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Sensitivity and selectivity determination of erythrosine in food sample by ionic liquid-liquid phase microextraction

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ABSTRACT

In this paper, a new method was developed to determinate the erythrosine in candy and tomato sauce using in-situ ionic liquid formation liquid-liquid microextraction (ISILF–LLME) followed by fluorescence detection. In this experiment, a little amount of the KPF_6 (as an ion-pairing agent) was join to the sample solution containing small number of the $[C_8MIM][Br]$ (as hydrophilic ionic liquid). The fine droplets of 1-octyl-3-methylimidazolium hexafluorophosphate $[C_8MIM][PF_6]$ was formed and a cloudy solution was obtained. The main factors influencing the extraction efficiency of ISILF–LLME including the selection of IL, amount of KPF_6, solution pH, the extraction time, and centrifugation time, were optimized. The effect of other synthetic food colorants for determination of erythrosine was evacuated. Under optimal conditions, erythrosine had good linearity, with a correlation coefficients of 0.9996, linear range from 0.2 to 80 ng mL^{-1}, and detection limits of 0.055 ng mL^{-1}. The spiked recovery of samples was from 91.2 to 102.3%. The results indicate that the developed method was successfully applied to analytical erythrosine in candy and tomato sauce.

KEYWORDS

Erythrosine;
In-situ ionic liquid formation;
Liquid–liquid microextraction;
Fluorescence;
Extraction efficiency.

INTRODUCTION

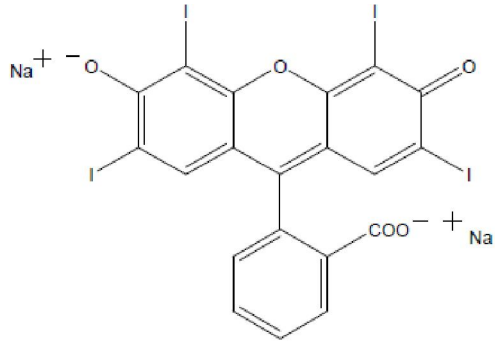
Ionic liquids (ILs) are ionic, non-molecular solvents with a melting point lower than 100°C. The most notable properties include their negligible vapor pressure, high chemical/thermal stability, and variable viscosity. Being recyclable, ILs are appealing as environmentally friendly solvents in the field of separation sciences. Meanwhile, ILs can be designed based on the required use. They have been used as extraction media and alternative to conventional volatile organic solvents because they have some good physical and chemical properties, such as non flammability, good solubility for organic and inorganic compounds, and environmental benignity[1-4].

Dispersive liquid–liquid microextraction (DLLME)[5-7] has been proposed recently. The appropriate mixture of extraction solvent and dispersant is rapidly added into a sample solution by syringe. The extraction solvent by dispersant is uniformity dispersed into the solution with a fine droplets form, enabling extraction solvent is easily to extraction the target compounds. When extraction is completed, achieve phase separation by centrifugation, and determinate of the target analytes in the

enriched phase are performed. The DLLME have many advantages which are easy operate, speed, inexpensive, and high preconcentration factors. The more important DLLME can be applied under batch conditions, at the same time extraction can be completed almost in a few seconds, this indicates that the extraction is faster and analytical is shorter time.

DLLME methods that ILs which is used as the extraction media and small amount of organic solvent or surfactant as the dispersant solvent have been reported. Such as temperature-controlled IL dispersive liquid-phase microextraction[8], ultrasound-assisted IL dispersive liquid-phase microextraction[9] and surfactant-assisted IL dispersive liquid-phase microextraction etc[10]. A new method of DLLME based on ILs called in-situ ionic liquid formation liquid–liquid microextraction (ISILF–LLME) has been developed[11-16]. This ISILF–LLME method the extractant phase is formed in the sample solution via a metathesis reaction between a non-water-soluble IL and an ion exchange reagent, thereby forming a water-immiscible IL. Homogeneously dispersed fine drops of the extractant phase are generated, and high enrichment factors are obtained with low extraction times because of high contact surface between the phases[16]. Compared with temperature-controlled IL DLLME and ultrasound-assisted IL dispersive liquid-phase microextraction, this new method avoids heating, ultrasound, and freezing process, thus, the new method fast and inexpensive. Moreover, the formation of a water miscible IL and finally extraction of target compounds are combined in one step which have no use a dispersive solvent, this make the whole process fast and easy to operate[11-15].

Safety issues of Food synthetic colorants are more and more attention, many countries have strict restrictions of its kinds and quality, however, due to the advantages in terms of price and stability of food synthetic colorants, the total usage of the world continues rise. But certain studies prove that commonly used food dyes are carcinogenic and toxic[17]. Therefore, accurate and reliable methods to determine dyes are required for the assurance of food safety[18-19]. Erythrosine (ERY) disodium 2-(2,4,5,7-tetraiodo-3-oxidooxoxanthen-9-y1) benzoate monohydrate



Scheme 1 : The structure of erythrosine

(Scheme 1) belongs to the xanthene class of dyes of highly water-soluble[20]. In China, ERY as a kind of colorant is permitted for use at a maximum limit of 0.05 g/kg of the product[21].

