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Simultaneous determination of 11 water-soluble dyes in food products and beverages by high performance liquid chromatography

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Abstract

A simple and inexpensive liquid chromatography diode array detector (LC-DAD) procedure has been developed to analyse food dyes in beverages, candies, jams, salted fish, Chinese sausage, and cake. A reverse stationary phase provided sufficient selectivity and chromatographic performance for the separation of 11 water-soluble dyes (tartrazine, amaranth, indigo carmine, ponceau 4R, sunset yellow, allura red, carmoisine, fast green FCF (Food green 3), brilliant blue, quinoline yellow, and indocyanine green). The samples were extracted with 1% ammonium solutions and acetonitrile, purified, and concentrated using a C_{18} solid-phase extraction (SPE) cartridge for beverages, and weak anion exchange SPE cartridge for solid samples. They were determined using a reverse-phase C_{18} column with gradient elution of 0.2% ammonium acetate buffer, and acetonitrile as the mobile phase. Multiple-specific wavelengths were used to monitor the dyes in the visible range to provide higher sensitivity and an expanded scope for a large number of analytes. The limit of detection and limit of quantification of the dyes were in the range of 0.2 - 0.5 and 0.5 - 1.0 µg/mL, respectively. The precision of the method ranged from 2.71 to 6.31%, while recovery ranged from 90.8 to 105.6%. The validated method was successfully applied to the quantitative analysis of 11 water-soluble dyes in 36 commercial products obtained from the local supermarket. Application to the analysis of beverages and food samples available to consumers proved that the described methods are suitable for the routine analysis of dyes in food products containing a broad range of dyes.

Introduction

Water-soluble dyes are widely used to modulate the appearance and overall sensory quality of foodstuffs. Based on previous survey studies on acute toxicity, chronic toxicity, substance decomposition, and purity, it is evident that the abuse of synthetic pigments can cause cancer (IARC, 1975; Feng *et al.,* 2011). To prevent excessive use of water-soluble dyes, some countries and regions have instituted laws and regulations to limit the types, purities, uses, and amounts permitted for use in foods and drinks. The use of synthetic colorants in foods has also been harmonised across the European Union by formulating the directive 94/36/EC (EU, 1994), in which the acceptable daily intake (ADI) values for colouring foodstuffs are as follows: tartrazine, 7.5 mg/kg; amaranth, 0.15 mg/kg; indigo carmine, 5.0 mg/kg; ponceau 4R, 0.7 mg/kg; sunset yellow, 4.0 mg/kg; allura red, 7.0 mg/kg; carmoisine, 4.0 mg/kg; fast green, 25.0 mg/kg; brilliant blue, 6.0 mg/kg; and quinoline yellow, 0.5 mg/kg. The United States Food and Drug Administration (FDA) also regulates the content and type of colours used. Although indocyanine green is not a food additive, it has been detected in trace amounts (0.5 - 2 mg/L) in some beverages in Vietnam. Determining these dyes is essential, and has been widely studied.

To date, several researchers have reported analytical approaches for the extraction and reliable identification and quantification of food dyes. Notably, thin-layer chromatography (Oka *et al.,* 1994), adsorptive voltammetry (Ni *et al.,* 1997), differential pulse polarography (Combeau *et al.,* 2002), derivative spectrometry (Berzas Nevado *et al.,* 1998; Vidotti *et al.,* 2005), and spectrophotometric methods in combination with chemometrics (Ni and Gong, 1997; Dinc *et al.,* 2002) have been used for analysis of food dyes. In the past several years, liquid chromatography (LC) has been shown to have considerable potential for the analysis of synthetic food colours in terms of qualitative and quantitative determination, as well as

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the speed of analysis (Yoshioka and Ichihashi, 2008; Zou *et al.,* 2013; Rejczak and Tuzimski, 2017). At present, LC using reverse-phase with ion pairing is the most preferred technique because it offers the best overall resolution and sensitivity. Ion-pair chromatography involves the addition of salt modifiers to the mobile phase. Suitable salts are selected to create large ions with opposite charges to the compounds to be analysed, thus generating reversible ion-pair complexes (Minioti *et al.,* 2007; Harp *et al.,* 2013). Among all the LC methods available for food colours, very few offer simultaneous quantitation and resolution of abundant water-soluble dyes in a variety of food commodities / matrices.

