis of the results was made using Student's t-test, and there was no significant difference



Scheme 1: Synthetic food dyes used in this study. (a) Tartrazine, E 102, CI 19140. (b) Allura Red AC, E 129, CI 16035. (c) Sunset Yellow FCF, E 110, CI 15985. (d) Quinoline Yellow WS, E 104, CI 47005.

Table 1: Optimized gradient program for the separation of four dyes by HPLC-UV, at a flow rate of 1.0 mL min^{-1}.

t (min)	A (%)	B (%)	C (%)
0–12	100	0	0
12–17.6	52.5	9.5	38
17.6–21	0	20	80
21–23	100	0	0

between the results. The experimental t-values were below the critical t values (2.78, significant level = 0.05). Therefore, matrix components were not expected to interfere in the determination of the food dyes, Sunset Yellow FCF, Allura Red AC, Quinoline Yellow WS, and Tartrazine.

3. Results and Discussion

3.1. Optimization of the Separation. In this study, a simple reversed-phase liquid chromatography (RP-LC) method to determine simultaneously four synthetic food dyes, Sunset Yellow FCF, Allura Red AC, Quinoline Yellow WS, and Tartrazine, in various foodstuffs is developed. The hydrophobicity of these dyes depends on the presence of the azo group and the quinoline ring. Normally, the retention time of the polar molecules is shorter than that of apolar molecules under reverse-phase liquid chromatography conditions [19]. However, the presence of acidic and alkaline groups in the compounds changes the elution sequence. Usually, acetonitrile and methanol were used to elute these types of food dyes in the literature [19, 20]. The use of a modifier is a common approach to separate ionic species in short analysis time. An inorganic electrolyte as a modifier is generally added to the mobile phase [19]. For this aim, acetic acid-ammonium acetate buffer (pH 5–7.5) has been recently used as a modifier for the purification and separation of many azo dyes by RP-HPLC [19, 20, 23–25]. The ammonium acetate concentration higher than 0.1 M was not suitable for water-soluble food dyes because of the "salting-out" effect

Table 2: Determination of the synthetic dyes in food samples ($N = 3$).

Sample	Food dye	Proposed method		Standard method (NMKL 130, 1989)		Legal limit mg kg^{-1} [31]
		Value found (mg kg^{-1})	RSD %	Value found (mg kg^{-1})	E%	
Gummy bears	E129	38.4 ± 0.6	1.6	37.2 ± 0.6	3.3	300
Yellow chewing gum	E102	127.5 ± 1.9	1.5	—	—	300
Red chewing gum	E129	70.0 ± 0.9	1.2	—	—	300
Orange chewing gum	E110	117.2 ± 1.1	0.9	—	—	10
Ice pops	E102	13.3 ± 0.2	1.7	—	—	150
Sweets candy 1	E129	19.9 ± 0.2	1.2	—	—	300
	E102	25.8 ± 0.3	1.0			300
Sweets candy 2	E129	57.6 ± 0.3	0.6	—	—	300

