Regular Article

ANALYTICAL AND BIOANALYTICAL CHEMISTRY RESEARCH

Published by the Iranian Chemical Society

Anal. Bioanal. Chem. Res., Vol. 4, No. 2, 213-225, December 2017.

An Ion-pair Dispersive Liquid-Liquid Microextraction for Simultaneous Determination of Synthetic Dyes in Ice Cream Samples by HPLC

Mohammad Faraji*, Banafshe Nasiri Sahneh, Rika Javanshir

Faculty of Food Industry and Agriculture, Department of Food science & Technology, Standard Research Institute (SRI), Karaj P.O. Box: 31745-139, Iran

(Received 22 December 2016, Accepted 28 April 2017)

An efficient, sensitive, and fast method was developed based on an ultrasound-assisted extraction followed by an ion-pair dispersive liquid-liquid microextraction (USAE-IP-DLLME) for the simultaneous determination of five commonly used synthetic sulfonate dyes (tartarazine, quinoline yellow, sunset yellow, azorubine and brilliant blue) in ice cream samples using high performance liquid chromatography. First, important parameters on USAE and samples clean-up were investigated and optimized. Then, some effective parameters on DLLME were studied and optimized. Under the optimum conditions, good linearity (0.5-1000 μg l^{-1}, > r^2 = 0.99) were obtained for the dyes. Limits of detection and limits of quantization were in the range of 0.01-0.05 μg l^{-1} and 0.03-0.15 μg l^{-1}, respectively. The recoveries of the five synthetic colorants ranged from 90.3-109.7%. Intra (1.4-6.4%) and inter-day precision (3.9-9.7%) expressed as relative standard deviation (RSD) at 10 and 100 μg l^{-1} levels less than 10% were also achieved. Finally, this method was applied successfully in determination of the colorants in the ice cream samples.

Keywords: Dispersive liquid-liquid microextraction, HPLC-UV-Vis, Ice cream, Synthetic dyes, Ultrasonic-assisted solvent extraction

INTRODUCTION

Today ice-cream plays the role of actual food and it is extensively used by people especially children. In addition to its digestive and metabolic qualities ice-cream has nutritive qualities and can also influence the mind for its organoleptic characteristics and for its importance as thermoregulator food in the fight against heat [1,2]. Beside many advantages associated with ice-cream consumption, one concern is the use of synthetic dyes in its formulation that can induce a risk factor. The use of any synthetic dyes in ice cream is not regulated under the current Iranian Food Act (IFA). According to ISIRI 2450 (5th revision) use of any synthetic dyes in ice cream is banned [3].

In recent years, synthetic dyes have been widely used in the food industry to compensate for the loss of natural colors of food during processing and storage, and to provide the desired colored appearance. Moreover, synthetic dyes are more colorfast, have greater stability and lower production cost in comparison with natural dyes [4-7]. However, the use of these colorants is strictly controlled by the legislation of different countries, because some of these substances pose potential risks to human health, especially if they are excessively consumed.

Some adverse health effects of using synthetic food colors are allergy and asthmatic reaction [8,9], DNA damage [10,11], hyperactivity [12] and carcinogenesis [13,14] *etc*. Thus, it is necessary to develop accurate and reliable analytical methods for the confirmative determination of synthetic food dyes in foodstuffs of various matrices to ensure food safety and consumer health.

Up to date, many analytical methods have been

*Corresponding author. E-mail: mfaraji@standard.ac.ir

developed using spectrometric determination [15-21], Raman spectroscopic [22], voltammetry [23,24], capillary electrophoresis [25,26], ion chromatography [27] and high performance liquid chromatography with diode array detection (HPLC-DAD) [7,28-39] or mass spectrometer detection (HPLC-MS) [36-40] for synthetic colorant determination in foodstuffs and beverages. These techniques require prior sample preparation and can be used anywhere from a simple dilution followed by filtration [28], liquid-liquid extraction (LLE) [20], solid phase extraction (SPE) [34], and cloud point extraction [15,17,21] up to more advanced techniques such as homogeneous liquid-liquid microextraction [31], in-tube electromembrane extraction [35], and dispersive liquid-liquid microextraction [29-31]. The complexity of the sample preparation will depend on concentration of the dyes and also the type of sample being analyzed.

The objective of the present work is to develop an ion-pair dispersive liquid-liquid microextraction method for determination of five common synthetic dyes (tartarazine, quinoline yellow, sunset yellow, azorubine, and brilliant blue) in some ice cream samples for the first time. Several experimental parameters that influence the extraction efficiency of the dyes are investigated and optimized. Finally, figures merits of the proposed method are compared with the previous published methods.

