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ORIGINAL ARTICLE

Comparison of High-Performance Liquid Chromatography and Thin Layer Chromatography Methods to Identify Tartrazine in Four Food Types

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ABSTRACT

Background: The use of tartrazine as an unauthorized synthetic color in food is a consumer health concern. In Iran, most studies have employed the Thin Layer Chromatography (TLC) to detect any fraud in food colors. Therefore, this study aimed to identify the presence of tartrazine in four types of foods commonly consumed by children in Shiraz, southern Iran using TLC and compare the results with those obtained through high-performance liquid chromatography (HPLC).

Methods: One hundred and fifty food samples with yellow and orange colors, including 20 samples of freeze pop, 25 samples of ice cream, 57 samples of jelly, 48 samples of candy, were collected from the supermarkets in Shiraz, Iran. In TLC method, color extraction was performed by the white wool method.

Results: According to the findings of the TLC method, 118 samples (78.67%) contained synthetic food colors, while 32 (21.33%) food samples did not. Among the samples, 8 (5.33%) contained only tartrazine, and 14 (9.33%) had tartrazine together with other synthetic colors. The HPLC revealed that 127 samples (84.64%) contained synthetic colors, and 23 samples (15.36%) did not. Only 11 (7.33%) samples had tartrazine, while102 samples (68.00%) had tartrazine in addition to other synthetic colors.

Conclusion: The finding of TLC and HPLC methods were differed due to the lower detection limit of the TLC method. Therefore, it is necessary to use more accurate methods such as HPLC to detect fraud in food colors.

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Introduction

Food colors play an essential role in consumer acceptance among food additives due to their impact on food appearance. Nowadays, synthetic food colors are widely used to substitute for natural food colors. These additives can conceal inferior or defective products, making foods more visually appealing (1, 2). Synthetic food colors are commonly used in a variety of foods, including snacks, beverages, and ice cream, due to their higher resistance to light, temperature, and oxidation (3). In the food industry, artificial azo dyes make up about 65% of the dyes. Tartrazine is an artificial synthetic azo dye commonly used in human food

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and pharmaceutical products (4).

According to some studies, synthetic colors are considered foreign substances by the body, similar to drugs and pollutants. After consumption, metabolic enzymes or intestinal flora may convert some of these colors into aromatic amines, which can then be absorbed or excreted (5, 6). Excessive use of such colors can endanger human health. Some studies have also shown that synthetic food colors such as sunset yellow and tartrazine can cause a range of allergic reactions, including skin rashes, asthma exacerbation, restlessness, sleep disorders, and aggravate hyperactivity in children with the condition of ADHD (7, 8). Various studies have shown that unauthorized synthetic food colors have been used illegally in foods. For example, studies conducted in 2015 in Arak city, Iran and 2017 in Sri Lanka showed that unauthorized synthetic food colors were used in food (2, 9).

Therefore, measuring and controlling the color of food products is mandatory in many countries and there are regulations for the use of synthetic food colors; for example, in Iran, according to Standard No. 740 of the Iran National Standards Organization (INSO), only seven synthetic food colors are allowed for use in foods. Tartrazine is classified as an unauthorized synthetic food color. In most countries, the possible adverse effects of such dyes are tested periodically (10-12). Analytical methods for detecting synthetic food color and its potential fraud in food industry include high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), electrochemical sensors, and spectrophotometry (4). The TLC method is less expensive and is qualitative for separating compounds in a mixture. However, in addition to identifying the mixture's components (qualitative), the HPLC method can also measure the amount of components quantitatively. As in Iran, TLC method is mostly used to detect the type of synthetic food colors and their fraud usage; therefore, this study aimed to compare the two methods of identifying tartrazine frauds by TLC and HPLC in four food products.

