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Fast Sensitive and Accurate Analysis of the Most Common Synthetic Food Colorants in 65 Egyptian Commercial Products Using New HPLC–DAD and UPLC-ESI–MS/MS Methods

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Abstract

Overexposure to food colorants above the allowed daily intake (ADI) level can provoke hyperactivity and other disturbed behaviors especially in children. Two new methods were developed to separate five synthetic colorants, which were Tartrazine (E102), Sunset Yellow (E110), Allura Red (E129), Carmoisine (E122), and Brilliant Blue (E133). They are labeled on a large variety of commercial food products in the Egyptian market without mentioning their definite concentrations. Therefore, there was a real need to determine these colorants with simple, accurate, and fast methods. This is the first study to determine these colorants in a wide variety of food products present in the Egyptian market. The HPLC approach with photodiode array detection was developed to quantify these colorants, on a C18 column, with a mobile phase composed of acetonitrile and water containing 1% ammonium acetate (pH 6.8), separation was carried out using a gradient program. The colorants were eluted and efficiently separated within 9 min. Then, as a complementary technique to HPLC, the UPLC-ESI–MS/MS approach was developed for identification and accurate mass measurement of the colorants found in high concentrations, the colorants were obtained simultaneously in negative mode, the run time was only 3 min. These developed methods were validated according to ICH recommendations and they were applied to analyze 65 food products including jelly powder, puddings, ice cream powders, concentrated soft drink powders, carbonated drinks, chewing gums, and sugar confectionery.

Keywords Egyptian food products · RP-HPLC–DAD · Synthetic colorants · UPLC-ESI–MS/MS

Introduction

Recently, food safety has become a vital issue to the general public, medical, nutritional experts, and food science researchers. Food additives are widely used to confer a desirable appearance and enhance the nutritional properties of commercial products (Ntrallou, 2020; Bordagaray, 2018; Bordagaray, 2019). Colorants are chemicals which are applied during development or processing of foods and soft drinks. However, some of these chemicals represent potential risks to human health and result in adverse effects like metabolic acidosis, tremors, and hyperpnoea (Sorouraddin, 2015), especially if they are used in exceeded amounts (Sierra-Rosales, 2017). Therefore, regulated use of these colorants in different food items must be ensured (Islam, 2019).

Children are the most vulnerable as they consume a lot of candies and drinks, especially those rich in synthetic colorants. Owing to their lower weight relative to adults, acceptable daily intake values (ADIs) are easily reached so

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that the adverse effects are more obvious in this population. Centered on agencies' international guidelines, such as the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA), all food colorants must be listed (Mazdeh, 2016). The analysis of food needs quick methods for routine control and high sample throughput. Chromatographic separation allows multiple compounds in complex matrices to be quantified simultaneously, many methods have been involved to improve separation efficiency and decrease the time of analysis (Lhotská, 2018).

The investigated synthetic colorants were (Tartrazine, Sunset Yellow, Allura Red, Carmoisine, and Brilliant Blue). Chemical structures, numbers of the European community (E numbers), and denominations of the color index (CI numbers) were represented in (Fig. S1). Upon observation, they were among the dyes most labeled in Egyptian food products such as ice cream, puddings, sweets, drinks, and chewing gums; however, their concentrations were not mentioned. Mentioning their concentrations is valuable to be sure that ADIs are not exceeded particularly by young children. This work aimed to monitor various Egyptian food products which are consumed daily especially by children, using fast simple methods. Chromatographic analysis was successfully performed on 65 food products.

Various analytical approaches have been reported to determine a wide variety of synthetic food colorants, like thin-layer chromatography (TLC) (Oka, 1994; de Andrade, 2014), adsorptive voltammetry (Ni, 1997), differential pulse polarography (Combeau, 2002), and electrophoresis (Cifuentes, 2006; Dossi, 2007; Prado, 2006). However, most of them require time-consuming pretreatment and cannot be applied to complicated color mixtures (Mazdeh, 2016). Different chromatographic methods have been reported including HPLC with UV detection (Demiralay, 2006; García, 2003; Lino, 2010; Saad, 2005; Techakriengkrai, 2007; Tfouni, 2002; Brazeau, 2018), ion chromatography with UV detection (Dossi, 2006), molecular absorption spectrophotometry (Cantarelli, 2009), GC using flame ionization detection (Dong, 2006, Wang,2006), flow injection analysis (FI) with the aid of UV detection (García-Jiménez, 2007; García-Jiménez, 2006). High-performance ion chromatography (Lucena, 2005), reversed-phase liquid chromatography (Garcıa-Falcón, 2005; Kirschbaum, 2003; Prado, 2002; Mathiyalagan, 2019), and ion-pair liquid chromatography (Fuh, 2002; Gianotti, 2005; González, 2003; Gallego, 2003; Ishikawa, 2003; Kiseleva, 2003). There were some methods to determine the studied colorants in presence of others in different matrices using HPLC with UV/Vis or photodiode array detection (Al-Degs, 2009; Enríquez-Gabeiras, 2012; Kirschbaum, 2006; Long, 2009; Olgun,2012; Serdar, 2009; Iammarino, 2019). HPLC coupled with UV/Vis or DAD detection is the most common approach as colorants are highly absorbed at the UV and/or visible wavelength. UPLC-MS/MS methods for analysis of colorants in foods have also been reported (Chen, 2014; Gao, 2015; Guerra, 2018; Tsai, 2013; Zou, 2013). Ultraviolet (UV) detectors were replaced by MS detectors as they provide more structural details and can adhere to the confirmatory process requirements set out in European Commission Decision 2002/657/EC. Recently, E102,E110, E122, E129, and E 133 were separated in the presence of some other colorants in food products consumed by children in Saudi Arabia using only an HPLC–DAD method and the method developed was not completely validated (Ahmed, 2021), while E102, E110, E129, and E 133 were separated in the presence of some other colorants in only chewing gums and soft drinks present in Korea using HPLC–DAD method and LC–MS/MS method, where the limit of detection of the HPLC–DAD method was almost similar to our proposed HPLC–DAD method while the limit of detection of our LC–MS/MS method was lower than the reported LC–MS/MS method (Jang,2021). Also, E102,E110, E129 were separated using HPLC–UV method in soft drinks, powder juice and candies (Al-Khateeb, 2021). Nguyen has separated E102,E110, E122, E129, and E 133 in the presence of other colorants in food products in Korea (Nguyen, 2021), using only HPLC–DAD method with longer run time and higher limit of detection than our method.