EXPERIMENTAL

Chemicals and Reagents

HPLC-grade methanol, acetonitril and chloroform were obtained from Merck Company (Darmstadt, Germany). Analytical grade tetra-butylammonium bromide (TBAB), ammonium acetate and acetic acid were purchased from Merck Company (Darmstadt, Germany). Water was purified using a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA). Brilliant blue (BB), quinoline yellow (QY), azorubine (Az) and sunset yellow FCF (SY) were purchased from Sigma-aldrich (Steinheim, Germany). Also, tartrazine (TT) was purchased from Megha International Company (Mumbai, India). All of the stock solutions (1000 mg l^{-1}) were prepared in water. Working solutions (20 mg l^{-1}) were prepared from stock solutions by diluting with water. 2.0 M TBAB solution was prepared by dissolution of proper amount of the reagent in water. Ammonium acetate buffer with concentration of 50 mM was prepared by dissolving proper amount of the salt in deionzed water. pH of the buffer was adjusted to 7.0 by 0.1 M HCl and/or 0.1 M NaOH and then was filtered. Carrez I solution was prepared by dissolving 15.0 g potassium hexacianoferrate in 100 ml deionized water, and for preparing 100 ml Carrez II solution, 22.0 g zinc acetate was mixed by 3.0 ml acetic acid in water.

Apparatus

The chromatographic analysis was carried out in a high-performance liquid chromatography from Knauer of Germany model EuroChrom consisting of a degasser, quaternary pump (model K1100), manual sample injector with 20 µl loop size and UV detector (model K2600) was controlled by EZChrom software. The HPLC operating mode was gradient, the injection volume was 20 µl and the column temperature was adjusted at room temperature. The chromatography column was a Supelcosil LC-18: 250 mm × 4.6 mm, 5 µm (Supelco, Bellefonte, PA, USA). Sample data collection was optimized to 18 min per sample with UV detection at wavelength of maximum absorption of the dyes, 420 nm (for TT and QY), 485 nm (for SY), 515 nm (for Az) and 625 nm (for BB). Mobile phase used was combination of methanol, acetonitril and ammonium acetate buffer (50 mM) pH = 6.7. Elution program is shown in Table 1. Under optimum condition, the retention times for TT (first peak), QY1 (second peak), SY (third peak), QY2 (fourth peak), Az (fifth peak) and BB (sixth peak) are about 3, 4, 6, 8, 11.3 and 12.2 min, respectively. The mobile phase was filtered through a 0.45 µm pore size filter (Merck Millipore, Billerica, Massachusetts, USA) and degassed by vacuum prior to use. Moreover, the mobile phase flow rate was set to 1.0 ml min^{-1}. A Cesil CE-7200 UV-Vis spectrophotometer (Cambridge, England) was applied for the absorbance measurements of the solutions. A 40 kHz and 0.138 kW ultrasonic water bath with temperature control (Tecno-Gaz SpA, Italy) was applied to solvent extraction. All of the pH measurements were performed with a WTW Inolab pH meter (Weilheim, Germany).

Sample Preparation

A total of 30 fruit flavour ice cream samples were

Table 1. Elution Program of the Dyes by HPLC

Time	%Phase A (80:10:10; ammonium acetate:acetonitril:methanol)	%Phase B (55:20:25; ammonium acetate:acetonitril:methanol)
0	100	0
3	100	0
4	0	100
10	0	100
11	100	0
18	100	0