Material and Methods

One hundred and fifty samples of four food items in orange and yellow colors, in which tartrazine could be used, were randomly collected from Shiraz supermarkets and transferred to the relevant laboratory, including 20 fruit ice creams, 57 jellies, 48 candies, and 22 freeze pops. All materials used in this study were purchased from Merck (Germany) and Sigma (USA), including concentrated acetic acid, 25% ammonium hydroxide, Kieselgel 60 F254 silica gel on 20×20 cm aluminum foil, n-butanol, white wool, hematocrit tubes, hamilton syringes, acetonitrile HPLC grade, methanol (HPLC grade), ammonium acetate, standard tartrazine dye, TLC chromatography tank (with volume of 25×7×25-cm^3) and also HPLC device (Waters company, USA) was equipped with a dual-piston pump, UV detector and degasser. Isolations were performed on a C-18 (250 mm×4.6 mm, 5.6 µm) chromatographic column, and the tartrazine dye wavelength was measured using a dynamic double beam UV-Vis spectrophotometer (Halo DB-20R).

According to Standard No. 2634 of the INSO, extraction was performed using the white wool method (13). First, sugar or flour coatings or other edible decorations were removed from the sample's surface. Our samples included lollipops, jellies, ice cream, and freeze pop, which were free of fat, protein, starch, or other decorations, so dye extraction was performed directly without any isolation and separation. To activate the silica gel plates, the plate was placed at 100°C for 8 minutes to remove moisture and activate the silica gel. After cooling the plates, staining was done according to Standard No. 2634, starting 3 cm above the edge of the plate with a distance of 1.5 cm from each other and a code was written under the marked stain to recognize each of the stains. The stain created by the tartrazine standard was used to differentiate other color stains.

Regarding TLC tank preparation and color detection step, the mobile phase, which included 10 volumes of n-butane (50 mL), 5 volumes of concentrated acetic acid (25 mL) and 6 volumes of distilled water (equivalent to 30 mL) was prepared inside the tank and placed under the hood the day before. The stained plate was placed inside the tank, and after the mobile phase rose to about 5 cm from left to the bottom of the plate, the plate was taken out of the tank. After drying, each stain retention factor (RF) was measured and compared to the RF of standard stain. Considering HPLC steps, the standard tartrazine solution was prepared in five concentrations of 10, 5, 2.5, 1.25, and 0.75 mg/L to create a calibration plot and stored at 4°C.

The mobile phase included acetonitrile, methanol, concentrated acetic acid and 25% ammonium hydroxide that were prepared in the proportions of 190 mL: 10 mL: 5600 µL: 8300 µL. The pH adjustments were made to provide solutions with a pH of 6 using NA_4OH. The injection volume of the sample was 20 µL, and the column temperature was kept at ambient room temperature. Mobile phase flow rates were selected to be 0.8 mL/min, then the calibration curve was plotted (Figure 1), and the tartrazine was detected using a UV detector with a wavelength of 420 nm (Figure 2). The identification of colors was made based on retention time.

