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Original Research Article

Development of an Ultrasound Assisted Ion Pair Based Surfactant-enhanced Liquid–liquid Microextraction Technique Combined with Spectrophotometry for Preconcentration and Determination of Ultra-Trace Amounts of Sunset Yellow in Food Samples

Mohammad Reza Jalali Sarvestani¹, Zohreh Doroudi^{2,*}

¹Young Researchers and Elite Club, Yadegar-e-Imam Khomeini (RAH) Shahr-e-Rey Branch, Islamic Azad University, Tehran, Iran

^{2*}Department of Chemistry, Yadegar-e-Imam Khomeini (RAH) Shahr-e-Rey Branch, Islamic Azad University, P.O. Box 18735-334 Tehran, Iran

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ABSTRACT

The present work deals with a fast, simple, and efficient technique to extract, preconcentrate, and measurement of sunset yellow in food specimens with acceptable recoveries based on liquid–liquid microextraction enhanced by surfactant with ultrasound assisted ion pair basis combined with spectrophotometry (UA-IPSE-DLLME). Box–Behnken design was used to optimize different effective experimental parameters on the extraction efficiency. Under the optimum conditions (pH = 6.5, volume of chloroform = 26 μL , concentration of Zephiramine = 0.33 mM and extraction time = 2.5 min), the calibration curve was found linear within the range of 0.5–55.0 ng mL^{-1} for Sunset Yellow. The limit of detection (LOD), preconcentration factor and the enrichment factor of the proposed method for sunset yellow was 0.13 ng mL^{-1} , 558 and 490 respectively. The selectivity of the proposed method over various interfering foreign species was also checked out and no serious interference was observed. At the end, the established method was successfully employed for the determination of sunset yellow at different edible real samples.

Keywords: Sunset Yellow; Ultrasound assisted ion pair based surfactant-enhanced liquid–liquid microextraction; Box–Behnken design; Microextraction

Introduction

Natural and synthetic azo dyes are widely used as coloring agents for foodstuffs, drugs and cosmetics [1]. In comparison with natural dyes, synthetic dyes show a lot of important advantages including high stability to light, pH and heat, higher solubility in water and lower production cost [2]. But, at high amounts synthetic dyes can have toxic effects on humans. Therefore, regulations laws, and acceptable daily intake (ADI) values control the use of synthetic dyes strictly [3]. Sunset Yellow (SY) as an azo dye is extensively utilized in drinks, foods, and pharmaceuticals to offer red or orange color to the products of these industries [4]. Considering the potential hazards to human beings, controls are essential for the existence and content of this dye [5]. The ADI value of SY is 2.5 mg kg^{-1} body weight/day [6]. Accordingly, it is essential to control and determine SY contents in foodstuffs with acceptable sensitivity and accuracy [7]. Until now, various analytical methods, including UV-Visible spectrophotometry [8], HPLC (high-performance liquid chromatography) [9], electrochemical method [10], mass spectrometry [11], and fluorimetry [12] have been developed for the determination of SY. However, some problems, such as complex matrices and very low dye concentration of samples, could arise in the determination process without pretreatment of the samples [13]. Therefore, analytical chemists have made great efforts to obtain higher sensitivity and selectivity. Hence, these analysis methods were coupled with preconcentration and extraction approaches like solid-phase extraction (SPE) [14, 15], dispersive liquid-liquid microextraction (DLLME) [16], cloud point extraction (CPE) [17, 18], liquid liquid microextraction [19], and membrane filtration [20]. DLLME is a fast and simple microextraction method in terms of using a disperser solvent and suitable extractant with a high miscibility in the extractant as well as aqueous phase like ethanol, methanol, acetone or acetonitrile [21]. To date, a large number of advanced approaches of DLLME were established and reported. A modification of classical DLLME in terms of surfactant, as disperser agent known as SA-DLLME (surfactant assisted emulsification dispersive liquid-liquid microextraction) was reported in 2010 [22]. The SA-DLLME or DLLME has mainly the disadvantage of incapability at extracting hydrophilic compounds into the extraction solvent. Techniques like ion pair-based surfactant-assisted microextraction (IP-SAME) can reduced this limitation [23, 24]. The surfactant contributes as a carrier agent in the ionic species transfer from the aqueous phase to the extracted phase by ion-pair creation [25, 26]. In IP-SAME, employment of the ultrasound energy can significantly improve the dispersion of