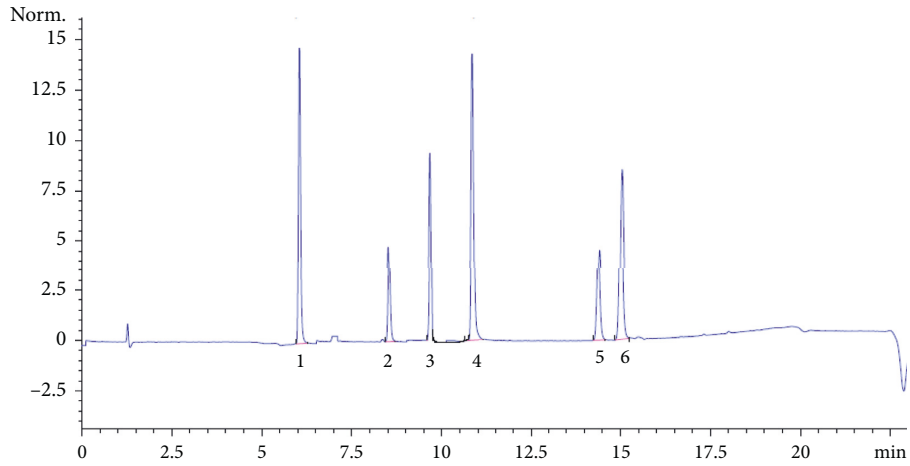


Figure 1: Chromatogram of a mixed standard dye solution by using the optimized gradient program in this study. Individual dye concentration was 1 mg L^{-1}.

[26]. That the acetate buffer solution as a modifier does not interact with the HPLC system and consistence for UV absorbance measurements are also advantages. The optimum pH was determined to be 7.5 for the mobile phase A because there are some examples of successful separation of water-soluble food dyes at this pH in RP-HPLC systems [19, 27–30].

A series of mixed standard solution (1, 2.5, 5.0, 10, 25, and 50 mg L^{-1}) containing the four water-soluble dyes was used to study the optimum conditions of separation. Many gradient elution programs were tested to obtain the shortest analysis time. The most suitable program was the gradient program shown in Table 1. The chromatogram of the mixture of four dyes (individual concentration 1 mg L^{-1}) is shown in Figure 1. The peaks and their corresponding t_R are shown in Table 3. As seen from Figure 1 and Table 3, there are three peaks for the mixture of Quinoline Yellow WS, and the t_R values of these peaks are 8.7, 14.6, and 15.1 min. These peaks are observed due to isomeric structures of Quinoline Yellow WS dye [22].

3.2. Validation of the Method. Table 4 gives LOD, dynamic ranges (limit of quantification-limit of linearity (LOQ-LOL)), calibration equations of mixed standard dye solutions, and coefficients of determination (R^2). The peak area

Table 3: Chromatographic data of the dyes with the optimized gradient program in this study.

Peak	Dye	λ_{max} (nm)	t_R
1	Tartrazine	472	6.1 ± 0.1
2	Quinoline Yellow WS I	417	8.7 ± 0.1
3	Sunset Yellow FCF	482	9.9 ± 0.1
4	Allura Red AC	507	10.9 ± 0.1
5	Quinoline Yellow WS II	417	14.6 ± 0.1
6	Quinoline Yellow WS III	417	15.1 ± 0.1

Table 4: Limits of detection (LOD), dynamic range (LOQ-LOL), calibration equations, and coefficients of determination (R^2) of all dyes.

Dye (C.I. numbers)	LOD (mg L^{-1})	LOQ-LOL (mg L^{-1})	Calibration equation	R^2
Tartrazine (E 102)	0.24	0.72–50	$y = 63.603x - 2.304$	0.999
Sunset Yellow FCF (E 110)	0.25	0.75–10	$y = 54.578x - 9.839$	0.999
Allura Red AC (E 129)	0.23	0.69–50	$y = 73.621x - 9.762$	0.999
Quinoline Yellow WS (E 104 I)	0.08	0.24–50	$y = 21.435x - 4.891$	0.999

of the compounds was used to calculate calibration equations. The dynamic ranges for the dyes are different from that of each other. The LOL value is 50 mg L^{-1} for all dyes, except for Sunset Yellow FCF. For this dye, the shorter linear range is obtained. The linearity limit is 10 mg L^{-1} for Sunset Yellow FCF. Detection limits for all dyes are nearly same (between 0.23 and 0.25 mg L^{-1}), except for Quinoline Yellow WS with 0.08 mg L^{-1}. The detection limit for Quinoline Yellow WS in Table 4 belongs to Quinoline Yellow WS I isomer.

Both the intra- and interday precisions were below 5% at a concentration level of 1.0 mg kg^{-1} for food matrices chosen, in case of all dyes.