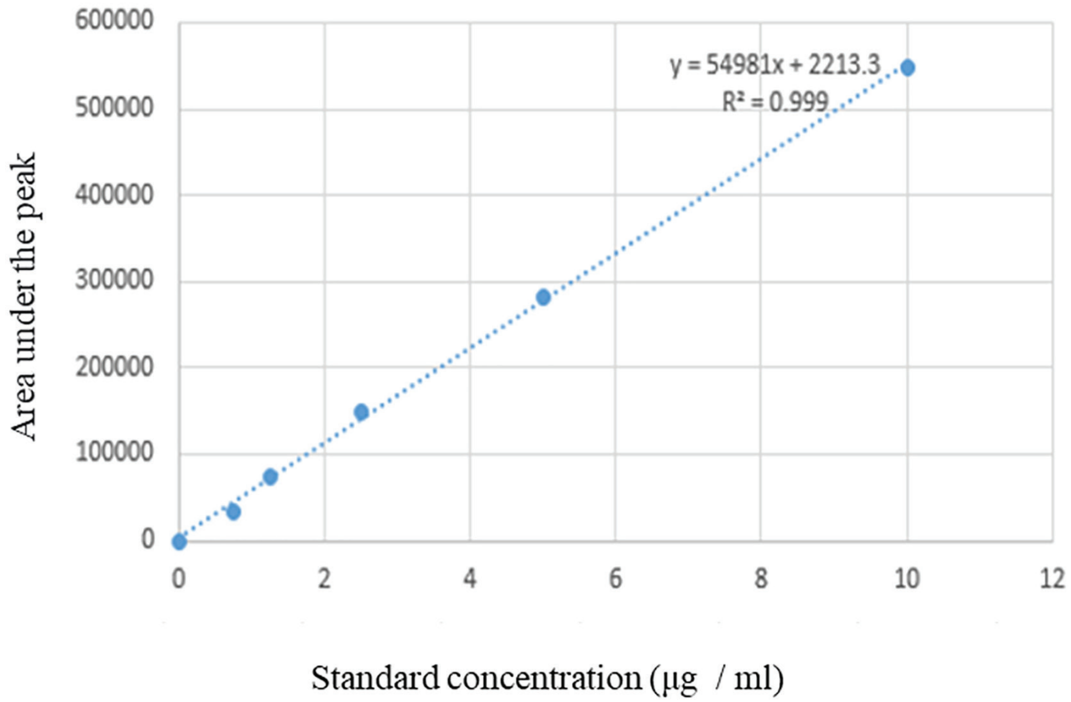

Figure 1: Tartrazine standard calibration curve.

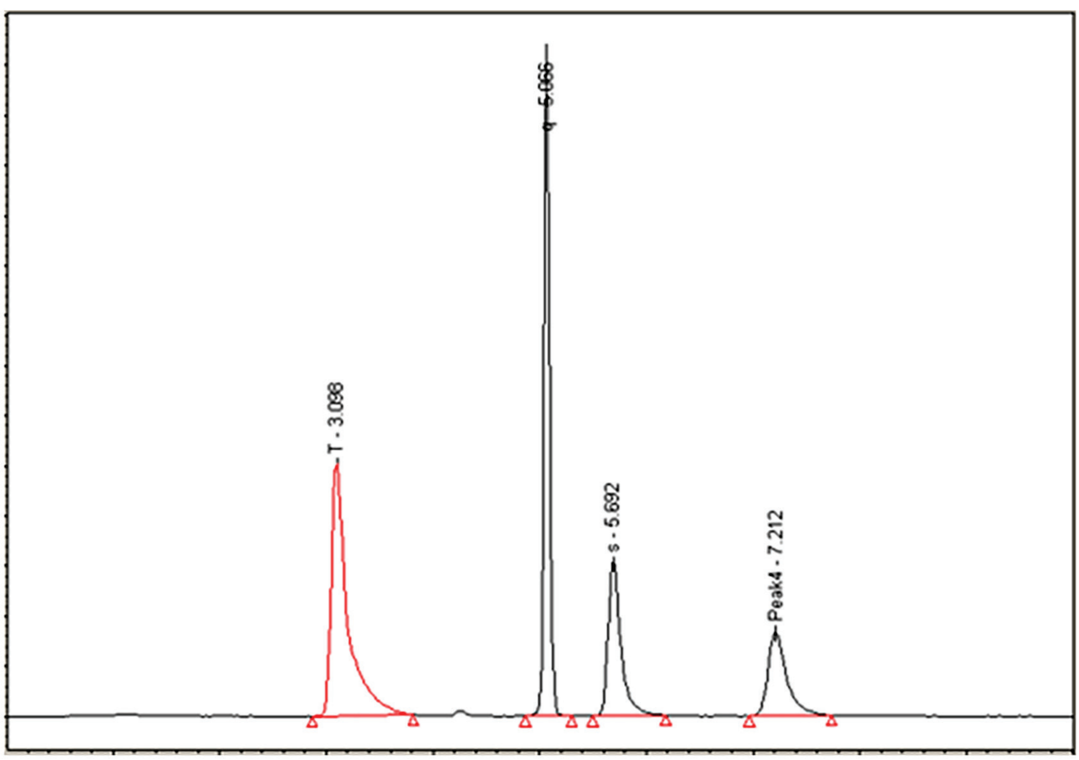

Figure 2: Tartrazine peak at 420 nm in HPLC.