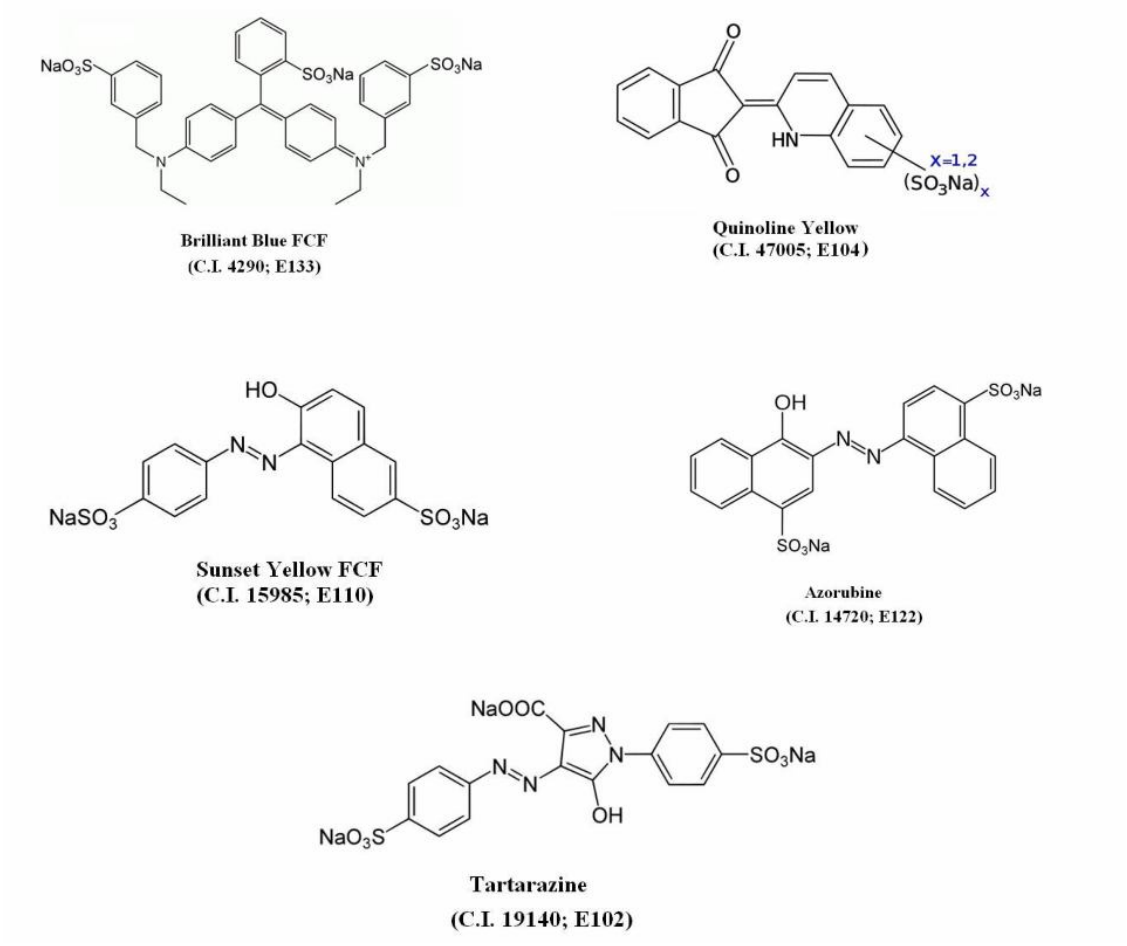

Fig. 1. Chemical structure, name, color index and number of the dyes used. C.I, color index.

purchased from three famous brands located in the Karaj, Iran. The samples were categorized as: blueberry (3), cranberry (3), orange (3), banana (3) sour green apple (3), melon (3), cherry (3), pomegranate (3), peach (3) and saffron (3).

The ice-cream samples were prepared according to Del Giovine's method (Del Giovine & Bocca 2003) with some modifications. Briefly, 2.0 g of homogenized ice-cream was weighted in a beaker and then 40 ml ethanol-ammonia-water solution (50:2.5:47.5) was added. Then, the suspension is placed in an ultrasonic bath for 15 min to improve dye release efficiency. Afterwards, 2.0 ml of each Carrez I and Carrez II solutions were added to the solution and the mixture was transferred to 50-ml centrifuge tube and diluted to 50.0 ml with double-distilled water. Further, it was centrifuged for 10 min at 5000 rpm. 1.0 ml of supernatant was transferred to a 15 ml screw-capped conical bottom glass vial and diluted up to 10 ml, then 300 µl of TBAB (2.0 M) was added to the vial. A mixture of 1.5 ml methanol (as disperser solvent) and 100 µl chloroform (as extraction solvent) was injected rapidly into the solution using a 5.0 ml syringe. After gently shaking, a cloudy solution which consisted of very fine droplets of chloroform dispersed into the aqueous sample was formed. The mixture was then centrifuged for 3 min at 5000 rpm, causing the dispersed fine droplets of the extraction phase to settle down to the bottom of the tube. The supernatant aqueous phase was discarded and the settled phase was quantitatively transferred to a 2 ml eppendorf vial and dried under a gentle nitrogen flow. Finally the residue was reconstituted with 50 µl of MeOH/ammonium acetate buffer 1:4, v/v and then was injected to HPLC.

RESULTS AND DISCUSSION

Separation of Dyes in HPLC

The structures, names and color index (C.I.) numbers of the dyes used in this study are shown in Fig. 1. Nature of the studied dyes is different from the polarity point of view. So, preliminary tests showed that getting good resolution in separation of the dyes is not possible in isocratic mode. In isocratic mode with high organic solvent ratio, resolution of TT, QY and SY is not suitable. On the other hand, with low organic solvent ratio retention times of Az and BB were too long. Therefore, to obtain good resolution for all of the studied dyes with reasonable run time, gradient elution was used (Table 1).

Optimization of Sample Pretreatment

Effect of extraction solvent composition, ultrasonic effect and carrez solution addition. Extraction of the dyes from the samples should be undertaken prior to chromatographic analysis. By reviewing the literature [25], in preliminary tests, three solvents were checked. The extract solvents include: Ethanol-ammonia-water 50:1:49, 50:2.5:47.5 and 50:5:45 (V/V/V). Results showed similar extraction efficiency in terms of recovery and reproducibility for the 5 dyes. In this study, the solution of 50:2.5:47.5 (V/V/V) was used in next experiments.

Then, in order to achieve highest efficiency in the release of dye from ice cream, the suspension is placed in an ultrasonic bath. Ultrasound assisted extraction (UASE) is an inexpensive, simple, and efficient extraction technique for solid and semi-solid samples. The enhancement in extraction obtained by using ultrasound is mainly attributed to the effect of acoustic cavitations and also exerts a mechanical effect, allowing greater penetration of solvent into the tissue, increasing the contact surface area between the solid and liquid phase. As a result, the solute quickly diffuses from the solid phase to the solvent [41]. Results showed that by applying ultrasonication, recovery for the 5 dyes could be increased up to 30% and also reproducibility could be improved.