A key stage preceding the determination of dyes is sample preparation, which is usually required for the preconcentration of target analytes and removal of the matrix components. Various analytical methods have been developed for the determination of food dyes, such as cloud point extraction (Karatepe *et al.,* 2017; Bişgin, 2018), solid-phase extraction (SPE) (Yu and Fan, 2016), and liquid-liquid microextraction (Bazregar *et al.,* 2018; Bişgin, 2019). SPE is a powerful sample preparation technique that allows rapid clean-up and enrichment of sample analytes prior to chromatographic analysis.

In the present work, we developed an easy and cost-effective liquid chromatography diode array detector (LC-DAD) method for the analysis of 11 dyes (Figure 1). The extraction of the analytes was carried out using C_{18} SPE or weak anion exchange (WAX) SPE. The procedures were preliminarily validated and applied to the analysis of different beverage and solid food samples such as candies, jams, salted fish, Chinese sausages, and cakes.

Materials and methods

Chemical and materials

All chemicals and reagents used in the experiments had analytical grade purity. Methanol, acetonitrile, and HPLC-grade water were purchased from Honeywell (North Carolina, USA). Ammonium acetate, formic acid, and ammonia solution (25%) were purchased from Merck (Darmstadt, Germany). Tartrazine, amaranth, indigo carmine, ponceau 4R, sunset yellow, allura red, carmoisine, fast green FCF, brilliant blue, quinoline yellow, and indocyanine green were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Standard preparation

Individual standard stock solutions of 2000 µg/mL of each dye category were prepared by weighing 100 mg of each compound, and dissolving them in a 50 mL volumetric flask using methanol and water in a 50:50 ratio (v/v). The volumetric flasks were sonicated for 1 min, and wrapped with aluminium foil to protect them from light. Stock solutions were stable for approximately six months when refrigerated. Working standard solutions were prepared daily by pipetting aliquots of stock solutions to give a concentration of 100 µg/mL.

Sample preparation

Candies, jams, and beverages

Extraction procedure

The candy (or jam) samples (1 g) were placed in a 50 mL centrifuge tube, and dissolved in 10 mL of water. The sample was then diluted to 20 mL with 6% ammonium acetate solution to give a 3% ammonium acetate loading solvent.

The beverage samples (1 mL) were diluted to 20 mL with 3% ammonium acetate solution. Carbonated liquid samples were degassed by ultra-sonication for 30 min before pipetting.

Clean-up procedure

The C_{18} SPE cartridge was conditioned with 6 mL of MeOH, and equilibrated with 6 mL of 3% ammonium acetate solution. Next, 20 mL of the prepared sample solution was loaded on the SPE cartridge at a flow rate of 1 mL/min, washed with 6 mL of 3% ammonium acetate solution, and eluted with 6 mL of methanol. The eluted solvent was evaporated and reconstituted with 1 mL of methanol and 0.2% ammonium acetate solution (5:95, v/v). It was then filtered through a 0.22 µm PTFE (polytetrafluoroethylene) filter, and transferred into a vial.

Salted fish, Chinese sausage, and cake

Extraction procedure

The solid sample (1 g) was accurately weighed and then extracted thrice with 3 mL of different mixtures of 1% ammonia solution and acetonitrile, in an ultrasonic bath for 15 min each time. The extracted solution was neutralised to pH 7 with 4.2 mL of 1% aqueous formic acid, and diluted to 20 mL with water.

Clean-up procedure

The WAX SPE cartridge was conditioned with 6 mL of methanol, followed by 6 mL of 0.1% formic acid, and equilibrated with 6 mL of water. All extracted solutions were loaded onto the SPE cartridge at a flow rate of 1 mL/min, washed with 3 mL of water and 3 mL of methanol, and eluted with a mixture of 5% ammonia and methanol. The eluted solution was evaporated to dryness under a nitrogen stream at 50°C.