Color measuring and identifying were performed by Analytical Quality Assurance (AQA) [the retention time, calibration data, relative standard deviation (RSD%)], limit of detection (LOD) and the limit of quantification (LOQ)) that were shown in Table 1. In sample preparation, first, all ice cream and freeze pops samples were liquefied at room temperature and jelly samples were liquefied in a water bath at 90°C. After homogenization, 10 mL of each sample was poured into a 25 mL volumetric balloon with 2 mL ammonium acetate, while it reached a volume of 25 mL with 50:50 deionized water and methanol. Solid samples (candy) were first pulverized and after homogenizing 5 grams, the volume was adjusted to 25 mL as described for liquid samples. Finally, all prepared samples were centrifuged at 5000 rpm for 5 minutes at 30°C, and after passing through a 0.22 µm syringe filter, 20 µL of each sample was injected into HPLC.

Table 1: Tartrazine dye Analytical Quality Assurance (AQA) data. *Recycling: Minimum and maximum recycling range.

Analytics name	Retention time in minutes	Calibration data						
		Wavelength (nm)	Correlation coefficient (r)	Equation for linear regression	* Recycling range (%)	RSD* % (n= 3)	LOD (mg/kg)	LOQ (mg/kg)
Tartrazine (E102)	3.09	420	0.999	y=54981x+ 2213.3	2/112-3/106	1.2-3	0.08	0.24

Table 2: Results of color analysis of samples by TLC method.

Product type	Containing a combination of tartrazine and other synthetic dyes (%)	Containing tartrazine (%)	Sample size
Freeze pop products	1 (4)	0	25
Fruit ice cream	0	0	20
Jellies	2 (3.51)	2 (3.51)	57
Lollipops	11 (22.92)	6 (12.5)	48
Total	14 (9.33)	8 (5.33)	150

Table 3: Results of color analysis of samples by HPLC method.

Product type	Containing a combination of tartrazine and other synthetic dyes (%)	Containing tartrazine (%)	Sample size
Freeze pop products	22 (88)	0	25
Fruit ice cream	1 (0.05)	9 (0.45)	20
Jellies	46 (80.7)	2 (3.51)	57
Lollipops	33 (68.75)	0	48
Total	102 (68)	11 (7.33)	150

Results

The results of the TLC analysis of the samples were shown in Table 2 revealing that out of 150 analyzed food items, 118 (78.67%) samples had additive color, while 32 (21.33%) food samples did not have any synthetic food color. Totally, 8 samples (5.33%) contained only tartrazine, and 14 samples (9.33%) used tartrazine mixed with other synthetic dyes. HPLC analysis showed that out of 150 tested samples, 127 (84.67%) samples had synthetic colors and 23 (15.33%) samples did not have any synthetic color. Eleven (33.7%) samples had tartrazine alone and 102 samples (68%) contained tartrazine mixed with other colors as described in Table 3. Most frauds were demonstrated to be performed in foods related to jelly, candy, ice cream and then freeze pop, respectively (Figure 3).