In our study, a reversed-phase high-performance liquid chromatography with diode array detector (RP-HPLC–DAD) method and a UPLC-ESI–MS/MS method were developed for the determination of five water-soluble synthetic food colorants (E102, E110, E129, E122, and E133) in a set of 65 food products present in the Egyptian market. These colorants were chosen owing to their abundance among food products in the local market in Egypt. The proposed methods included a simple pretreatment procedure for the samples and allowed the detection of colorants at very low concentrations. It is worth mentioning that this study is considered to be the first one to determine the concentrations of the synthetic food colorants (E102, E110, E129, E122, and E133) in a wide variety of food products such as sweets, drinks, ice cream powders, puddings, and chewing gums in the Egyptian market, which are consumed mainly by children, and to compare the results with international limits.

Experimental

Materials and Reagents

High purity standard of Tartrazine (E102) (99.1% purity), Sunset Yellow (E110) (98.1% purity), Allura Red (E129) (98.1% Purity), Carmoisine (E122) (98.0% purity), and Brilliant Blue (133) (98.5% purity) were obtained from Sigma-Aldrich. HPLC-grade methanol was obtained from Fisher Scientific. Ammonium acetate (98%) and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich. The ultra-pure water was prepared by distillation in glass and passage through a Milli-Q water system, passed through a 0.45-μm membrane

filter, and degassed for 30-min in an ultrasonic bath. Food samples were purchased from Egyptian supermarkets.

RP-HPLC–DAD Analysis

The HPLC separation and quantitation were achieved on Waters LC 2695, (Milford, MA, USA) with a gradient pump using low-pressure mixing system, vacuum membrane degasser, an autosampler with a 100 µl sample loop and a capacity of 120 vials, and a Waters module 2996 photodiode array detector. The column C18 (100 mm × 4.6 mm (i.d.)) packed with Inertsil ODS-3 V (5-µm particle diameter, GL Sciences, Tokyo, Japan) and an HPLC column oven (DALIAN REPLETE®, Hong Kong) was used. Waters Empower software was used for Data acquisition. The mobile phase consisted of eluent A, water containing 1% ammonium acetate (with pH 6.8, adjusted with ammonium hydroxide); and eluent B, acetonitrile. RP-HPLC–DAD assay was carried out by constructing a gradient elution program of 5% B at 0–3 min, 10% B at 3–9 min, 40% B at 9–9.5 min, and 70% B at 9.5–12 min. In the end, a 3 min equilibrium phase to the column was made to recover first condition of 95% A: 5% B for the next run. The acquisition wavelength of DAD was set to scan between 200 and 800 nm. At column temperature 25 °C, all determinations were performed. The injected volume was 20 µL and the run time was 12 min. The flow rate set at 1.0 mL min^{-1}. The mobile phase was filtered through a 0.45-µm membrane filter and degassed under vacuum prior to use.

UPLC-MS/MS Analysis

Separation was made on a Waters 3100 instrument (Milford, MA, USA) with a binary solvent manager pump, autosampler, and thermostatic column compartment. An API 5000 triple quadrupole (TQ detector, Acquity UPLC, USA) was the tandem mass spectrometer. A UPLC acquity® BEH shield RP18 column (150 mm × 2.1 mm, 1.7 µm) was used. Mass lynx software (Version 4.1) was used for data analysis and system operation. UPLC/MS/MS assay was carried out using an isocratic system with a flow rate of 0.3 mL min^{-1}. Acetonitrile: 4 mM ammonium acetate (80:20, v/v) was the mobile phase. By electrospray ionization mass spectrometry, mass spectra were obtained simultaneously in negative mode (ESI/MS). The injected volume was 2 µl through a 3-min run time.

The tuning parameters were set for detection of the investigated colorants optimally: desolvation temperature, 400 °C; ion source temperature, 120 °C; capillary voltage, 3.5 kV; desolvation gas flow, 600L/h. The proper collision energy, cone voltage and representative product ions values for these five colorants were listed in Table 1.

Preparation of Standards of Colorants and Calibration

Dissolving 10.0 mg of pure coloring agents into 10.0 mL of methanol was used to prepare individual standard stock solutions containing each colorant. The solutions were freshly prepared, tightly closed to amber colored volumetric flasks, and kept at 4 °C. For calibration of HPLC method, these stock solutions were diluted with methanol to obtain working standard solutions of suitable concentrations within the concentration range (0.5–50 µg mL^{-1}). For calibration of UPLC-MS/MS method, accurate volumes of each of E102, E110, E129, E122, and E133 stock solutions were transferred into volumetric flasks (10 mL) and diluted to volume with methanol to make synthetic mixtures in the concentration range (0.01–5 µg mL^{-1}) of each compound.