In continue, effect of addition of the Carrez solutions was evaluated. Bento et al. were used Carrez solutions to precipitate proteins present in the samples in determination of synthetic colorants in yogurt in order to overcome matrix complexity [32]. Therefore, 1.0 ml and 2.0 ml of each Carrez solution were tested. Results showed that most of the interference proteins present in ice cream samples are precipitated in the presence of 2.0 ml of each Carrez solution.

Optimization of DLLME Parameters

The enrichment factor (EF) was defined as the ratio between the analyte concentration in the sedimented phase (C_{sed}) and the initial concentration of analyte (C_0) in the sample, in Eq. (1):

$$EF = \frac{C_{sed}}{C_0} \quad (1)$$

EF is obtained from comparing peak areas of the analytes achieved in two cases, direct injection of standard solution of the selected dyes in extraction solvent, and injection of sedimented phase having enriched analytes.

Extraction recovery (ER) is defined as the percentage of the total analyte amount (n_0) which is extracted into the sedimented phase (n_{sed}), in Eq. (2):

$$ER = \left(\frac{n_{sed}}{n_0}\right) \times 100 = \left(\frac{C_{sed} \times V_{sed}}{C_0 \times V_0}\right) \times 100 = \left(\frac{V_{sed}}{V_0}\right) \times EF \times 100 \quad (2)$$

Which V_{sed} and V_0 are the volumes of the sedimented phase and water sample, respectively.

Extraction Mechanism and Optimization of TBAB Amount

Due to the strong hydrophilic property of the sulphonated azo dyes (anionic compounds), their extraction into organic solvents is very low. Under this circumstance, ion-paring could be good solution to overcome this problem [15,17]. Therefore, tetrabutylammonium bromide (TBAB) as cationic reagent was used. To guarantee the sufficient reaction of the dyes, TBAB should be adequate. The effects of TBAB concentrations on extraction efficiency were therefore studied in detail (Fig. 2a). The extraction efficiency of the dyes increased obviously with increasing TBAB concentration from 10 to 60 mM. Further increasing the TBAB concentration beyond 60 mM excess had no significant effects on the extraction efficiency. Also, results showed that in the absence of TBAB, extraction efficiency of the dyes is near zero. So, 60 mM TBAB was selected for the next studies.

Effect of pH

The pH of solution is a significant factor affecting the extraction performance of the analytes with ionizable groups during DLLME process [42]. For the optimization of the pH of sample solution, a set of experiments were conducted in which the pH of the aqueous sample before DLLME was adjusted to 2, 4, 6, 8 and 10 (adjusted by 0.1 M HCl for acidic and 0.1 M NaOH for basic solutions). The results (Fig. 2b) indicated that in pHs < 4 and pHs > 8, extraction efficiency is low, but at pH in the range of 4-8 extraction efficiency is maximum. The reason for this could be that condition for ion-pair formation of the dyes is not suitable at pHs < 4 and pHs > 8. The obtained results are in agreement with Pourreza's research [15]. In acidic conditions (4 > pH) sulfonic groups of the dyes could be protonated, and on the other hand, in basic conditions (8 < pH) charge density of ammonium group of the TBAB could be reduced under either of these conditions leading to decreasing ion-paring. Therefore, considering most foods are weak acids or neutral, further experiments were done at pH 5.0.

Selection of Extraction Solvent and its Volume

Choosing an appropriate extraction solvent is of primary importance for most extractions. In DLLME, the extraction solvent has to meet some requirements. It should have a higher density than water, a low solubility in water, and high extraction capability for the target analytes, and also should form a stable two-phase system in the presence of a dispersive solvent when injected to an aqueous solution [42]. Based on these criteria, four conventional extraction solvents including carbon tetrachloride, chloroform, tetrachloroethylene, chlorobenzene were studied. The obtained results (Fig. 2c) show that chloroform has a superior efficiency in comparison with the other tested solvents. Such high efficiency for chloroform could be interpreted by high polarity of this solvent in comparison with the other tested solvents which getting good extraction efficiency in extraction of polar analytes [42]. So, it was selected as the extraction solvent in further experiments.

The suitable volume of extraction solvent was investigated using 1.0 ml ethanol with different volumes of chloroform (60, 80, 100, 120 and 150 µl). Results showed that the recovery of the dyes was increased at first by increasing the extraction solvent volume and reaching to its maximum at 100 µl and then remained constant. Due to evaporation and reconstitution up to fix volume, further increase in the extraction solvent volume did not lead to decrease enrichment factor due to dilution effect. Consequently, 100 µl of chloroform was chosen for further experiments.

Fig. 2a.