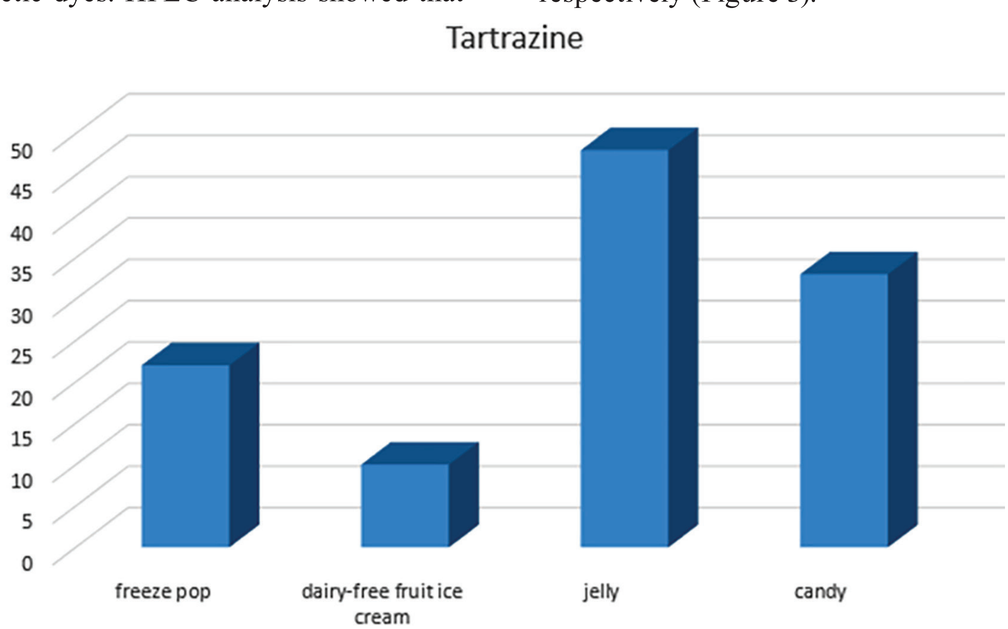

Figure 3: Comparison of tartrazine frauds in the analyzed samples.

Discussion

Asthma-like symptoms, the weakened immune system, and the aggravation of hyperactivity in children can be caused by excessive consumption of synthetic food colors. Tartrazine, as an unauthorized synthetic color, is widely used nowadays (14). According to the present study results, it was observed that 127 samples (84.67%) had synthetic colors and 23 samples (15.33%) did not have any synthetic food colors. Also, 113 samples (75.33%) contained unauthorized synthetic food colors. Kermani *et al.* in Bojnourd, Iran (2015) conducted a study on 90 samples of colored candies. Out of 90 samples of candies, 16 samples (17.78%) had natural colors and 74 samples (82.3%) had synthetic colors (15).

Another study conducted in Shahr-e Kord, Iran by Hafshjani *et al.* (2014) examined the colors used in ice cream, saffron liquids, candy, confectionery tea, kebab chicken, etc. Their findings showed that 33.8 % of the samples had synthetic food colors. Also, the highest use of synthetic colors was for confectionery tea with an average of 56.2% and the lowest for ice cream with an average of 7.1%, as reported in the present study, which found the least synthetic colors in ice cream. In their study, the most synthetic colors used belonged to tartrazine with an average of 67.7%, and the lowest amount of tartrazine was reported for ice cream, which is consistent with the results of the present study (16). Arast *et al.* in Qom (2010) examined 398 food samples to identify synthetic colors; while the results showed that 52% of the samples did not have synthetic colors. Twenty-six point seven percent of the samples had unauthorized synthetic colors, 21.3% had authorized synthetic colors and the most widely used color was yellow (17).

Regarding the comparison of the TLC and HPLC methods to identify tartrazine fraud in four categories of snacks consumed by children, it was determined that more than half of the samples were analyzed by the HPLC method containing tartrazine, but in the TLC method, out of 25 samples of analyzed freezed pop products, only one sample with tartrazine dye was detected. Among the analyzed jellies by the TLC method, 4 jellies were found to contain tartrazine. The results of HPLC analysis of the jellies showed that 48 jelly samples contained tartrazine. Among the analyzed candies, 17 candies were identified by TLC as containing tartrazine, and in HPLC, 33 samples of 38 candies had tartrazine. As the results showed, none of the fruit ice creams had tartrazine according to the TLC method, but in the HPLC method, 10 out of 20 fruit ice cream samples had tartrazine. Ten samples of candy analyzed by TLC methods had no synthetic colors, so were re-analyzed by HPLC. The results were similar to the TLC results, which showed that no synthetic colors were used in these 10 samples. In general, according to Iranian National Standard No. 740, the use of tartrazine dye in food is illegal.