Preparation of Commercial Samples

All of the sixty five tested samples were brought from the Egyptian markets and categorized to four categories: jelly powders, puddings, ice cream powders (A), concentrated fruit-flavored soft drinks powders (B), carbonated fruit-flavored drinks (C), chewing gums, candy smartiz, and sugar confectionery(D). Category A samples were numbered from A 1 to A28, category B samples were numbered from B 29 to B43, category C samples were numbered from B 44 to B51, and category D samples were numbered from B 52 to B65.

Table 1 The optimum UPLC-MS/MS parameters and selected typical fragment ions for the studied synthetic colorants

Colorant	Precursor ion (m/z)	Production (m/z)	Dwell (sec)	Cone (V)	Coll. energy (eV)	Ion mode
E102	233.02	210.91	0.036	15	10	ESI -
E133	373.32	169.99	0.036	30	35	ESI -
E110	203.05	170.74	0.036	25	25	ESI -
E129	225.17	206.76	0.036	25	35	ESI -
E122	228.06	220.96	0.036	25	30	ESI -

Samples Analyzed by RP-HPLC–DAD Technique

Treatment of Carbonated Fruit-Flavored Drinks

Twenty-five milliliters of the sample were adequately transferred to a 50-mL volumetric flask and 20 mL of HPLC water was added. The samples were subjected to sonication for 15 min and completed with HPLC water to the mark. After filtering through 0.45-µm disposable syringe filters, 20 µl of the solutions were injected.

Treatment of Concentrated Fruit-Flavored Soft Drink Powders

Two grams of each sample was weighed and diluted with 30 mL HPLC water in a 50-mL volumetric flask. The samples were subjected to sonication for 15 min and completed to the mark with HPLC water. After filtering through 0.45-µm disposable syringe filters, all solutions were injected (20 µl).

Treatment of Jelly Powder, Ice Cream Powder, Puddings, and Mehalabia

Two grams of each sample was weighed and diluted with HPLC water in a 25-mL volumetric flask. The sample solutions were subjected to sonication for 15 min and completed with HPLC water to the mark. After filtering through 0.45-µm disposable syringe filters, all solutions were injected (20 µl).

Treatment of Chewing Gums, Candy Smartiz, and Sugar Confectionary

Five grams of each sample was weighed then sliced and transferred to a beaker containing 5 ml of 1% ammonia solution (Khanavi, 2012). Twenty-five milliliters of HPLC water was added, the sample solutions were subjected to sonication for 30 min, filtered through a folded filter paper, and the filtrate was collected in a 50-ml volumetric flask and completed with water. After filtering through 0.45-µm disposable syringe filters, the solutions were injected (20 µl).

Samples Analyzed by UPLC-MS/MS Technique

The selected samples (A5, A11, A13, A19, B31, B40, B41, B42, B43, and D 53) were treated as follows: accurately, 0.5 gm of each sample was weighed and dissolved in 10 mL of water (50 mg/mL), except savory red sampleD53) was diluted to 5 mg/mL, as it is highly concentrated and vortexed for 1 min. All solutions were subjected to sonication for 30 min and further diluted by HPLC water. All sample solutions were filtered by a 0.22-µm syringe filter then injected.

Results and Discussion

Optimization of the Separation

Optimization of the HPLC–DAD Method

Many mobile phase compositions have been tried, methanol and phosphate buffer (50:50, v/v); methanol and phosphate buffer (70:30, v/v), methanol and ammonium acetate buffer (80:20, v/v); methanol and ammonium acetate solution 1% (w/v) (70:30,v/v); and acetonitrile and ammonium acetate buffer (20:80, v/v). The separation was poor for these trials. E122 and E133 colorants were not well separated and the peaks E102 and E110 were forked. Isocratic elution was not appropriate and gradient elution was then tried to achieve better separation. The use of gradient elution to separate colorants was also present in previous methods (Kirschbaum, 2003; Serdar, 2009; Iammarino, 2019; Filiz, 2019; Ahmed, 2021, Jang, 2021). Different amounts of ammonium acetate in the mobile phase were tested. Experiments and a reported method (Minioti, 2007) revealed that the most effective amount for ammonium acetate is 1% (w/v). Different pH values for the ammonium acetate buffer were also examined (6, 6.8, 7.5, and 7.9), and increasing pH to more than 7 leads to poor resolution. Several time programs with different initial percentage of acetonitrile were tested, increased acetonitrile concentration to more than 10%, resulted in insufficient separation and characteristic overlap of E122 and E133 colorants, while lower acetonitrile concentration (about 5%) resulted in excessive tailing and longer retention time of E133. Different column temperatures were examined (25 ºC, 30 ºC, and 35 ºC) and no significant effect was observed upon increasing temperature more than 25 ºC. The best results were achieved by using mobile phase consisting of water containing 1% ammonium acetate at pH 6.8 and acetonitrile using gradient elution program and column temperature kept at 25 °C. Optimum resolution, consistent baseline separation of the studied compounds, was achieved by the previous condition (Fig. 1). The chromatographic conditions used allowed us to achieve the separation of colorants within 9 min.