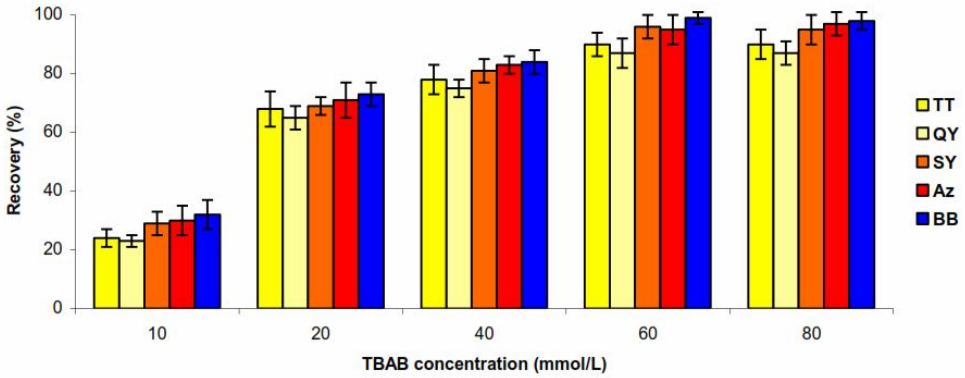

Fig. 2b.

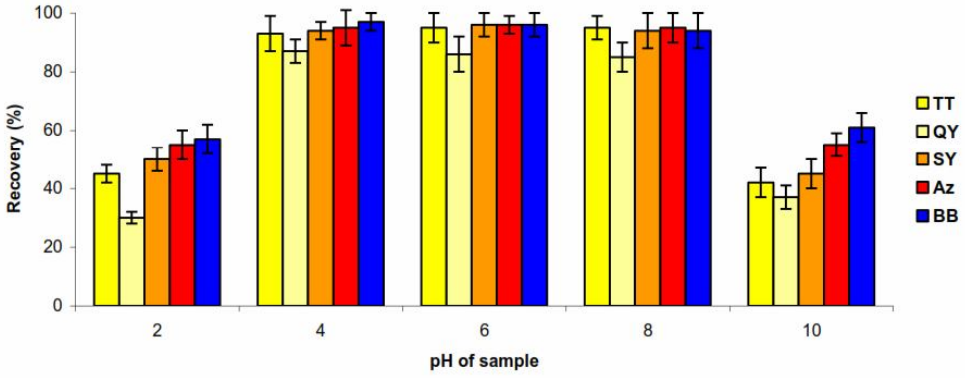

Fig. 2c.

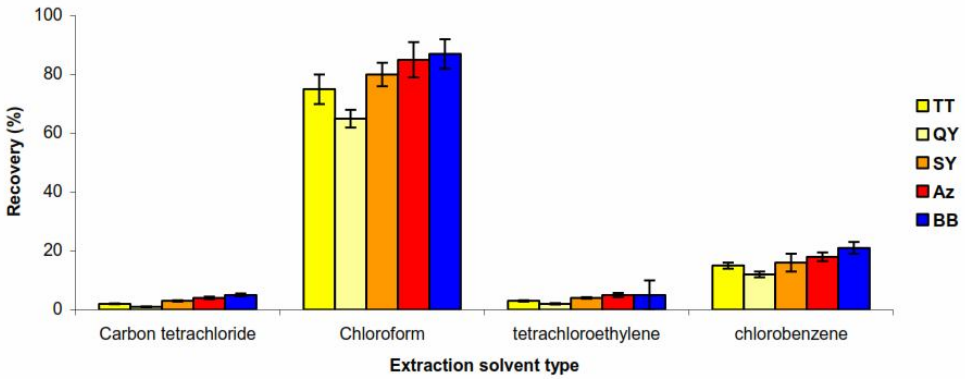

Fig. 2. Effect of TBAB amount on extraction efficiency of the dyes (Fig. 2a), effect of the pH of the solution on extraction efficiency of the dyes (Fig. 2b), and effect of extraction solvent type on extraction efficiency of the dyes (Fig. 2c).

Table 2. Analytical Characteristics of the UASE-DLLME-HPLC-Vis Method

Dyes	RSD%				LOQ	LOD	Linear range	R^2
	Inter-days (n = 6)		Intra-day (n = 6)		($\mu g\ l^{-1}$)	($\mu g\ l^{-1}$)	($\mu g\ l^{-1}$)	
	10 ($\mu g\ l^{-1}$)	100 ($\mu g\ l^{-1}$)	10 ($\mu g\ l^{-1}$)	100 ($\mu g\ l^{-1}$)				
TT	8.3	7.3	5.3	3.3	0.09	0.03	0.5-1000	0.9928
QY	9.7	8.2	6.4	4.7	0.15	0.05	0.5-1000	0.9915
SY	7.5	5.4	3.7	2.5	0.03	0.01	0.5-1000	0.9984
Az	6.4	4.8	5.2	3.4	0.06	0.02	0.5-1000	0.9977
BB	4.6	3.9	2.7	1.4	0.03	0.01	0.5-1000	0.9909

RSD: relative standard deviation; LOQ: limit of quantification; LOD: limit of detection.