extractant solvent as fine droplets within the aqueous phase. Consequently, the contact surface area within the organic and aqueous phases enhances considerably which leads to the facilitation of the analyte mass transfer in to the extractant solvent, improvement of extraction efficiency and reducing of the extraction time [27]. Among the established instrumental analytical methods, UV-Visible spectrophotometry is one of the most straightforward, economical and practicable techniques. Moreover, sophisticated operators are not needed for the implementation of the analysis procedure.

In this respect, an UA-IPSE-DLLME (ultrasound assisted ion pair based surfactant-enhanced dispersive liquid-liquid microextraction) technique was coupled with UV-Visible spectrophotometry in this work for the determination of SY determination, in this research. At first, a Box-Behnken design was used for optimizing all of the effective experimental parameters. Then the selectivity, repeatability and the performance of the proposed technique for the determination of SY at food specimens were scrutinized.

Experimental

Reagents and standard solutions

All chemicals used in this work were analytical reagent grade and double-distilled water was used throughout. Sunset Yellow (disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate), chlorobenzene (C_6H_5Cl), chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), dichloromethane (CH_2Cl_2), sodium hydroxide (NaOH), and sodium chloride (NaCl), acetic acid (CH_3COOH), boric acid (H_3BO_3) and phosphoric acid (H_3PO_4) were obtained from Merck chemical company (Darmstadt, Germany). Tetradecyl dimethylbenzylammonium chloride dihydrate (Zephiramine) and hexadecyl trimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich. Universal buffer solutions were prepared by mixing phosphoric, acetic, and boric acid [28].

A stock solution of SY ($1000 \mu\text{g mL}^{-1}$) was prepared by dissolving 0.100 g of SY dye in water and diluting to 100 mL in a volumetric flask. The solutions were stored in a refrigerator at 4°C ; at this temperature, SY was stable for at least 1 month. Fresh working standard solutions were obtained by appropriate dilution of the stock solution and were stable during the day. A solution of cationic surfactant (2.0×10^{-2} M) Zephiramine was prepared by dissolving accurately 0.664 g of Zephiramine in water and diluting to 100 mL in a volumetric flask.

Apparatus and software

All absorbance measurements were obtained using a Hewlett-Packard 8453 diode array spectrometer controlled by a Hewlett-Packard computer, between 400 and 700 nm digitized every 1 nm. A model 780 digital Metrohm pH meter equipped with a combined glass–calomel electrode was used for the pH adjustments. The centrifuge was performed by a Sigma 3K30). An ultrasonic (VGT-1740QTD, Taiwan) water bath with a temperature control and a digital timer was used to emulsify the extraction solvent. The experimental design was performed with Minitab Version 19.

Analytical procedure

For the UA-IPSE-DLLME, 4.0 mL of the buffered solution (pH 6.5) and 1.0 mL NaCl 20% was inserted to 5.0 mL sample solution with different concentration of SY, and put in a 12 mL screw cap glass test tube with a conical bottom. 26 μL of chloroform as an extractant solvent and 165 μL 2.0×10^{-2} M Zephiramine as emulsifier were introduced into the sample solution. At this stage, the conical tube was sonicated for 2.5 min at predetermined temperature (25 ± 2 °C) to allow complete extraction. The emulsion was then disrupted via centrifuging for 3 min at 3000 rpm to sediment the organic phase at the bottom of the tube. A syringe was used to remove the upper aqueous phase. The sedimented phase was dried by passing through nitrogen gas. Ultimately, the residue was dissolved in 500 μL water. The solution absorbance was calculated at 483 nm. A blank solution (no SY) was also considered to the same process and determined along with the specimens.