The residue was reconstituted with 1 mL of methanol and 0.2% ammonium acetate solution (5:95, v/v), filtered through a 0.22 µm PTFE filter, and transferred into a vial.

Instrumentation and chromatographic conditions

Method development, quantification, and validation studies were performed on an Agilent LC 1100 series (Agilent Technologies, Mississauga, ON, Canada) equipped with a G-1310A pump, G-1316A column thermostat, G-1313A autosampler, and G1315B diode array detector.

Chromatographic separation was achieved using a Kromasil C_{18} column (150 × 4.6 mm I.D, 5 µm). The mobile phase consisted of acetonitrile (A) and 0.2% aqueous ammonium acetate (B). The gradient elution program was as follows: 0 - 2 min, 5% A; 2 - 4 min, linear gradient 10% A; 4 - 9.5 min, linear gradient 25% A; 9.5 - 11.5 min, linear gradient 30% A; 11.5 - 15 min, linear gradient 35% A; 15 - 15.5 min, linear gradient 98% A; 15.5 - 16.5 min, hold 98% A; 16.5 - 17 min linear gradient 5% A; 17 - 20 min, 5% A. Analyses were performed by injecting 10 µL at a flow rate of 1 mL/min. Agilent Technologies ChemStation software was used to monitor and control all analytical conditions, and to reprocess the chromatographic data. Five different wavelengths were used to identify the dyes: 415, 515, 610, 630, and 775 nm.

Optimization of the sample preparation

The extraction methods, including vortex mixing and sonication, were evaluated. For effective solvent extraction, 1% aqueous ammonia, 1% aqueous ammonium acetate, and a mixture of these solutions with methanol, acetonitrile, and water were examined. To optimise the SPE procedure, a series of SPE cartridges including C_{18} (Silicycle), HLB (Hydrophilic-Lipophilic-Balanced, Waters), WAX (Waters), and PEP-2 (Cleanert) was evaluated by spiking the standard sample solutions.

Method validation

The proposed method was validated for selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy according to Association of Official Analytical Chemists (AOAC, 2002) guidelines.

Results and discussion

Optimization of sample preparation

Extraction method

The extraction solvent may not be able to penetrate some solid samples such as dried fruit, Chinese sausage, and salted fish because they have a dry and hard covering. Therefore, we examined the extraction efficiencies of vortex mixing and sonication with 1% aqueous ammonia as the extraction solution for 5 min. The results showed that the recovery of dyes using the vortex mixing method was lower than that obtained from sonication, especially for dried samples.

To extract dyes from food products, previous studies have used methanol-aqueous ammonium acetate (Brazeau, 2018), methanol-aqueous ammonia (Harp *et al.,* 2013), ethanol-aqueous ammonia (Yoshioka and Ichihashi, 2008), ethanol-aqueous ammonia-water (Zou *et al.,* 2013), and water (Minioti *et al.,* 2007) as extraction solvents. For beverages, candies, and jams, a mixture of water and sampling weight has been used, and a ratio of 10:1 was found to be the best choice.

For salted fish, Chinese sausage, and cake, we first examined the extraction efficiencies of three solutions: 1% aqueous ammonia, 1% aqueous ammonium acetate, and water. It was found that aqueous ammonia (1%) gave the highest recoveries of the tested dyes, with the exception of indocyanine green (62.4%), which is the most hydrophobic dye in our study. Based on these results, we used the mixture of 1% aqueous ammonia with methanol and acetonitrile in several ratios to assess dye recoveries. The results showed that the recovery of indocyanine green increased when the ratio of organic solvent was increased, and was more than 95% in 60% acetonitrile and 80% methanol. However, tartrazine had the best recovery with the lowest organic solvent ratio. Therefore, we developed a three-step extraction procedure, in which the ratio of the organic solvent increased from 0 to 50%, and then to 60% (Table 1). The recovery of dyes showed fairly similar results in acetonitrile and methanol; acetonitrile gave more efficient protein and polysaccharide precipitation than methanol in the same ratio (Zhao and Juck, 2018), and acetonitrile did not break up the hydrogen bonding between the analytes and SPE sorbents. Therefore, the mixture of 1% aqueous ammonia and acetonitrile was chosen as the optimum extraction solvent. With high starch-containing matrices such as cake, using 1% aqueous ammonia as the extraction solvent gave high recoveries of all eleven dyes without using α-amylase to hydrolyse the polysaccharide links present in the matrices. Hence, the proposed extraction method was simpler than the research reported by Brazeau (2018).