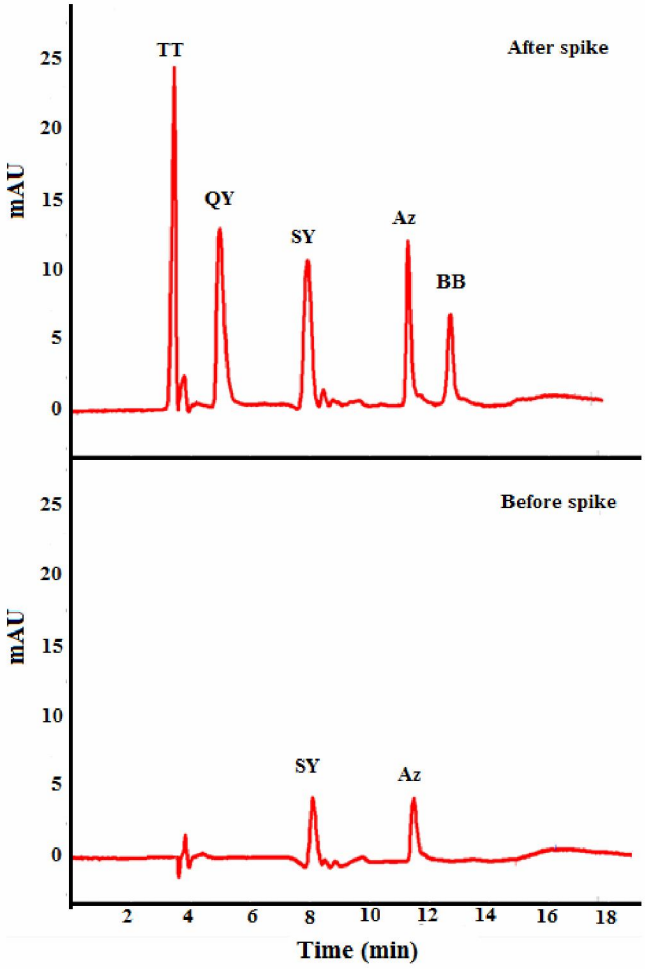

Fig. 3. HPLC-Vis chromatogram (λ = 420 nm) of orange flavor ice cream sample before and after spike of 50 $\mu g\ Kg^{-1}$ of mixture standard solution of the 5 dyes.

Selection of Disperser Solvent and its Volume

The main criterion for the selection of the disperser solvent is the miscibility with both the extraction solvent and the aqueous sample in order to induce the phenomena of dispersion. Usually, the selection of a dispersive solvent is limited to the solvents such as acetonitrile, methanol, acetone and ethanol, which are miscible with both water and extraction solvents [42]. Obtained results showed that among tested solvents, methanol has a good recovery respect to other dispersive solvents. So, methanol was selected for the subsequent studies.

For the optimization of the dispersive solvent volume, the experiments were performed by using different volumes (0.5, 1.0, 1.5 and 2.0 ml) of the dispersive solvent. The results indicated that with increasing the volume of methanol, the extraction efficiency increased first, and then decreased for all the analytes. The reason for this could be that at a low volume of methanol, a cloudy state was not formed well, thus giving a low recovery; at higher volume of methanol, the solubility of the ion-pair in water was increased, leading to a decreased extraction efficiency because of a decrease in the distribution coefficient. Based on the experimental results, 1.5 ml of methanol was chosen.

Effect of Ionic Strength

Generally, addition of salt enhances the extraction of analytes, because the salting-out effect can reduce the solubility of the analytes in water and forced much of them onto the organic phase [20]. On the other hand, in DLLME methods, volume of the sediment phase increases by increasing the ionic strength, because of the decrease in the solubility of the extraction solvent and also viscosity of the aqueous phase increases results in a decrease in diffusion coefficients of the analytes [42] which both of them lead to decrease extraction efficiency. To investigate the effect of salt on the extraction efficiency of DLLME, NaCl was added in the range of 0-15% (w/v). Results revealed that salt addition has a significant effect on the extraction efficiency of DLLME as the peak response was found to decrease as the ionic strength increased. These results revealed that the second phenomenon is predominant. Moreover, in extraction methods with ion-pairing mechanism usually salt ions could be interfered by analyte in ion-pair formation [43]. Therefore, no salt was added for further experiments.

Method Performance

The figures of merit in the proposed UASE-DLLME method including linear dynamic range (LDRs), limits of detection (LODs), limits of quantification (LOQs), EF and ER% for the extraction of the dyes from 10 ml aqueous solutions were investigated under optimum conditions. The calibration graphs were linear in the range from 0.5-1000 μg l^{-1} (n = 8) for all of the dyes with good determination coefficient (R_2 > 0.99). LODs and LOQs values were calculated based on signal to noise ratio of three (LOD = 3 S/N) and signal to noise ratio of ten (LOQ = 10 S/N), respectively. LODs were obtained in the range of 0.02-0.05 μg l^{-1} and LOQs were obtained between 0.06 and 0.15 μg l^{-1}. The EFs and ER% for all of the dyes as defined in section 2.4 were obtained in the range of 160-190 and 80-95%, respectively. The precision of the proposed method was evaluated in terms of repeatability (RSD% ≤ 6.4, n = 6) and reproducibility (RSD% ≤ 9.7, n = 6) at 10 and 100 μg l^{-1} levels of each dye.