Preparation of real samples

The samples such as fruity candy, smarties, jelly powder, and orange soft drink were bought from the local supermarkets in Tehran (Tehran, Iran). Appropriate amounts (1.0 gr) of samples were dissolved in deionized water. Ultrasonication was performed for 5 min to degase the carbonated drink and remove the carbon dioxide. The samples were completely dissolved through a warming procedure (50°C, 20 min). After dissolving, membrane filter (0.45 μm) was used to filter the solutions and diluting to 100 mL was performed for the filtrated sample solutions in a volumetric flask. Under the proposed approach, an aliquot of the solutions was treated for UA-IPSE-DLLME and following determination of SY.

Multivariate optimization

Chemometric tools like response surface methodology (RSM) that are developed on the basis of statistical design of experiments (DOEs) have been widely used for the optimization of effective parameters on the analytical techniques, in the recent decade [29-31]. In these methods, the several factors affecting the response can simultaneously optimize by considering the interaction between them. Because the optimum situations inferred from the univariate investigations deviates from the correct results of the multivariate optimization If a considerable interaction observed between the variables. Various factors can influence the extraction efficiency of the UA-IPSE-DLLME method. Therefore, using Box–Behnken design (BBD), the interactions between the independent variables (sample pH, concentration of the surfactant, volume of extraction solvent and ultrasound emulsification time) were optimized and enhanced on the highest extraction yield of SY from food samples. The Box–Behnken design is a second-order multivariate method in terms of a three-level partial factorial designs. Box–Behnken is a rotatable, spherical, or nearly rotatable that consists of a central point and with the mid-points of the edges of the variable space. The number of tests (N) needed for developing BBD is described as $N = 2k(k - 1) + C_0$, in which k represents the number of factors and C_0 shows the number of central points [32]. Thus, 27 trials were conducted to optimize these 4 variables at 3 levels (high, medium, and low) in the present BBD. The experiments were repeated three times at the central point for error estimation.

Results and Discussion

In the pharmaceutical and food industries, SY is widely used in order to give an orange color to their processed products. At first, in order to find the maximum absorption wavelength (λ_{max}) of SY and to check the influence of surfactant on it, the absorption spectrum of SY before and after extraction and preconcentration was obtained and the results showed that the λ_{max} of SY is 483 nm and the existence of surfactant does not have any influence on its the highest absorption wavelength. In this regard, the absorbance calculations were carried out at this wavelength.

Selecting the extraction solvent

One of the key factors that play an important role in the efficiency of a UA-IPSE-DLLME method is selecting an adequate extractant solvent. In this technique, the chosen extractant solvent should not be soluble in water, its density must be higher than water and it should have

an excellent extraction capability for the desired analytes. In this respect, the performance of different halogenated solvents including dichloromethane (CH_2Cl_2), carbon tetrachloride (CCl_4), chloroform (CHCl_3) and chlorobenzene ($\text{C}_6\text{H}_5\text{Cl}$) as the possible extractant solvent were investigated and their emulsification and extraction efficiency were evaluated and the obtained results are presented in Fig. 1. It is observed that chloroform has the highest extraction recovery among the examined solvents. Hence, chloroform was chosen as the extractant solvent.