Optimization of SPE procedure

To increase the sensitivity and selectivity of the method and protect the HPLC column against

Table 1. Recovery (%) of 5 µg/mL of colour additives spiked in salted fish using several extraction solvents.

Extraction solvent	H_2O	1% NH_4OH/H_2O	1% CH_3COONH_4/H_2O	MeOH:1%NH_4OH/H_2O			ACN:1%NH_4OH/H_2O		
				40:60	60:40	80:20	40:60	60:40	80:20
Tartrazine	29.6	97.6	45.2	96.2	91.6	88.4	93.6	87.8	79.6
Amaranth	13.5	97.7	75.4	97.1	93.2	91.6	95.8	92.5	85.9
Indigo carmine	16.6	102.6	68.2	96.2	91.9	86.4	94.3	90.6	88.3
Ponceau 4R	nd	100.5	78.3	98.3	94.1	87.3	97.5	94.2	91.5
Sunset yellow	35.6	98.3	82.6	102.3	96.8	97.2	97.5	99.8	97.1
Allura red	nd	92.6	80.9	97.8	94.3	96.9	99.7	98.9	96.9
Carmoisine	nd	99.8	55.2	100.1	99.8	101.5	94.3	97.4	99.6
Fast green FCF	nd	98.2	nd	98.2	99.8	97.6	96.9	95.2	98.4
Brilliant blue	nd	99.1	nd	97.6	98.5	99.6	101.2	96.8	99.4
Quinoline yellow	nd	97.5	nd	103.2	100.4	98.3	98.5	100.2	97.2
Indocyanine green	nd	62.4	nd	78.2	85.6	96.7	87.1	95.6	98.1

nd: not detected.

matrix interference, SPE technology was used to concentrate and purify target analytes. The retention capacities of C18 and HLB SPE cartridges for candies, jams, beverages, and WAX and PEP-2 cartridges for other solid samples were evaluated.

Candies, jams, and beverages

To investigate the performance of SPE, a mixed standard solution in water was introduced to the C_{18} and HLB SPE cartridges, and eluted with methanol. All dyes were not retained by the C_{18} and HLB sorbents except quinoline yellow, fast green FCF, brilliant blue, and indocyanine green, which were more hydrophobic than others with anionic form in all pH ranges. As the highly polar sulfonic acid functionalities of the dyes resulted in low retention in reversed-phase sorbents, the cationic ion pair reagents, ammonium acetate and ammonium formate were added to equilibration, loading, and washing solvents in several concentrations (0.5 - 6%). This step improved the retention capacities of C_{18} and HLB cartridges with dye additives. In this case, the dye existed in the anionic form. It was found that the recovery of dyes was low (< 50%) when the concentration of the ion pair reagent was low (2%). Further, ammonium acetate was more suitable than ammonium formate, which presented a lower pH of ~4 and led to indocyanine green precipitation. With 3% ammonium acetate in the loading solvent, the C_{18} and HLB cartridges showed similar recovery of the 11 dyes ranging from 85.2 to 107.3%, and satisfied the routine analysis requirements (Table 2). As the HLB cartridge had both lipophilic and hydrophilic retention characteristics (Thurman and Mills, 1998), the washing solvent was optimized to eliminate polar matrix interferences such as sugar, polysaccharide, and other water-soluble food additives. In addition, as the C_{18} cartridge provided low retention capacity of polar interferences, it was more suitable than HLB for purifying analytes from polar sample matrices. Using the C_{18} column instead of HLB and the WAX column for polar sample preparation was cost-effective because the C_{18} column is three times cheaper than WAX and HLB.