3.3. Application to Real Samples. The method developed was applied to foodstuffs samples purchased from local market and supermarkets. The preparation of samples was given in Section 2.4. Ice pops, a sample of gummy bears, three samples of chewing gum, and two samples of sweets candy were analyzed ($n = 3$), and the results are summarized in Table 2. Both Allura Red AC and Tartrazine were detected in sweets candy I sample. All results were below the legal limits [31].

Data Availability

The chromatographic data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

We thank the Scientific Research Projects Unit of Karadeniz Technical University (Project No: 9727) for the financial support of this work.

References

[1] M. Oplatowska-Stachowiak and C. T. Elliott, "Food colors: existing and emerging food safety concerns," *Critical Reviews in Food Science and Nutrition*, vol. 57, no. 3, pp. 524–548, 2017.

[2] K. Rovina, P. P. Prabakaran, S. Siddiquee, and S. Md Shaarani, "Methods for the analysis of Sunset Yellow FCF (E110) in food and beverage products- a review," *Trends in Analytical Chemistry*, vol. 85, pp. 47–56, 2016.

[3] T. Güray, "Spectrophotometric determination of sunset yellow (E-110) in powdered beverages and pharmaceutical preparations after cloud point extraction method," *Journal of The Turkish Chemical Society, Section A: Chemistry*, vol. 5, no. 2, pp. 479–492, 2018.

[4] Y. Gao, C. Li, J. Shen, H. Yin, X. An, and H. Jin, "Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved," *Journal of Food Science*, vol. 76, no. 6, pp. T125–T129, 2011.

[5] B. Weiss, "Synthetic food colors and neurobehavioral hazards: the view from environmental health research," *Environmental Health Perspectives*, vol. 120, no. 1, pp. 1–5, 2012.

[6] L. E. Arnold, N. Lofthouse, and E. Hurt, "Artificial food colors and attention-deficit/hyperactivity symptoms: conclusions to dye for," *Neurotherapeutics*, vol. 9, no. 3, pp. 599–609, 2012.

[7] F. Gultekin and D. Kumbul Doguc, "Allergic and immunologic reactions to food additives," *Clinical Reviews in Allergy and Immunology*, vol. 45, no. 1, pp. 6–29, 2013.

[8] M. O. Elhkim, F. He´raud, N. Bemrah et al., "New considerations regarding the risk assessment on tartrazine: an update toxicological assessment, intolerance reactions and maximum theoretical daily intake in France," *Regulatory Toxicology and Pharmacology*, vol. 47, no. 3, pp. 308–316, 2007.

[9] O. E. Thomas and O. A. Adegoke, "Toxicity of food colours and additives: a review," *African Journal of Pharmacy and Pharmacology*, vol. 9, no. 36, pp. 900–914, 2015.

[10] S. Kobylewski and M. F. Jacobson, "Toxicology of food dyes," *International Journal of Occupational and Environmental Health*, vol. 18, no. 3, pp. 220–246, 2012.

[11] S. Arefin, M. S. Hossain, S. A. Neshe, M. M. Or Rashid, M. T. Amin, and M. S. Hussain, "Tartrazine induced changes in physiological and biochemical parameters in Swiss albino mice, *Mus musculus*," *Marmara Pharmaceutical Journal*, vol. 21, no. 3, pp. 564–569, 2017.

[12] EC, "Directive of the European Parliament and of the council 94/36/EC of June 30, 1994 on colours for use in foodstuffs," *Official Journal*, vol. L237, no. 13, 1994.

[13] A. Kaur and U. Gupta, "The review on spectrophotometric determination of synthetic food dyes and lakes," *Gazi University Journal of Science*, vol. 25, no. 3, pp. 579–588, 2012.

[14] G. Yentür, M. Yaman, and A. Bayhan, "Studies conducted for the quantity determination of synthetic dyes added into some foodstuffs," *Journal of Food*, vol. 23, no. 3, pp. 195–199, 1998.