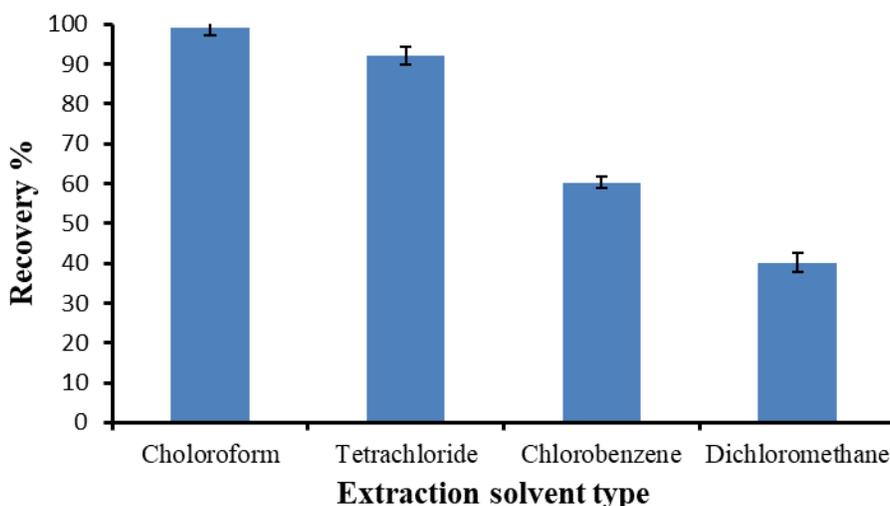


Fig. 1. Effect of extraction solvent on the recovery of Sunset Yellow.

Extraction conditions: concentration of Carmoisine, 20 ng mL^{-1} ; extraction temperature, $25 \text{ }^\circ\text{C}$; extraction time, 2.5 min; sample pH, 6.5; concentration of Zephiramine, $3.3 \times 10^{-4} \text{ M}$; extraction solvent, $26 \mu\text{L}$; concentration of NaCl, 2% w/v

The influence of surfactant nature

The nature of the utilized surfactant can play a main role in the analyte extraction and preconcentration efficiency. In fact, the employed surfactant act as an emulsifier which can expedite emulsifying the organic extractant solvent in to the aqueous medium. In this research, the performance of two cationic surfactants including CTAB and Zephiramine as the emulsifier were assessed and the acquired experimental findings indicated the recovery value is higher in the case of Zephiramine and this surfactant is better than CTAB. Therefore, Zephiramine was chosen as an optimum surfactant for further works.

The impact of adding salt

Adding salt to the aqueous phase can decline the organic analytes solubility in the water and improve the analyte transfer to the organic phase. In this respect, salt addition is generally employed in extraction methods to ameliorate the analyte extraction recovery (especially the high polar analytes). To assess the influence of salt addition on the extraction efficiency of SY, the concentration of NaCl was varied from 0 to 5% w/v and the findings indicate by augmenting the concentration of NaCl from 0 to 2% the extraction efficiency improved considerably. However, further addition of sodium chloride does not have any effect on the extraction efficiency. Consequently, 2.0% (w/v) was considered as the optimized concentration of sodium chloride.

The influence of temperature

Due to the fact that both mass transfer and emulsification processes can be influenced by temperature, this parameter can have a remarkable effect on the extraction efficiency. In this respect, the impact of temperature on the extraction process was assessed in the range of 25 to 45 °C. The obtained results demonstrated the temperature does not have any tangible influence on the extraction efficiency. Therefore, further experiments were performed at the room temperature (25 ± 2 °C) for the convenience of the work.

Box–Behnken analysis

Some preliminary experiments were implemented in order to choose the used ranges and levels in the next tests. The experimental ranges for independent variables included concentration of the surfactant (X_1 : 0.10–0.50 mmol L⁻¹), the sample solution's pH (X_2 : 4.0–8.0), the volume of the extraction solvent (X_3 : 15–30 µL) and ultrasound emulsification time (X_4 : 1–4 min). Box–Behnken experimental design was statistically employed for evaluation and optimization of the important variables. Based on the Box-Behnken matrix, 27 experiments comprising 3 replicates at the central point were randomly performed to reduce the bias of uncontrolled variables.

Using Analysis of variance (ANOVA), the significance of each factor and interaction terms was determined (Table 1). If the P-value in the ANOVA table is lower than 0.05, it can be deduced that the statistical importance of a parameter is at 95% confidence level. The model p-value of 0.0000 for the quadratic model indicates that it is significant. The correlation coefficient

(($R^2=0.9763$ and adjusted $R^2=0.9614$) is a good standard for expressing the fitting quality of the polynomial model equation.