A chromatogram was established with scanning in the wavelength range from 200 to 800 nm, continuously. The spectral data were very useful to confirm peak purity. The absorption maxima of the studied colorants were 426 nm, 482 nm, 508 nm, 517 nm, and 629 nm for E102, E110, E129, E122, and E133, respectively. Quantitation was achieved at 388 nm, where reasonable linearity ranges, LOD, and LOQ were achieved for all of the colorants studied. The average retention time ± standard deviation for E102, E110, E129, E122, and E133 were found to be 3.561 ± 0.02, 6.533 ± 0.04, 7.109 ± 0.05, 8.394 ± 0.07, and 8.864 ± 0.06 min, respectively, for seven replicates. The first to be eluted from the

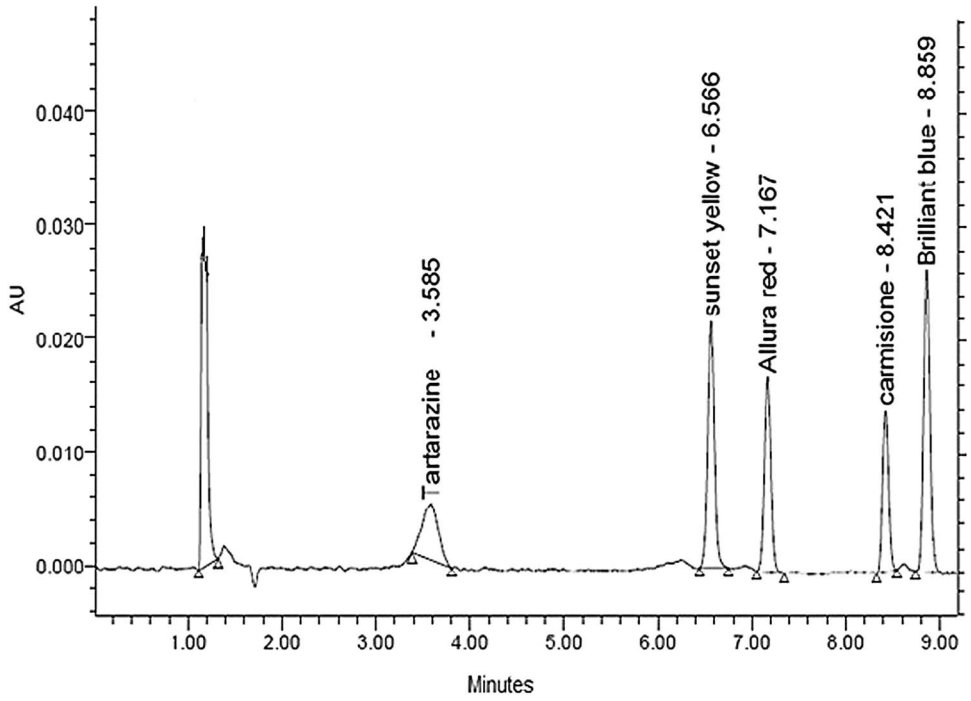

Fig. 1 HPLC chromatogram of 20 µl injection of laboratory prepared mixture of the five colorants, quantitation was achieved at 388 nm

Table 2 System suitability results of the HPLC method developed for the analysis of E102, E110, E129, E122, and E133

Parameters	E102	E110	E129	E122	E133
Retention time (min)	3.561	6.533	7.109	8.394	8.864
%RSD of retention time ($n=7$)	0.56	0.61	0.69	0.83	0.68
Capacity factor (K')	2.26	4.97	5.51	6.65	7.05
Selectivity (α)	2.21	1.11	1.21	1.06	
Resolution (R_S)	7.035	3.223	6.165	2.153	
Symmetry factor (T)	1.1	1.03	1.02	1.03	1.05

chromatographic column were the azo compounds, followed by non-azo compounds. The last synthetic colorant eluted was E133 which is derived from triphenyl methane and has an apolar character. For that, this compound interacted longer with the column and was the last to be eluted. The selectivity of the HPLC method was demonstrated in Table 2.

Optimization of the UPLC-MS/MS Method

In the LC–ESI–MS/MS system, selection of the mobile phase composition is of high importance because it must offer an acceptable compromise between good chromatographic elution, separation, analytes ionization, and efficient desolvation of charged species in the MS detector. Satisfactory identification was performed with a mobile phase consisting of acetonitrile: 4 mM ammonium acetate (80:20 v/v) using isocratic elution (Fig. 2). The optimum flow rate was 0.3 mL min^{-1}. These followed conditions, allowed us to quantify the studied colorants in 3 min.

Under these operating conditions, E102, E110, E129, E122, and E133 were negatively charged due to their chemicophysical properties. They undergo deprotonation due to the presence of acidic sites. For this reason, MS detection was performed in the negative ESI mode.

Structural Analyses of E102, E110, E129, E122, and E133 by UPLC-ESI–MS/MS

Upon observing the structural analyses of the studied colorants, E102 exhibited an (M-H)− ion at m/z 233.02 and eluted at 0.75 min, its MS/MS spectrum produced fingerprint fragment ions at m/z 210.91 and 197.58 (Fig. S2), E110 exhibited an (M-H)− ion at m/z 203.05 eluted at 0.79 min, its MS/MS spectrum produced fingerprint fragment ions at m/z 170.74, 155.78, and 106.68 (Fig. S3), E129 exhibited an (M-H)− ion at m/z 225.17 and eluted at 0.79 min, its MS/MS spectrum produced fingerprint fragment ions at m/z 235.92, 206.76, 200.03, 181.05, 171.75, and 135.98 (Fig. S4); E122 exhibited an (M-H)− ion at m/z 228.06 and eluted at 0.79 min, its MS/MS spectrum produced fingerprint fragment ions at m/z 220.96, 206.08, 169.98, and 79.69 (Fig. S5); and E133 exhibited an (M-H)− ion at m/z 373.32 and eluted at 0.78 min, its MS/MS spectrum produced fingerprint fragment ions at m/z 481.40, 333.33, and 169.99 (Fig. S6).