Salted fish, Chinese sausage, and cake

The retention capacities of two functional groups including secondary-quaternary amines of WAX SPE cartridge and amide of PEP-2 cartridge with dyes containing two or three sulfonic acid groups (Figure 1) were examined. A mixed standard solution was applied at pH 7 to WAX and PEP-2 cartridges. The results showed that the WAX cartridge retained all the dyes. Although the PEP-2 cartridge showed retention of quinoline yellow, brilliant blue, and indocyanine green, it could not retain other dyes (Table 2). This result indicated that the basicity of the SPE functional groups affected the retention capacity. The amine groups have an ion pair electron localised on the nitrogen. Protonation of nitrogen takes place while the ion pair electron of the amide group is delocalised between the nitrogen and oxygen through resonance. Consequently, amides are less basic than amines (Figure 2). As the pK_a of WAX functional groups was ~9.5, the pH of the loading solution was adjusted from 2 to 7 by adding 1% aqueous formic acid. The results showed that indocyanine green was precipitated at pH < 5 of the loading solutions, leading to poor recovery. Further, the high concentration of ammonium formate, which was formed upon addition of formic acid to ammonia solution, decreased the recovery of tartrazine. Therefore, pH 6 - 7 of the loading and washing solutions was optimal, and a two-fold dilution step before loading the samples for SPE was ideal.

Table 2. Recovery (%) of 5 µg/mL of colour additives spiked in various loading solvents after applying in four different SPE cartridges.

Loading solvent	C_{18}		HLB		WAX		PEP-2	
	Water	3% ammonium acetate	Water	3% ammonium acetate	pH 7	pH 4	pH 7	pH 4
Tartrazine	nd	106.5	nd	94.0	103.2	98.2	nd	nd
Amaranth	nd	102.4	nd	96.1	100.0	101.3	nd	nd
Indigo carmine	nd	90.9	nd	85.2	98.1	93.2	nd	nd
Ponceau 4R	nd	93.7	nd	91.3	94.1	96.5	nd	nd
Sunset yellow	nd	97.5	nd	91.9	88.8	89.2	nd	nd
Allura red	nd	97.5	nd	93.7	90.3	85.6	nd	nd
Carmoisine	nd	95.7	nd	88.8	84.5	76.2	nd	nd
Fast green FCF	32.6	92.1	49.8	88.1	90.1	79.3	nd	10.2
Brilliant blue	25.3	97.9	14.6	93.7	93.6	87.9	38.2	30.6
Quinoline yellow	12.1	105.7	28.2	101.5	98.2	90.4	43.5	29.1
Indocyanine green	28.3	107.3	51.5	105.2	100.4	42.7	75.6	35.6

nd: not detected.

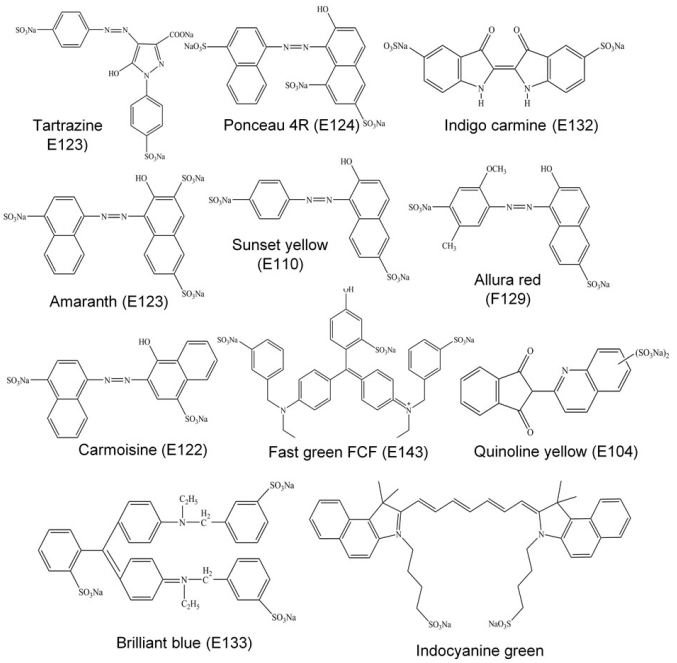

Figure 1. Chemical structures of the 11 food dyes assessed in the present work.