Table 1. Analysis of variance (ANOVA) for response surface quadratic model

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	10	0.400182	0.040018	65.83	0.000
Linear	4	0.163697	0.040924	67.32	0.000
X ₁ - conc. surf.	1	0.027456	0.027456	45.16	0.000
X ₂ - pH	1	0.070380	0.070380	115.77	0.000
X ₃ - Vol.	1	0.054378	0.054378	89.45	0.000
X ₄ - Time	1	0.011482	0.011482	18.89	0.001
Square	4	0.215305	0.053826	88.54	0.000
X ₁ *X ₁	1	0.196216	0.196216	322.77	0.000
X ₂ *X ₂	1	0.063579	0.063579	104.59	0.000
X ₃ *X ₃	1	0.012980	0.012980	21.35	0.000
X ₄ *X ₄	1	0.042682	0.042682	70.21	0.000
2-Way Interaction	2	0.021180	0.010590	17.42	0.000
X ₂ *X ₃	1	0.018360	0.018360	30.20	0.000
X ₂ *X ₄	1	0.002820	0.002820	4.64	0.047
Error	16	0.009727	0.000608		
Lack-of-Fit	14	0.007981	0.000570	0.65	0.750
Pure Error	2	0.001746	0.000873		
Total	26	0.409909			

$R^2 = 97.63$; adjusted $R^2 = 96.14$; predicted $R^2 = 91.82$.

DF, degree of freedom; SS, sum of squares; MS, mean square.

The adjusted R^2 value (0.9614) showed that this model does not explain only 3.86% of the overall variation. Therefore, the value of correlation coefficient ($R^2 = 0.9763$) represents good

relation between the predicted and experimental values of the response. To demonstrate the association between responses and input variables, experimental data with a mathematical equation of the second order polynomial were fitted (Eq. 1).

From the ANOVA investigation, it was found that the “Lack of Fit p-value” of 0.750 implies the Lack of Fit is not significant relative to the pure error and it clarified the statistical significance of the quadratic model for the response. To statistically analyze the experimental data, it is essential to suppose that the data are resultant from a normal distribution. The normal probability plot of residuals that is presented at Fig. 2 demonstrates that a linear association exists between them with a relatively high correlation coefficient which implies the normal distribution of error around the appropriate applicability and mean value of the experimental data prognostication confirming the normality assumption within the fitted model.

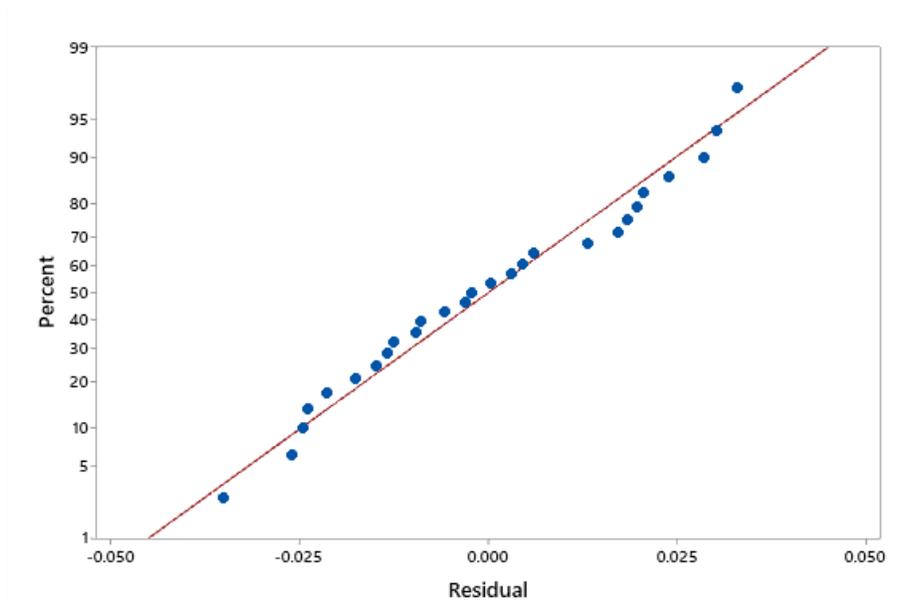


Fig. 2. The normal probability plot of residuals

Response Surface Methodology

Using four factors at three levels BBD, the effect of process variables was investigated including concentration of the surfactant, pH of the sample solution, the volume of the extractant solvent, and ultrasound emulsification time on the UA-IPSE-DLLME of SY. From the developed model, analysis of results by response surface methodology (RSM) was assessed to plot the response versus factors and to determine the interaction between the optimal levels and factors, for which