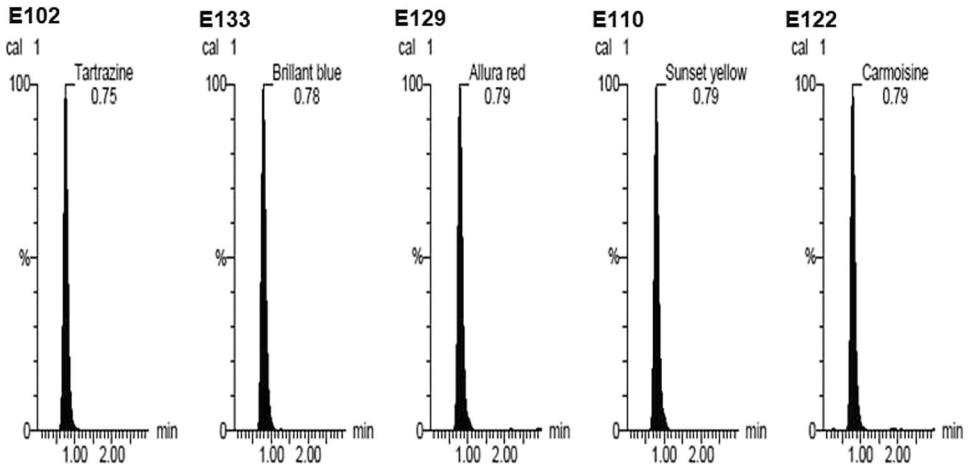

Fig. 2 UPLC-ESI–MS/MS chromatograms of 2 µl injection of the five colorants

Validation of the Methods

Linearity

The linearity of the methods proposed was tested by evaluating the various concentrations of each compound. Nine concentrations were chosen in the 0.5–50 µg mL^{-1} range for the HPLC–DAD process, while concentrations in the range 0.01–5 µg mL^{-1} were used for the tested colorants in the UPLC-MS/MS range. Three injections were made for each concentration. The high value of the correlation coefficient was used to assess the calibration graphs' linearities. The regression parameters for the methods obtained by the least square treatment of the results were shown in Table 3.

Precision and Accuracy

Analysis of three concentration levels of working solutions of each compound on the same day determined the intra-day accuracy and also precision (each concentration was repeated three times). Inter-day accuracy and precision were measured on three consecutive days by evaluating the three concentration levels of working solutions. The acceptability of the data included accuracy stated as relative error (RE %) and precision stated as relative standard deviation (RSD %). Tables S1 and S2 summarized the results of intra-day and inter-day precision and accuracy.

The maximum measured relative standard deviation of the measurements was approximately 2.1% for UPLC-MS/MS and 2.6% for HPLC–DAD, suggesting the excellent precision of the analytical methods proposed at both repeatability and intermediate levels.

Limits of Detection and Quantification

According to ICH recommendations (ICH, 2005), the limit of detection (LOD) and the limit of quantification (LOQ) is determined as the ratio of 3.3 and 10 standard deviations of the blank (n = 9), respectively, and the slope of the calibration line. Table 3 presented the detection and quantitation limits calculated for E102, E110, E129, E122, and E133.

Table 3 Characteristic parameters for the regression equations of the proposed HPLC–DAD and UPLC-MS/MS methods for determination of E102, E122, E110, E129, and E133

Colorant	Linearty range (µg mL^{-1})	Calibration equation	LOD (µg mL^{-1})	LOQ (µg mL^{-1})	R^2
HPLC-DAD					
E102	0.5-50	Y=94149.9X-4933	0.111	0.370	0.9999
E110	0.5-50	Y=10555X-403.8	0.150	0.500	0.9996
E129	0.5-50	Y =7576.9X -161.43	0.042	0.140	0.9997
E122	0.5-50	Y =6808.9X -257.31	0.067	0.223	0.9999
E133	0.5-50	Y =10489X +294.5	0.067	0.223	0.9999
UPLC-MS/MS			LOD(ng mL^{-1})	LOQ(ng mL^{-1})	
E102	0.01–5	Y=2.7814X+397.28	1.308	3.964	0.9996
E110	0.01–5	Y=2.0377X+147.77	1.570	4.760	0.9998
E129	0.01–5	Y =1.7819X +210.25	1.833	5.554	0.9994
E122	0.01–5	Y =2.8166X +219.12	0.403	1.220	0.9997
E133	0.01–5	Y =6.1596X +68.669	1.168	3.538	0.9999

Table 4 Concentrations of the five synthetic colorants among sixty five real food samples of four categories using the HPLC–DAD method