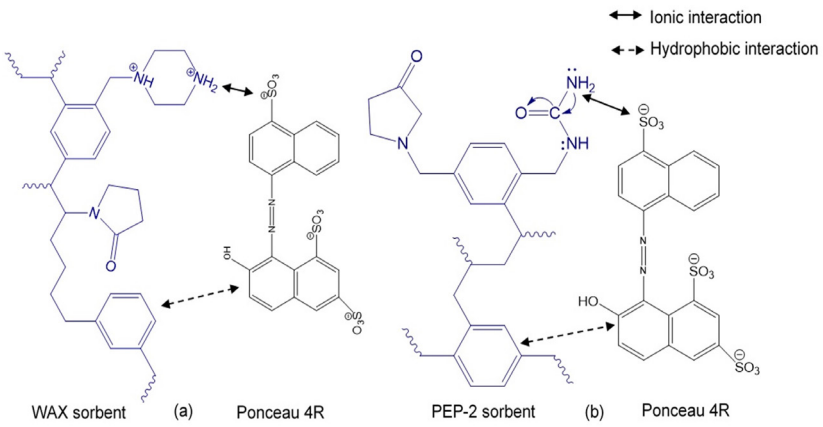

Figure 2. Retention mechanism between (a) WAX sorbent and Ponceau 4R, and (b) PEP-2 sorbent and Ponceau 4R.

Optimization of chromatographic conditions

Identification of 11 water-soluble dyes was based on their retention times (t_R), UV-Vis spectra, and co-injection with standards. As the maximum absorbances of the dyes were similar and depended on their colour, the yellow dyes (tartrazine, sunset yellow, and quinoline yellow) were quantified at 415 nm, red dyes (ponceau 4R, amaranth, allura red, and carmoisine) at 515 nm, blue dyes (fast green FCF and brilliant blue) at 630 nm, indigo carmine at 610 nm, and indocyanine green at 775 nm. Optimization of chromatographic conditions were performed using two compositions of mobile phases (methanol-buffer (1) and acetonitrile-buffer (2) systems) in gradient mode with 1% ammonium acetate as buffer. The results showed that system 1 could not separate allura red from carmoisine ($R_s < 1.2$) and fast green FCF from brilliant blue. Additionally, with system 1, indocyanine green was retained too long (over 30 min in 100% methanol). System 2 provided good resolution of all analytes, albeit with low retention, which was inconvenient for separating analytes from interferences of complex matrices. Moreover, the peak shape of the first peak tartrazine was symmetrical. Based on these results, we examined several concentrations of ammonium acetate (0.5, 1.0, 2.0, 3.0, and 4.0%) to improve the retention capacity and peak shape. The results showed that higher resolution ($R_s > 2.5$), better peak shape ($0.8 < A_s < 1.2$), and available analysis time (20 min) were obtained using 2% ammonium acetate. With 3 and 4% ammonium acetate, the analytes were retained too long on the column, resulting in peak tailing and decreased intensity. The representative chromatograms of the sample and standard (Figure 3) showed that all analytes were eluted with highly symmetrical peaks and the analysis time was 20 min, which was shorter than the analysis time of 50 min, reported by Harp *et al.* (2013) to determine seven dyes.

Method validation

The matrices used for the validation studies were clear candy, beverage, salted fish, and cake; each containing no dyes. Samples of the matrices were spiked with various concentrations of the mixed standard solution for the validation study.

System suitability

System suitability was tested by performing six replicate injections and determining the theoretical plate number (N), resolution (R_s), symmetry factor (A_s), and repeatability (relative standard deviation (RSD) of retention time and area) of the analyte of interest. The %RSD values of the area and retention time were less than 2%, indicating the precise analysis of the 11 dyes by this system. All results showed that the proposed method met these requirements.