the results are shown in Fig. 3. Concentration of the used surfactant plays a key role in the emulsification microextraction method. Micelle is a molecular aggregation of surfactant molecules and the minimum concentration of the surfactant that is required for the formation of micelle in the solution is defined as critical micelle concentration (CMC). The obtained findings showed that by increasing the concentration of Zephiramine higher than its CMC (3.7×10^{-4} M) in the specimen solution, the extraction efficiency decreases gradually. This phenomenon is observed because when the surfactant concentration over goes the CMC, a fraction of analyte molecules can merge in to the micelles and as a consequence, the analyte solubility enhanced in the sample aqueous solution. pH is the next parameter that has sharp effects on the extraction efficiency because it can influence both ion pairing and extraction steps. To assess the influence of pH on the extraction efficiency the sample pH solution was varied form 4.0-8.0. As can be seen from the surface plots in Fig. 3, when the pH of solution increases from 4 to 6.5, the extraction recovery improves tangibly but, by further increasing of the pH value of the solution to more than 7, the response declines significantly because in higher pH values the OH⁻ ions compete with analyte to create an ion pair with Zephiramine. Moreover, the obtained responses indicated the SY extraction efficiency reached the highest value when the chloroform volume is 26 μ L. However, by further incrementing the volume of extractant solvent the extraction efficiency decreased gradually. Time in this kind of extraction is explained as the time interval between adding the extraction solvent and the end of sonication before the centrifugation onset. Time possesses a key influence on the both emulsification and mass transfer processes. The obtained results indicated the extraction efficiency improved by incrementing the extraction time to 2.5 minutes, but by further incrementing the extraction time the extraction efficiency decreased gradually. The calculated values for the critical point for extraction of SY are pH = 6.5, volume of chloroform = 26 μ L, concentration of Zephiramine = 0.33 mM and extraction time = 2.5 min.

Analytical figures of merit

After optimizing the effective experimental parameters, the linear range, repeatability, preconcentration factor (PF) and limit of detection (LOD) of the developed extraction technique was evaluated. The calibration curve for determination of SY was linear within the range of 0.5–55.0 ng mL⁻¹ ($R^2 = 0.9994$). The limit of detection is explained as $LOD = 3S_b/m$, in which S_b denotes the standard deviation of 10 the blank signals after 10 replications and m is the obtained

calibration curve's slope. For a specimen with 10 mL volume, it was 0.13 ng mL^{-1} . The accuracy, precision, and stability were assessed by measuring the absorption of 20.0 ng mL^{-1} samples at 5 various times over a single day and on 5 succeeding days respectively. Inter and intra-day accuracies of the technique were acceptable with a relative standard deviation (RSD) of 1.7% and 2.2% for determining SY. Ultimately, the preconcentration factor of the method which is determined as the ratio of the maximum sample volume and the minimum ultimate volume was 588 and enrichment factor (EF) (determined from the ratio of the calibration curves' slopes acquired with and without pre-concentration) of 490 for SY was calculated.