Application and Recovery

To evaluate the application of the proposed method for real samples, this method was successfully applied to some ice cream samples. Sample preparation was performed for the ice cream samples according to Section 2.3. To investigate the matrix effects, one sample of each flavour of ice cream samples were spiked with proper amounts of the analytes (50 μg l^{-1}), and the relative recoveries were subsequently calculated (Table 3). The results obtained showed that the different flavours used for the ice cream samples had no effect on the extraction efficiency. The proposed method shows the high relative recoveries for all ice cream samples from 90.3% to 109.4%, which ensures the accuracy of the amount of analytes detected in non-spiked ice cream samples. Then, all of the ice cream samples were analyzed and the dye content of the samples was calculated by considering sample preparation steps. The obtained results are shown in Table 4. The average TT, QY, SY, Az and BB concentration of the 30 ice cream samples ranges between N.D-7.6, N.D-8.7, N.D-13.7, N.D-14.7 and N.D-7.5 mg Kg^{-1}, respectively. Based on the obtained results, Az is the most abundant synthetic dye in ice cream samples alone or in combination with other synthetic dyes. Chromatograms of an orange flavour ice cream sample

Table 3. Results of Food Dyes Recovery Study

Sample name	C_{added} ($\mu g\ Kg^{-1}$)	Recovery (%)[a]				
		TT	QY	SY	Az	BB
Blueberry flavor ice cream	50	102.5	104.8	102.6	91.9	91.9
Cherry flavor ice cream	50	103.0	109.2	105.6	95.1	95.1
Banana flavor ice cream	50	109.0	107.6	90.9	97.6	97.6
Cranberry flavor ice cream	50	108.0	101.2	105.6	92.7	92.7
Saffron flavor ice cream	50	99.7	102.7	107.8	93.4	93.4
Melon flavor ice cream	50	93.4	96.6	107.0	109.4	109.4
Sour green apple flavor ice cream	50	100.6	100.4	91.3	99.6	92.6
Pomegranate flavor ice cream	50	90.3	101.8	91.0	94.8	94.8
Orange flavor ice cream	50	93.9	93.2	96.00	100.4	100.4
Peach flavor ice cream	50	90.9	93.9	91.2	96.6	96.6

[a]RSD values for three replicate measurements for all of the tested samples were less than 10%.

before spike (Fig. 2a) and after spike at the concentration level of 50 $\mu g\ l^{-1}$ (Fig. 2b) for the analytes is shown in Fig. 2. Two sulphonated azo dyes (sunset yellow FCF and azorubine) were found in the sample. The low detection limits allowed the accurate determination of the dyes in ice cream samples at low concentrations. Regarding the obtained results, 73% of the tested samples have synthetic dye which is not regulated under the current Iranian Food Act. according to ISIRI 2450 (5th revision) [3].

Comparison of the Proposed Method with Previously Reported Methods

The proposed method was compared with a variety of methods that were recently reported in the literature for extraction and determination of the food colors. The distinct features of the proposed method are summarized in Table 2. Evaluation of the data showed that, UASE-DLLME has a short extraction time, higher extraction efficiency, higher pre-concentration factor, lower LODs and lower solvent consumption in comparison with SPE [16], CPE [15,17,21] and SALLE [20] methods. Considering the instrumentation, compared to the spectrophotometric methods, when multi channel HPLC-Vis was applied, more analytes could be determined at their λ_{max} with good accuracy. In contrast, LODs (more sensitive) and separation power of LC-MS methods are better than HPLC-Vis techniques. However, multichannel HPLC-Vis techniques are more popular, simpler and time and cost-saving compared to LC-MS techniques [39]. Moreover, a high sensitivity, high efficiency, simplicity, rapidity, moderate cost, and less consumption of organic solvent indicate that the extraction based on the UASE-DLLME can be a promising approach in the field of dyes analysis from solid complicated matrices.

CONCLUSION

An ultrasonic assisted solvent extraction (UASE)

Table 4. Concentration of the Food Colorants in the Samples

Sample	Colorants (mg kg^{-1})				
	TT	QY	SY	Az	BB
Blueberry flavor brand 1	N.D	N.D	N.D	N.D	7.5
Blueberry flavor brand 2	4.8	N.D	N.D	N.D	6.4
Blueberry flavor brand 3	N.D	N.D	N.D	N.D	N.D
Cherry flavor brand 1	N.D	N.D	N.D	4.8	N.D
Cherry flavor brand 2	N.D	N.D	N.D	N.D	N.D
Cherry flavor brand 3	N.D	N.D	N.D	7.2	N.D
Banana flavor brand 1	5.2	N.D	N.D	N.D	N.D
Banana flavor brand 2	N.D	3.1	N.D	4.7	N.D
Banana flavor brand 3	N.D	N.D	N.D	N.D	N.D
Cranberry flavor brand 1	N.D	N.D	N.D	3.8	N.D
Cranberry flavor brand 2	N.D	N.D	N.D	4.7	N.D
Cranberry flavor brand 3	N.D	N.D	N.D	3.6	N.D
Saffron flavor brand 1	3.1	N.D	N.D	N.D	N.D
Saffron flavor brand 2	4.8	N.D	N.D	N.D	N.D
Saffron flavor brand 3	7.6	N.D	N.D	N.D	N.D
Melon flavor brand 1	N.D	N.D	N.D	N.D	N.D
Melon flavor brand 1	N.D	N.D	N.D	N.D	N.D
Melon flavor brand 1	N.D	N.D	N.D	N.D	N.D
Sour green apple flavor brand 1	N.D	8.7	N.D	N.D	4.2
Sour green apple flavor brand 1	N.D	2.1	N.D	N.D	5.3
Sour green apple flavor brand 1	N.D	3.4	N.D	N.D	3.9
Pomegranate flavor brand 1	N.D	N.D	N.D	8.4	N.D
Pomegranate flavor brand 2	N.D	N.D	N.D	6.7	N.D
Pomegranate flavor brand 3	N.D	N.D	N.D	9.5	N.D
Orange flavor brand 1	N.D	N.D	5.6	14.7	N.D
Orange flavor brand 2	3.2	N.D	13.7	5.6	N.D
Orange flavor brand 3	N.D	N.D	2.7	N.D	N.D
Peach flavor brand 1	N.D	N.D	8.1	3.7	N.D
Peach flavor brand 2	N.D	N.D	N.D	N.D	N.D
Peach flavor brand 3	N.D	N.D	N.D	N.D	N.D