Selectivity

Selectivity of the method indicates the ability to measure the analyte response in the presence of matrix interferences. Several samples of the four matrices (candy, beverage, salted fish, and cake) were analysed to determine the blank samples. Method selectivity was tested using HPLC to compare the retention time and UV-Vis spectra of each standard reference compound with that of the peaks of spiked and blank samples. This analytical method was able to distinguish dyes from other inferences in the matrix. The peak purity of the 11

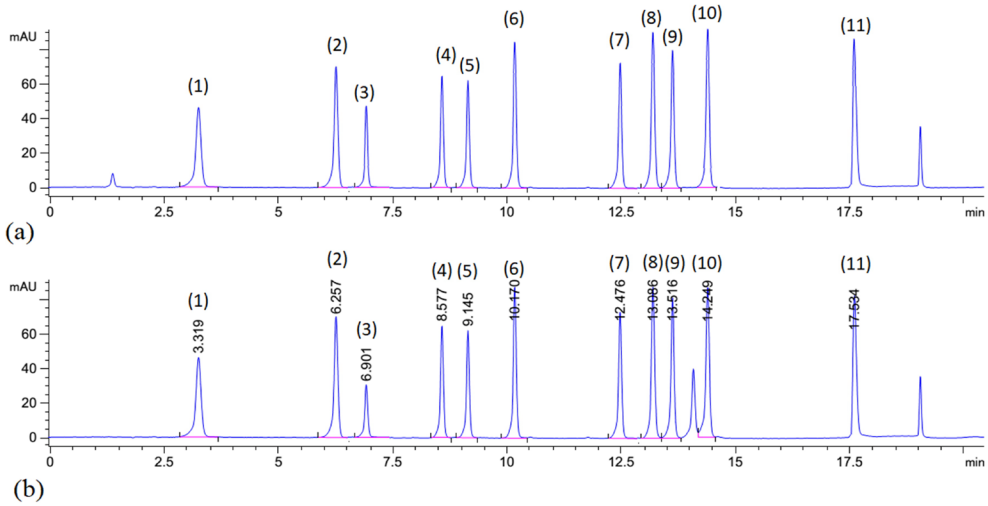

Figure 3. HPLC chromatograms of the (a) mixed colour standard solution, and (b) spiked blank sample. (1) Tartrazine, (2) Amaranth, (3) Indigo carmine, (4) Ponceau 4R, (5) Sunset yellow, (6) Allura red, (7) Carmoisine, (8) Fast green FCF, (9) Brilliant blue, (10) Quinoline yellow, and (11) Indocyanine green.

compounds was > 99.9%, as obtained from the spectrum overlaying the graphs of three-point purity detection.

Linearity, limit of detection, and limit of quantification

Under the optimized analytical procedure, linearities were evaluated from the peak area responses and six concentration levels of spiked samples (5 - 50 µg/mL). The results of the regression equation and square correlation coefficients (R^2) are summarised in Table 2. The LOD of indigo carmine was 0.5 µg/mL, and LOQ was 1 µg/mL, whereas the LOD of other dyes was 0.2 µg/mL, and LOQ was 0.5 µg/mL.

Precision

The precision of the method was verified by repeatability and intermediate precision. The repeatability precision was expressed as the mean RSD, and calculated from repeated determinations (n = 6) across the four matrices, tested with dye recoveries at 5 µg/mL. The intermediate precision was calculated from the recoveries of dyes from replicate sample determinations on a different day. The overall intra-day and inter-day RSDs were below 10%. The full intra-day and inter-day precision data of the cake matrix are shown in Table 3.

Accuracy

The four matrices tested were spiked with mixed standard solutions of all dyes at three concentration levels (5, 10, and 15 µg/mL). The developed method had good accuracy, with an overall recovery from 90.8 to 105.6%. The accuracy data of the cake matrix are summarised in Table 2. Considering the results of the recovery test, the method was deemed accurate.

Table 3. Precision, accuracy, and calibration parameters of the cake matrix.