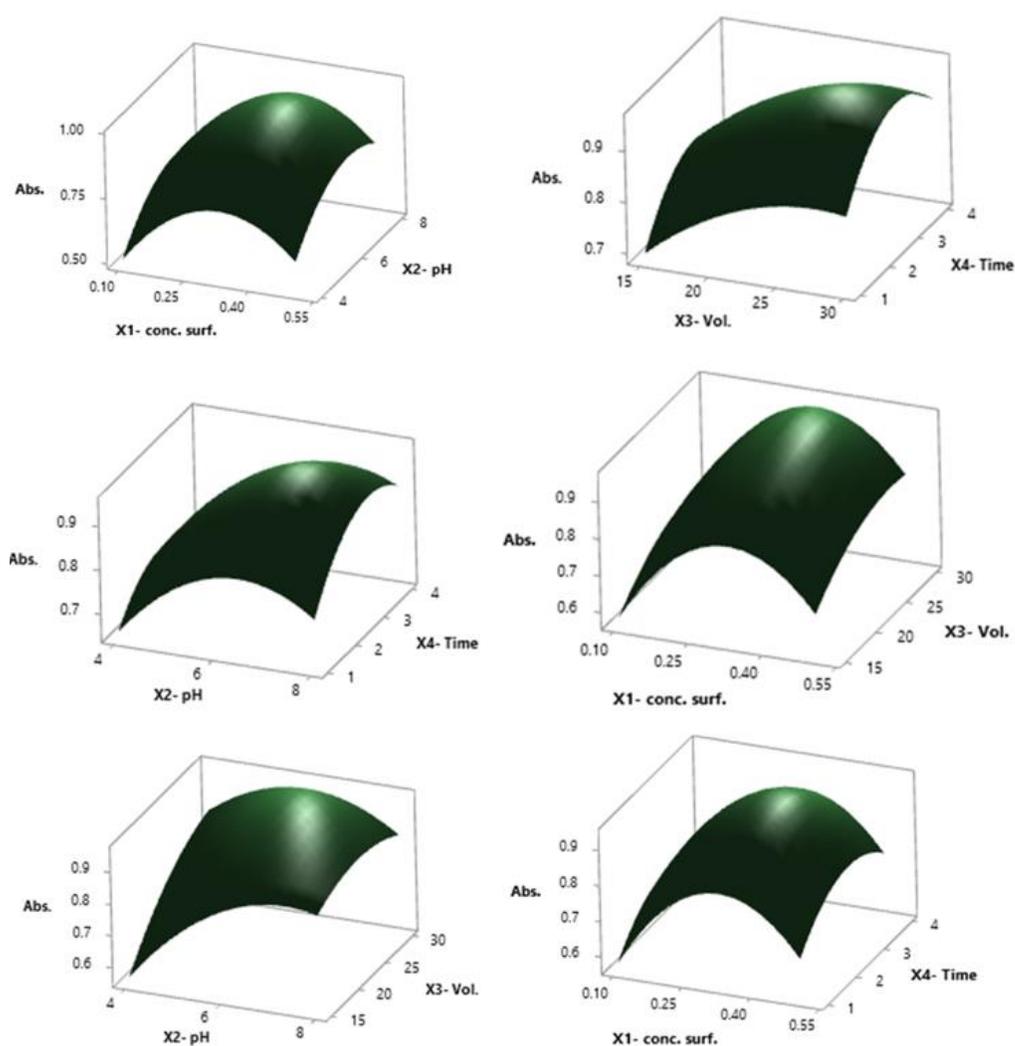


Fig. 3. Three-dimensional response surface plots representing the effect of process variable on absorbance: (X₁) Concentration of Zephiramine; (X₂) Sample pH; (X₃) Volume of chloroform; and (X₄) Extraction time

Interference studies

The selectivity of an analytical technique has an important effect on the accuracy the obtained results. In this respect, the effect of various anionic and cationic species on the analytical response of the designed method was investigated. For this purpose, a 20 ng mL⁻¹ solution of SY was prepared and different amounts of the interfering species were introduced to the solution and the absorption of the sample was measured in the presence of other interfering species. Then the tolerance limit that was determined as the highest amount of the interfering species causing an error not higher than $\pm 5\%$ in the determination of SY was calculated for the studied interfering species. The obtained results showed that the tolerance limit for glucose, fructose, Ca²⁺, Zn²⁺, Mg²⁺, Na⁺, K⁺, NH₄⁺, PO₄³⁻, NO₃⁻, Cl⁻, Br⁻ and F⁻ is 1000, for Co²⁺, Pb²⁺, Ni²⁺, Cd²⁺, Mn²⁺, Fe³⁺ and SO₄²⁻ is 300. Therefore, the proposed method has an admissible selectivity towards SY over a wide range of ionic species.