Table 5. Comparison of UASE-DLLME with other Previously Published Methods

Matrix	Dyes	Sample preparation	LR ($\mu g\ l^{-1}$)	LOD ($\mu g\ l^{-1}$)	EF	RSD (%)	Instrumentation	Ref.
Ice cream	Tartarazine, quenoline yellow, sunset yellow, carmoisine, brilliant blue	UASE-DLLME	0.5-1000	0.01-0.15	160-190	< 6	HPLC	This work
Water	Allura red	SPE	10-6000	2.35	250	< 7	spectrophotometer	[16]
Beverage and jelly samples	Amaranth	CPE	20-1600	13	Not reported	3.17	spectrophotometer	[15]
Soft drinks, sweet and gelatin stuffs	Sunset yellow	CPE	20-452	5.0	80	1.49	spectrophotometer	[21]
Candy, jelly gum, soft drink, mineral water	Sunset yellow	ISS-SPE	10-750	2.1	51.8	≤ 1.24	spectrophotometer	[44]
Jelly, fruity pastille, Smarties, candy	Sunset yellow, Allura Red, Brilliant Blue FCF	CPE	20-4000	7-10	10	3.4-4.2	spectrophotometer	[17]
Solid juice and jelly powder	Sunset yellow, methylene blue	SALLE	200-7000	60-70	Not reported	3.8	spectrophotometer	[20]
soft drinks, sugar- and gelatin-based confectionery	Tartrazine, Amaranth, Sunset Yellow, Allura Red, Ponceau 4R, and Erythrosine	IL-DLLME	0.05-1.0 to 300-2000	0.015-0.32	Not reported	Not report	HPLC	[30]
Condiments	Chrysoidin, safranine O, auramineO and rhodamine B	MSPD-HLLME	20-60 to 2000	6.7-26.8	Not reported	8.2	HPLC	[31]
Fizzy drink, fruit juice s, black tea, and fruit jelly	Amaranth, Ponceau 4R, Allura Red, and Carmoisine	in-tube electro-membrane extraction	1.0-800	0.1-1.0	25-32	5.2	HPLC	[35]
Beverages, fishery, meat, vegetable, bakery, additives and diet supplements	17 Synthetic dyes[a]	Solvent extraction- SPE	5-300 $mg\ Kg^{-1}$	Not report	Not reported	3.6	HPLC	[32]
Beverages, syrup, candies, gelatin candies and preserved fruit	34 Water-soluble synthetic dyes[b]	Solvent extraction	0.05-20	0.01-0.05	Not reported	1.4-6.4	LC-MS	[39]

LR: Linear range; RSD: Relative standard deviation; LOQ: Limit of quantification; LOD: Limit of detection; UASE: Ultrasound assisted solvent extraction; DLLME: Dispersive Liquid-Liquid Microextraction; SPE: Solid Phase Extraction; CPE: Cloud Point Extraction; ISS-SPE: insitusurfactant-basedsolid phase extraction; SALLE: Solvent Assisted Liquid-Liquid Microextraction; IL-DLLME: Ionic liquid-Dispersive Liquid-Liquid Microextraction; MSPD-HLLME: matrix solid-phase dispersion homogeneous liquid-liquid microextraction.

followed by ion-pair based dispersive liquid-liquid microextraction (DLLME) coupled with multichannel HPLC-Vis was developed for the sensitive simultaneous determination of five synthetic food colourants in ice cream samples. This method provides good precision, a wide linear range, and detection limits at the μg kg^{-1} level. UASE greatly increased the dyes release and extraction efficiency, resulting reduced extraction time. Addition of Carrez solutions after UASE leads to obtain simpler sample matrix. Next DLLME step leads to more clean-up and preconcentration of the dyes which is favorable in their trace analysis. The present method is attractive due to its simplicity, analytical precision, and considerable time saving. The results from validation indicate that the propose method could be extended to other analytes and other types of solid and semisolid samples.

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