[15] M. Üstün Özgür and İ. Koyuncu, "The simultaneous determination of Quinoline Yellow WS (E-104) and Sunset Yellow (E-110) in syrups and tablets by second derivative spectrophotometry," *Turkish Journal of Chemistry*, vol. 26, pp. 501–508, 2002.

[16] N. Mahmoodi, M. Faraji, and P. Ziarati, "Simultaneous determination of sunset yellow and carmoisine in orange flavored soft drink samples by high-performance liquid chromatography," *IOSR Journal of Applied Chemistry*, vol. 9, no. 8, pp. 79–83, 2016.

[17] F. I. de Andrade, M. I. Florindo Guedes, I. G. Pinto Vieira et al., "Determination of synthetic food dyes in commercial soft drinks by TLC and ion-pair HPLC," *Food Chemistry*, vol. 157, pp. 193–198, 2014.

[18] M. González, M. Gallego, and M. Valcárcel, "Determination of natural and synthetic colorants in prescreened dairy samples using liquid chromatography-diode array detection," *Analytical Chemistry*, vol. 75, no. 3, pp. 685–693, 2003.

[19] K. S. Minioti, C. F. Sakellariou, and N. S. Thomaidis, "Determination of 13 synthetic food colorants in water-soluble foods by reversed-phase high-performance liquid chromatography coupled with diode-array detector," *Analytica Chimica Acta*, vol. 583, pp. 103–110, 2007.

[20] E. Dinç, A. H. Aktaş, and O. Üstündağ, "New liquid chromatographic-chemometric approach for the determination of sunset yellow and tartrazine in commercial preparation," *Journal of AOAC International*, vol. 88, no. 6, pp. 1748–1755, 2005.

[21] D. C. Harris, *Quantitative Chemical Analysis*, W. H. Freeman and Company, New York, NY, USA, 2007.
[22] NMKL Method No: 130, 1989, https://www.nmkl.org.
[23] F. Z. Mazdeh, Z. Moradi, G. Moghaddam et al., "Determination of synthetic food colors, caffeine, sodium benzoate and potassium sorbate in sports drinks," *Tropical Journal of Pharmaceutical Research*, vol. 15, no. 1, pp. 183–188, 2016.
[24] W. D. A. Siqueira Bento, B. P. Lima, and A. P. S. Paim, "Simultaneous determination of synthetic colorants in yogurt by HPLC," *Food Chemistry*, vol. 183, pp. 154–160, 2015.
[25] M. S. Garcia-Falcon and J. Simal-Gandara, "Determination of food dyes in soft drinks containing natural pigments by liquid chromatography with minimal clean-up," *Food Control*, vol. 16, no. 3, pp. 293–297, 2005.
[26] F. A. Ozdemir Olgun, B. Demirata Ozturk, and R. Apak, "Determination of synthetic food colorants in water-soluble beverages individually by HPLC and totally by Ce(IV)-oxidative spectrophotometry," *Food Analytical Methods*, vol. 5, no. 6, pp. 1335–1341, 2012.
[27] M. Chen, D. Moir, F. M. Benoit, and C. Kubwabo, "Purification and identification of several sulphonated azo dyes using reversed-phase preparative high-performance liquid chromatography," *Journal of Chromatography A*, vol. 825, no. 1, pp. 37–44, 1998.
[28] K. Rovina, S. Siddiquee, and S. M. Shaarani, "Extraction, analytical and advanced methods for detection of Allura Red AC (E129) in food and beverages products," *Frontiers in Microbiology*, vol. 7, pp. 1–13, 2016.
[29] M. Kucharska and J. Grabka, "A review of chromatographic methods for determination of synthetic food dyes," *Talanta*, vol. 80, no. 3, pp. 1045–1051, 2010.
[30] M. Šuleková, A. Hudák, and M. Smrčová, "The determination of food dyes in vitamins by RP-HPLC," *Molecules*, vol. 21, no. 10, p. 1368, 2016.
[31] http://mevzuat.basbakanlik.gov.tr.