Real samples analysis

The applicability of the suggested technique to real food samples was evaluated and fruity candy, jelly powder, smarties and orange soft drink were extracted utilizing the UA-IPSE-DLLME process. Recovery experiments were performed by spiking the samples prior to the UA-IPSE-DLLME with the addition of known amounts of SY. The results are presented in Table 2 and show that the developed this method is used for the accurately SY determination in these specimens.

Table 2. Real sample analysis

Samples	Added concentration (ng mL ⁻¹)	Founded concentration (ng mL ⁻¹)	Recovery (%) (n = 5)
	0.0	18.4 ± 0.4 ^a	–
orange soft drink	5.0	23.1 ± 0.8	98.7
	10.0	27.9 ± 0.6	98.2
	0.0	12.5 ± 0.2	–
Smarties	5.0	17.4 ± 0.4	99.5
	10.0	23.1 ± 0.6	102.6
	0.0	18.3 ± 0.3	–
orange jelly	5.0	23.4 ± 0.9	100.4
	10.0	28.0 ± 0.5	98.9
	0.0	7.3 ± 0.4	–
Fruity candy	5.0	12.7 ± 0.5	103.2
	10.0	18.1 ± 0.6	104.6

^aStandard deviation

Comparison with other preconcentration techniques

A comparison was made between the established UA-IPSE-DLLME technique and other approaches reported in the literature (Table 3). The analyte's limit of detection was lower compared to the other preconcentration/ separation. In general, the current technique shows a low detection limit and high enrichment factor. Moreover, a high sensitivity, high efficiency, rapidity, simplicity, low cost, and less consumption of organic solvent indicate that the extraction based on the UA-IPSE-DLLME can be a promising approach in the field of dyes analysis in food samples.

Table 3. Comparison of analytical parameters of the proposed UA-IPSE-DLLME method with some of the methods reported in the literature

Sample preparation	Detection	LOD ^a (ng mL ⁻¹)	LR ^b (ng mL ⁻¹)	EF ^c or PF ^d	RSD ^e (%)	Remarks	Ref.
CPE by Triton X-100 and Trioctylamine	UV-Vis	0.5	20–452	33.3	1.49	Sensitive but PF is low, required large volumes trioctylamine to increase the efficiency	[33]
CPE by Brij 58	UV-Vis	7.8	10 – 4000	Not reported	1.44	LOD is high, time consuming, sensitive	[34]
CPE by Triton X-100	UV-Vis	9	0.020–4000	Not reported	3.5	LOD is high, time consuming, used an extra chemical	[17]
IL-DLLME	HPLC	0.015	0.05–300	Not reported	Not reported	Expensive and required large volumes of ionic liquid,	[35]
SPE	UV-Vis	0.66	0.00–10000	80	4.5	Time consuming, repeatability is low	[7]
DM- μ -SPE	HPLC	0.3	1–500	420	3.2	Expensive, high enrichment factor.	[36]
UA-IPSE-DLLME	UV-Vis	0.13	0.5-55	558	<2.2	High enrichment factor, sensitive, simple, low cost, and ecofriendly	This work

a. Limit of detection.

b. Linear dynamic range.

c. Preconcentration factor.

d. Enrichment factor.

e. Relative standard deviation.

ILDLLME Ionic liquid-Dispersive Liquid-Liquid Microextraction

CPE Cloud point extraction.

DM- μ -SPE dispersive magnetic micro-solid-phase extraction

UA-IPSE-DLLME ultrasound assisted ion pair based surfactant-enhanced liquid-liquid microextraction

CONCLUSIONS

A simple, inexpensive, and applicable ultrasound assisted ion pair based surfactant-enhanced liquid–liquid microextraction combined with spectrophotometry for determination and preconcentration of SY in food specimens has been developed. Using multivariate technique, the processes in analytical chemistry were optimized. It is a fast, effective, and economic method allowing the optimization of more than one variable at the same time. In this method, no toxic solvent dispersion was used and the extraction solvent volume was very low. The established process has the main advantages including good accuracy, rapidness, low-cost and being environmental friendly with high pre-concentration factor can be effectively utilized to preconcentration and determination of sunset yellow in real samples without interferences ions.

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