Colour	Recovery (%)			Precision (5 µg/g) (n = 6)		Calibration curve (n = 3)		
	Low-level (5 µg/g)	Mid-level (10 µg/g)	High-level (15 µg/g)	Intra-day RSD (%)	Inter-day RSD (%)	Slope (± SD)	y-intercept (± SD)	R^2 (± SD)
Tartrazine	100.2	100.1	101.0	3.45	2.71	16.64 ± 0.86	11.82 ± 4.11	0.9978 ± 0.0021
Amaranth	94.2	94.9	97.9	4.10	6.23	13.85 ± 0.63	-1.31 ± 0.27	0.9989 ± 0.0008
Indigo carmine	96.4	104.5	104.13	7.00	6.31	16.04 ± 1.63	-0.65 ± 0.03	0.9963 ± 0.0043
Ponceau 4R	101.6	99.8	101.1	1.97	4.7	11.13 ± 0.42	-1.19 ± 0.31	0.9998 ± 0.0003
Sunset yellow	102.4	101.1	101.4	1.44	5.54	14.10 ± 0.50	-0.11 ± 0.01	0.9998 ± 0.0002
Allura red	102.8	102.3	99.3	1.81	3.21	15.74 ± 0.58	-2.56 ± 1.23	0.9998 ± 0.0001
Carmoisine	97.8	99.8	99.6	2.54	4.32	15.81 ± 0.67	5.17 ± 0.71	0.9998 ± 0.0002
Fast green FCF	101.2	100.6	101.8	2.03	2.03	44.37 ± 1.55	5.82 ± 0.89	0.9998 ± 0.0001
Brilliant blue	100.2	100.1	101.0	1.56	2.71	40.61 ± 1.73	8.22 ± 1.27	0.9998 ± 0.0001
Quinoline yellow	96.8	99.5	100.0	2.45	5.89	12.32 ± 0.81	-3.85 ± 0.48	0.9996 ± 0.0002
Indocyanine green	93.6	98.9	100.2	1.42	3.55	45.40 ± 2.16	2.16 ± 0.35	0.9997 ± 0.0004

Note: Calibration from three different analytical days.

Table 4. Concentration of dyes in tested food products.

Matrix	Result		
	Dye detected	Frequency (n)	Concentration range (µg/g)
Beverage (n = 6)	Allura red	2	30.4 - 120.3
	Ponceau 4R	3	9.4 - 12.8
	Tartrazine	3	6.1 - 1495.2
	Sunset yellow	4	13.5 - 35.8
Fruit jam (n = 6)	Ponceau 4R	4	25.3 - 70.9
	Sunset yellow	3	18.6 - 22.5
Dried fruit (n = 6)	nd	nd	nd
Salted fish (n = 6)	Sunset yellow	6	12.3 - 17.8
Chinese sausage (n = 6)	Allura red	5	25.6 - 30.5
	Ponceau 4R	3	6.2 - 18.9
	Tartrazine	4	40.0 - 80.6
Cake (n = 6)	Sunset yellow	2	10.9 - 33.6
	Ponceau 4R	2	7.49 - 11.7
	Brilliant blue	4	12.75 - 72.71

nd: not detected.

Method application

The proposed method was applied to determine the content of dyes in thirty-six commercial products, from six food matrices (beverage, fruit jam, dried fruit, salted fish, Chinese sausage, and cake). Data from Table 4 show that five out of the 11 dyes were detected (tartrazine, ponceau 4R, sunset yellow, allura red, and brilliant blue) in a wide concentration range (6.1 - 1495.2 µg/g). The most common dye in these products was sunset yellow (15 of 36 products). Beverages had the highest dye content, which was 1495.2 µg/mL of tartrazine. In contrast, no dyes were detected in dried fruit.

Conclusion

In the present work, we developed a cost-effective and reliable analytical method for food dyes, which exhibited good linearity (R^2 > 0.995), precision (2.71 - 6.31%), and high recovery (90.8 - 105.6%). The dyes were fully extracted from several matrices using 1% aqueous NH_4OH and acetonitrile, purified by SPE on a C_{18} column (for beverages, candy, and jam) and WAX cartridge, and simultaneously determined by HPLC with a diode array detector. In 36 commercial products, covering six food matrices, tartrazine, ponceau 4R, sunset yellow, allura red, and brilliant blue were quantified in a wide concentration range of 6.1 - 1495.2 µg/g. The results proved that the described methods are suitable for routine analysis of dyes in food products with a wide concentration range. The relatively inexpensive HPLC-DAD technique may be a useful and alternative platform to LC-MS/MS. It is also worth noting that the scope of the analytical procedures developed in the present work can be easily expanded.

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