Sample type	Sample code	Product name	Colorant declared on the label	Colorant found	Concentration (mg/kg of final product)
Jelly powder, Mehalabia, puddings, and Ice Cream (A)	A1	Dreem Jelly "Strawberry flavor"	E102	E102	98.700
			E122	E122	73.421
	A2	Tag El-Melouk Jelly "Strawberry flavor"	E122	E122	14.935
				E110	8.756
	A3	Holw El-Sham Jelly "Berry flavor"	E133	E129	1.435
	A4	Dreem Jelly "Green apple flavor"	E102	E102	80.557
			E133	E133	2.666
	A5	Dreem Jelly "Tangerine flavor"	E110	E110	53.460
			E102	E102	113.470
	A6*	Dreem Jelly "Cherry &Cola flavor"	E122	E122	7.254
			E102	E102	19.750
			E110	NI	
			E133	NI	
	A7	Dreem Jelly "Apricot flavor"	E110	E110	50.135
	A8	Holw El-Sham Jelly "Apricot flavor"	E110	E110	48.809
	A9	Bait Food Jelly "Apricot flavor"	E110	E110	48.071
	A10	5-Minutes Jelly "Strawberry flavor"	E102	E102	19.151
			E122	E122	41.353
	A11	Green's Jelly "Lemon Flavor"	E102	E102	102.744
	A12	Green's Jelly "Orange Flavor"	E110	E110	31.067
	A13	Green's Jelly "Strawberry flavor"	E129	E129	120.254
	A14*	Green's Jelly "Strawberry &Banana flavor"	E129	E129	79.765
			E102	NI	
	A15	Diva Ice Cream "Mango flavor"	E102	E102	41.975
			E110	E110	61.844
	A16	Diva Ice Cream "Strawberry flavor"	E102	E102	8.722
			E122	E122	22.795
	A17*	Diva Ice Cream "Chocolate flavor"	E133	NI	
			E102	NI	
			E122	NI	
			E110	E110	2.127
				E129	4.380
	A18	Holw El-Sham Ice Cream "Mango flavor"	E102	E102	40.313
			E110	E110	31.836
	A19	5-Minutes Ice Cream "Mango flavor"	E102	E102	118.289
			E129	E129	2.344
			E110	E110	21.476
	A20	5-Minutes Ice Cream "Pistachio flavor"	E133	E133	0.1668
			E102	E102	4.576
				E129	3.376
	A21	Bait Food Pudding Powder "Vanilla flavor"	E102	E102	23.192

Table 4 (continued)

Sample type	Sample code	Product name	Colorant declared on the label	Colorant found	Concentration (mg/kg of final product)
			E110	E110	1.023
				E129	52.240
	A22	Bait Food Pudding Powder "Strawberry flavor"	E102	E102	13.638
			E122	E122	20.502
	A23	Alamia Mehalabia "Apricot flavor"	E102	E102	3.499
			E110	E110	1.144
	A24*	Easy Food Mehalabia "Strawberry flavor"	E110	NI	
	A25	Holw El-Sham Mehalabia "Milk with Banana"	E102	E129	1.319
	A26	Holw El-Sham Custard	E110	E110	1.603
	A27	Holw El-Sham Mehalabia "Orange Flavor"	E110	E110	4.229
			E102	E102	6.924
	A28	Holw El-Sham Mehalabia "Apricot flavor"	E110	E110	10.529
				E102	7.783
Concentrated soft drink powders (B)	B29	JANGO "Mango flavor"	E102	E102	58.569
			E110	E110	3.941
	B30*	My Chef "Amar-EL-din"	E102	NI	
			E110	E110	2.548
				E129	0.9193
	B31	Al-Diafa "Mango flavor"	E102	E102	112.711
			E110	E110	12.149
	B32	Do roots "Orange Flavor"	E110	E110	3.839
			E102	E102	38.243
	B33	Tolido "Mango flavor"	E110	E110	11.530
			E102	E102	11.158
	B34*	Fruty "Hibiscus flavor"	E133	NI	
			E129	E129	42.580
			E122	E122	57.926
	B35	Fruty "Mango flavor"	E110	E110	27.377
			E102	E102	26.476
			E102	E102	26.476
	B36	Fruty "Orange Flavor"	E110	E110	30.401
			E102	E102	78.661
	B37	TANG "Mango flavor"	E110	E110	94.642
			E129	E129	2.159
	B38	TANG "Amar El-Deen flavor"	E110	E110	60.085
	B39	TANG "Pineapple flavor"	E110	E110	3.119
			E102	E102	38.828
	B40	TANG "Orange flavor"	E102	E102	125.446
			E110	E110	29.662
			E129	E129	2.826
	B41	Again Plus "Orange flavor"	E110	E110	56.109
			E102	E102	120.147
	B42	Again Plus "Mango flavor"	E110	E110	116.707

Table 4 (continued)