Since tartrazine is an unauthorized food color in Iran, the industry uses a minimal amount of this color in their products to prevent fraud detection. Therefore, it could be the reason why the TLC method did not detect tartrazine fraud, and on the other hand, tartrazine was one of the colors widely available at a reasonable price. In ice cream, due to the low stability and brightness of natural colors in small quantities usage, and changes in taste in high quantities usage, therefore industry owners are turning to synthetic colors. Synthetic colors in minimal amounts along with natural colors can create a desired appearance. The difference between the two detection methods can be attributed to the low concentration of tartrazine which is below the TLC limit of detection, or the overlap of tartrazine with other natural or synthetic colors.

Barani *et al.* (2019) used the HPLC method to detect synthetic food color fraud instead of saffron in 160 grilled chicken samples due to the overlap of crocin stains with tartrazine stains in the TLC method. The results showed that 25.62% of the samples were stained with saffron and 74.38% were stained with synthetic food color, of which 10% of the staining with synthetic colors was related to tartrazine (18). Kucharska *et al.* (2009) studied three chromatographic methods, including TLC, HPLC and high-performance thin-layer chromatography (HPTLC), to identify synthetic food colors. Their results showed that each of the three methods could be used successfully to analyze synthetic food colors, but none were entirely ideal. Although the TLC method is the most efficient method for determining the type of colors in terms of simplicity and low cost, but in this method, some natural and artificial colors may have the same maximum absorption and Rf coefficient, and this can cause some problems in color differentiation and recognition (19).

Conclusion

The findings indicated that tartrazine color was used illegally in a wide range of analyzed samples despite the prohibition of tartrazine usage in the food industry. This unauthorized food color is used in the food industry in minimal quantities and in combination with other permitted colors. As mentioned, tartrazine can cause problems for some sensitive people with their health. Due to the differences in the detection results of tartrazine by TLC and HPLC, it is necessary to use more practical and accurate methods such as HPLC to investigate the fraud of color additives, especially in foods consumed by sensitive groups. Although significant differences were found in the detection of tartrazine between these two methods, TLC is still practical and cost-effective, especially for mass screening and routine analysis, which are costly and time-consuming.

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None to declare.

Authors' Contribution
HR: Data collection, Data analyses and interpretation, writing the original draft, Reviewed and approved the manuscript. RA: Data analyses and interpretation, Review and editing the manuscript. EB: Methodology, Investigation, Reviewed and approved the manuscript. SMM: Consulting for Data analyses and interpretation, Reviewed and approved the manuscript.

Conflicts of Interest
The authors declare no conflict of interest.