Sample type	Sample code	Product name	Colorant declared on the label	Colorant found	Concentration (mg/kg of final product)
			E102	E102	122.160
				E129	6.220
	B43	7-Days Juice "Tamarind flavor"	E102	E102	1.934
			E110	E110	107.375
			E133	E133	2.175
				E122	27.650
Carbonated drinks (C)	C44	Fanta "Orange flavor"	E110	E110	39.402
	C45*	Fanta "Green apple flavor"	E133	NI	
			E102	E102	24.942
	C46	Fanta "Strawberry flavor"	E110	E110	16.389
	C47	Schweppes "Tangerine flavor"	E110	E110	29.144
			E102	E102	53.697
	C48	Schweppes "Pomegranate flavor"	E122	E122	16.140
				E110	0.690
	C49	Mirinda "Orange flavor"	E110	E110	42.220
	C50	Mirinda "Strawberry & Kiwi flavor"	E129	E129	54.247
				E110	0.722
	C51*	Mirinda "Green apple flavor"	E102	E102	19.985
			E133	NI	
Chewing gums, candy smartiz, and sugar confectionery (D)	D52*	Savory "Pineapple culinary essence"	E102	E102	27.717
			E110	NI	
	D53	Savory "Red food coloring"	E122	E122	213.696
				E129	35.699
	D54	Savory "Chocolate food coloring"	E102	E102	41.070
			E122	E122	111.580
	D55	Halls "Menthol flavor"	E133	E133	1.297
	D56	Halls "Cherry flavor"	E122	E122	5.757
	D57	Batook "Chewing gum"	E102	E102	33.777
			E110	E110	7.777
			E129	E129	4.935
			E133	E133	0.177
				E122	3.460
	D58	Chiclets "Tutti Frutti gums"	E110	E110	1.374
				E133	0.731
	D59*	Mentos 3-D "Watermelon, Pineapple,Melon flavor"	E133	NI	
	D60	M&M "Peanut"	E133	E133	12.227
	D61	Marbella coated chocolate substitutes	E133	E133	0.025
			E110	E110	2.384
			E129	E129	1.750
	D62*	Green Life Candy	E122	NI	
	D63	Mr. Jello fruity Jello	E133	E133	15.681
	D64*	Popping Candy	E110	NI	
			E129	E129	7.063
			E102	NI	

Table 4 (continued)

Sample type	Sample code	Product name	Colorant declared on the label	Colorant found	Concentration (mg/kg of final product)
			E133	NI	
	D65*	Supper magic "Bubble gum"	E133	NI	
			E110	E110	0.813
			E102	NI	
			E122	E122	31.530

NI not identified

*Samples where colorants were labeled but not identified

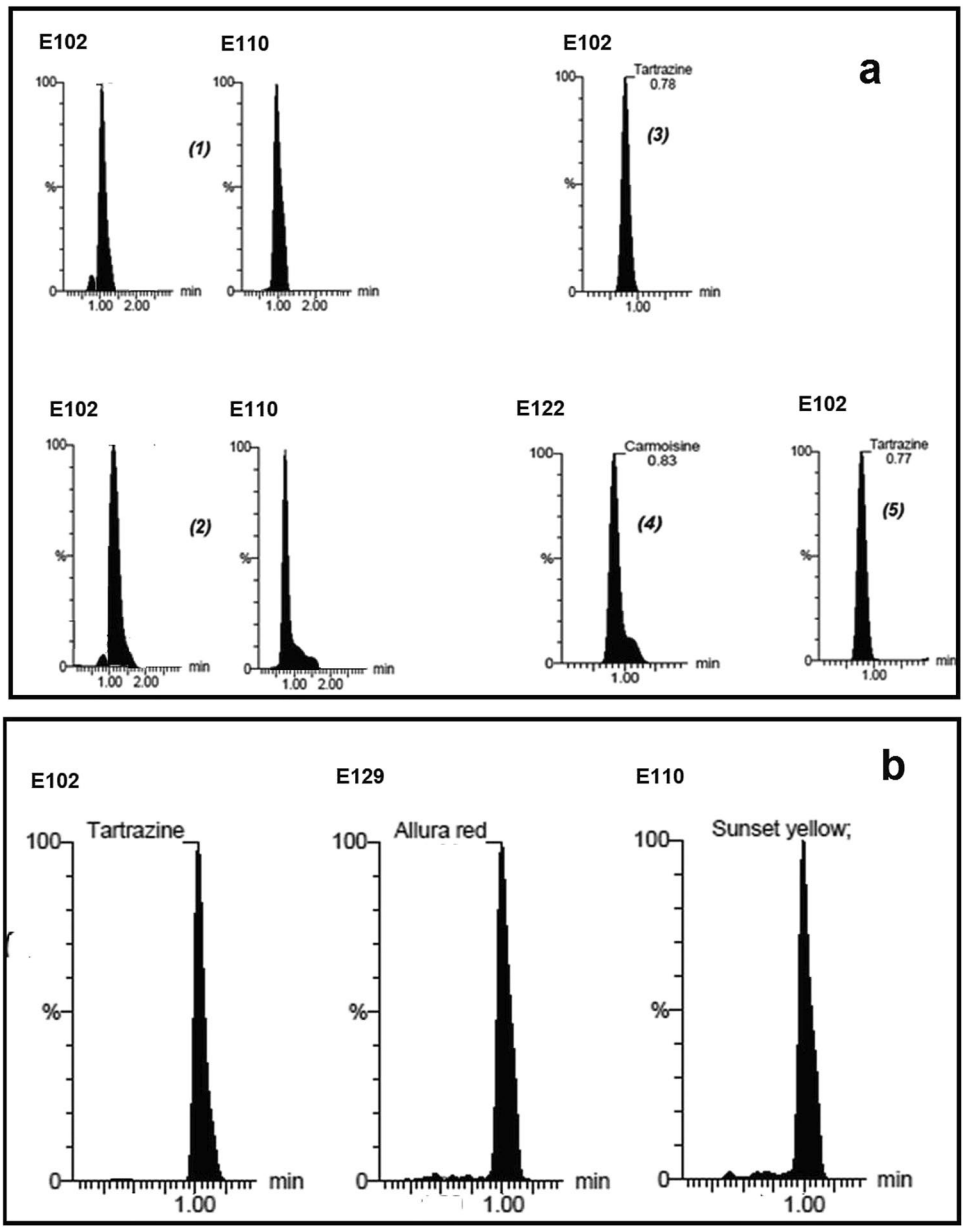

Fig. 3 **a** Examples of typical chromatograms: (1) E102 & E110 in sample B42. (2) E102 & E110 in sample B41. (3) E102 in sample A19. (4) E122 in sample D53. (5) E102 in sample B40; **b** identified colorant (E129) in sample B42

Selectivity

The selectivity of the methods was established by preparing five mixtures of the studied colorants within the linearity range at different concentrations. The mixtures were analyzed according to previously discussed procedures. Appropriate recoveries (Table S3) were generated, revealing the good selectivity of the methods proposed to concurrently analyze E102, E110, E129, E122, and E133.

Analytical Solution Stability

The analysis demonstrated that the standard colorants solutions were stable for about 3 h at room temperature, for 1 week if kept at 4 °C and for 1 month when kept in the freezer at – 20 °C.

Application to Real Samples

Analysis of Food Samples Using HPLC–DAD Method

The developed HPLC approach was efficiently applied to determine E102, E110, E129, E122, and E133 in sixty five food products which are consumed daily in Egypt especially by children, including carbonated fruit-flavored drinks, concentrated fruit-flavored soft drinks, chewing gums, candy smartiz, puddings, jellies, ice cream powders, and sugar confectionery. Upon reviewing literature for determination of these colorants in relatively similar matrices, the previous HPLC method (Al-Degs, 2009) for determination of only E129, E110, and E102 in commercial soft drinks showed long elution time of 10 min, while the reported method (Olgun, 2012), E102 and E110 and some other colorants were separated in beverages at very long retention time of 14 min and 19 min with the same limit of detection of our developed method and the previous method (Brazeau, 2018) needed a complicated sample pretreatment. This study is considered the first one to be conducted to determine the concentrations of the synthetic food colorants (E102, E110, E129, E122, and E133) in a huge amount of food products in Egypt and to compare the results with international limits with simple sample treatment, good sensitivity and short elution time.

The concentrations obtained by the developed HPLC method for the investigated colorants were shown in Table 4. The concentrations of colorants varied from 0.0252 mg kg^{-1} (sample D61) to 213.696 mg kg^{-1} (sample D53). A strange fact is that some manufacturers report that their product contains a certain colorant while a different colorant was detected and quantified by our method (sample A3). Furthermore, some products contained excess colorants which were not labeled (samples A2, A17, A20, A21, A25, A28, B30, B42, B43, C48, C50, D53, D57, D58, and D61). Some colorants were labeled but not identified as in the samples A6, A14, A17, A24, B30, B34, C45, C51, D52, D64, and D65.

Analysis of Some Selected Samples Using UPLC-MS/MS Method

Products which contain very high concentrations of colorants above ADI levels were investigated and characterized by UPLC-ESI–MS/MS technique. Ten food samples (A5, A11, A13, A19, B31, B40, B41, B42, B43, and D53) were selected. Table S4 summarized the results of the ten samples and Fig. 3 showed five food products containing high concentrations of synthetic colorants. According to FDA, ADI of the studied colorants were used to calculate the maximum allowed amount for children weighed 15–25 kg and adults weighed 60–70 kg per day. The data were given in Table S5.

Fig. 3 **a** Examples of typical chromatograms: (1) E102 & E110 in sample B42. (2) E102 & E110 in sample B41. (3) E102 in sample A19. (4) E122 in sample D53. (5) E102 in sample B40; **b** identified colorant (E129) in sample B42

The sample D53 contained the highest concentration among the others, definitely 199.6 mg/Kg of E122 colorant which is not allowed by FDA due to its harmful effects on human health as it has an adverse effect on activity and attention in children. Also, samples B40, B41, B42, and A19 acquired concentrations of colorants above the legal limit. Also in sample B42, the manufacturer reported that the product containing only E102 and E110, and the analysis confirmed the presence of one more synthetic colorant, E129 (Fig. 3). This revealed that our study is suitable for confirmation of the identity and quantity of colorants in foods.

These results have shown that there is a strong need to track the concentrations of synthetic colorants used in food products in order to protect public health from the significant adverse effects of such chemicals and to raise awareness among both consumers and policy makers.

Conclusion

At present, there are many food colorants added to food products in order to attract consumers especially children. Some individuals are sensitive to such food colorants; the names of the colors present in the food product must be the same as those on the food product label and the quantity of colorants must be specified in order to ensure food safety. For that, it is necessary to provide for a simplified procedure that allow the determination of these colorants. The current study provided a RP-HPLC–DAD approach for the

identification and determination of food colorants definitely Tartrazine E102, Allura Red E129,Sunset Yellow E110, Brilliant Blue E133, and Carmoisine E122 in a set of 65 Egyptian food products from different producers, in which they were well separated in only 9 min with simple sample preparation procedures. In addition a UPLC-ESI–MS/MS approach was developed and utilized as highly precise, sensitive confirmatory method, which could be applied successfully to suspicious marketed products.

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Data Availability All data generated or analyzed during this study are included in this published article and its supplementary information file.

Declarations

Competing interests The authors declare no competing interests.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in this study.

Conflict of interest Eman A. Abdel Hameed declares that she has no conflict of interest. Ghada H. Abd-ElHamid declares that she has no conflict of interest. Omayma M. El-Darder declares that she has no conflict of interest. Amany K. Ibrahim declares that she has no conflict of interest. Randa A. Abdel Salam declares that she has no conflict of interest. Ghada M. Hadad declares that she has no conflict of interest. Mohamed A. Abdelshakour declares that he has no conflict of interest.

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