References
1 Rovina K, Prabakaran PP, Siddiquee S, et al. Methods for the analysis of Sunset Yellow FCF (E110) in food and beverage products-a review. *TrAC Trends Analytic Chem*. 2016;85:47-56. DOI:10.1016/j.trac.2016.05.009.
2 Rezaei M, Safar Abadi F, Sharifi Z, Karimi F, Alimohammadi M, Susan Abadi RA, et al. Assessment of synthetic dyes in food stuffs produced in confectioneries and restaurants in Arak, Iran. *Thrita*. 2015;4. DOI:10.5812/thrita.22776.
3 Qi P, Lin ZH, Chen GY, et al. Fast and simultaneous determination of eleven synthetic color additives in flour and meat products by liquid chromatography coupled with diode-array detector and tandem mass spectrometry. *Food Chem*. 2015;181:101-10. DOI: 10.1016/j.foodchem.2015.02.075. PMID: 25794727.
4 Rovina K, Siddiquee S, Shaarani MDS. A review of extraction and analytical methods for the determination of tartrazine (E 102) in foodstuffs. *Crit Rev Anal Chem*. 2017;47:309-24. DOI:10.1080/10408347.2017.1287558. PMID: 28128965.
5 Yamjala K, Nainar MS, Ramisetti RR. Methods for the analysis of azo dyes employed in food industry–a review. *Food Chem*. 2016;192:813-24. DOI:10.1016/j.foodchem.2015.07.085. PMID: 26304415.
6 Kuno N, Mizutani T. Environmental Health PA. Influence of synthetic and natural food dyes on activities of CYP2A6, UGT1A6, and UGT2B7. *J Toxicol Environ Health A*. 2005;68:1431-44. DOI:10.1080/15287390590956588. PMID: 16009655.
7 de Andrade FI, Guedes MIF, Vieira ÍGP, et al. Determination of synthetic food dyes in commercial soft drinks by TLC and ion-pair HPLC. *Food Chem*. 2014;157:193-8. DOI:10.1016/j.foodchem.2014.01.100. PMID: 24679770.
8 Macioszek VK , Kononowicz AK. The evaluation of the genotoxicity of two commonly used food colors: Quinoline Yellow (E 104) and Brilliant Black BN (E 151). *Cell Mol Biol Lett*. 2004;9:107-22. DOI:10.21275/ART20173280. PMID: 15048155.
9 Rajapaksha GKM, Wansapala MAJ, Silva ABG. Research. Detection of synthetic colours in selected foods & beverages available in Colombo district, Sri Lanka. *Int J Sci Res*. 2017;6:801-8. DOI:10.21275/ART20173280.
10 740 OII. Permitted food additives- Food colors-List and general specifications. 2019.
11 Minioti KS, Sakellariou CF, Thomaidis NS. Determination of 13 synthetic food colorants in water-soluble foods by reversed-phase high-performance liquid chromatography coupled with diode-array detector. Anal Chim Acta. 2007;583:103-10. DOI:10.1016/j.aca.2006.10.002. PMID: 17386533.
12 Rao P, Bhat R, Sudershan R, et al. contaminants. Exposure assessment to synthetic food colours of a selected population in Hyderabad, India. *Food Addit Contam*. 2004;21:415-21. DOI:10.1080/02652030410001668772. PMID: 15204542.
13 Organization INS. Permitted food additives-Synthetic food colors in foods-Identification by thin layer chromatography-Test method. 2013.
14 Kiani M, Ezati P, Pourmohammadi B, et al. Prevalence of colors of used in Saffronal foods of Yazd using Thin Layer Chromatography (TLC) in 2015. *Navid No*. 2016;19:1-7. DOI: 10.22038/nnj.2016.7371.
15 Kermani M, Rajabizadeh A, Langarizadeh M, et al. Dye Concentration in Colored Candies in Bojnourd City in 2016. *Health Develop J*. 2020;7:155-63. DOI:10.22062/JHAD.2020.91284.
16 Alipour Hafshejani F, Mahdavi Hafshejani F. Determine the prevalence of food contamination to synthetic colors with thin layer chromatography

in Shahrekord. *J Shahrekord Uni Med Sci*. 2016;17:103-12.

17 Arast Y, Mohamadian M, Noruzi M, Ramuz Z. Surveillance on artificial colors in different confectionary products by chromatography in Qom. *Zahedan J Res Med Sci*. 2013;15:62-64.

18 Barani A, Tajik HJIJoFP. Simultaneous determination of saffron and synthetic dyes in ready-to-cook Iranian barbecued chicken by HPLC. *Int J Food Proper*. 2019;22:1608-14. DOI:10.1080/10942912.2019.1666870.

19 Kucharska M, Grabka JJT. A review of chromatographic methods for determination of synthetic food dyes. *Talanta*. 2010;80:1045-51. DOI:10.1016/j.talanta.2009.09.032. PMID: 20006052.