For now national standard methods for the determination of dyes are thin layer chromatography, high-performance liquid chromatography (HPLC) with gradient elution, and oscillopolarography in China[21]. For now have developed several methods of detect food color erythrosine have been proposed, such methods include resonance Rayleigh scattering (RRS)[18-19], high-performance liquid chromatography (HPLC)[22-25], chemiluminescence (CL)[26], spectrophotometry[27-28], capillary electrophoresis (CE)[17, 29, 30], and fluorescence[31]. But some of them are laborious and time consuming. Fluorescence is considered as the most convenient analytical technique in food analysis, because of its inherent simplicity, high sensitivity, and availability in most quality-controlled. Therefore, more significance to further develop higher sensitive fluorescence analytical methods for the evaluation of ERY.

In the present study, ISILF–LLME followed by fluorimetric determination was applied to determine ERY in candy and tomato sauce. To our knowledge, the use of ISILF–LLME combined with fluorescence has not been applied in the analysis of ERY. Influence of various experimental factors including the selection of IL, amount of KPF_6, solution pH, the extraction time, were optimized.

EXPERIMENTAL

Apparatus

Spectrofluorimetric measurements were made

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using a Cary Eclipse Fluorescence spectrophotometer (Agilent Technologies, USA). Spectrofluorimeter equipped with a xenon lamp and a computer with Cary Eclipse 1.1 software. All the measurements tested using a 0.5 mL quartz cell thermostated at 25.0±0.5 °C, with 5.0 nm bandwidths for the excitation and emission monochromators. pH was adjusted using a pH meter (Model pHS-3C, Shanghai Tianda Apparatus Ltd.).. A Model TDZ4-WS centrifuge (XiangYi Centrifuge Instrument Co. Ltd., China) was employed to make quickly obtain the phase-separation. The temperature was controled using a SHA-B constant-temperature shaker (Changzhou Guohua Electric Appliance Co. Ltd., China)..

Materials

The stock solutions of the colorants ERY (CI Food Red 14; 0.1 mg/mL) were purchased from the Chinese National Research Center for Certified Reference Materials (Beijing, China). Working solutions were used before by appropriate dilutions of the stock standards solutions with double-distilled water. The ILs were purchased from Cheng Jie Chemical Co. Ltd of Shanghai. (Shanghai, China). All reagents used were of analytical reagent grade. All standard solutions were stored at 4°C and their temperature was adjusted to ambient conditions prior to use. Throughout the experiment used double-distilled water.

Extraction procedure

Using a 15 mL screw-cap conical-bottom graduated plastic centrifugal tube placed a homogeneous sample solution of 10 mL containing the analytes (pH 11.0). 100 µL of IL using a 250 µL syringe injected into the sample solution. When 40 mg of KPF_6 was added, a cloudy solution formed. After standing for 3 min and was centrifuged for 10 min at a rate of 3500 rpm (1685×g). Using a 10ml pipette removed the upper aqueous solution. Then, the IL residue enriched with analytes added to acetone and then mixed, generating a volume of 200 µL. The acetone solution was transferred into quartz cells. finally, the fluorescent intensity of ERY was measured. The calibration graph was constructed similar to the studied dye solutions of known concentrations.

Preparation of the sample solution

All of the samples, including candy and tomato sauce, were purchased from a local market. Using 10 mL of double-distilled water dissolved suitable amounts (0.1–1g) of the samples. The candy and tomato sauce solutions were warmed at 50 °C for 10 min to completely dissolve the candy and tomato sauce in water and were sonicated for 10 min at room temperature. The samples solutions were diluted to 50 mL in a volumetric flask using NaOH solution and the pH of the solutions was adjusted to 11.0. using a folded Xinhua paper filter (No. 102) filtered these samples solutions, and the filtrates were collected after discarded the first 15 mL.

Calibration of the preconcentration factor and extraction recovery percentage

To evaluate the performance of the proposed method, we determined the extraction recovery percentage (ER%) and preconcentration factor (EF) using HPLC[25] and calculated them based on Eqs. (1) and (2):

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

where C_{sed} represent the concentration of the analytes in the enriched phase, C_0 represent the initial concentration of analytes in the sample solution.

$$\mathbf{R} = \frac{\mathbf{C_{sed}} \times \mathbf{V_{sed}}}{\mathbf{C_0} \times \mathbf{V_{aq}}} \times \mathbf{100\%} = \mathbf{EF} \times \frac{\mathbf{V_{sed}}}{\mathbf{V_{aq}}} \times \mathbf{100\%} \quad \textbf{(2)}$$

where V_{sed} and V_{aq} are the volumes of the enriched phase and sample solution, respectively.

RESULTS AND DISCUSSION

Optimization of extraction parameters

In this experiment, several mian factors, including the Selection of IL, amount of KPF_6, sample Solution pH, the extraction time and centrifugation time, were studied to achieve the best extraction efficiency for ERY. Approximately 10 mL of water was added to 70 ng mL^{-1} of ERY to research of the extraction efficiency of the ISILF–LLME under different extraction parameters. All the experiments were per-

formed in triplicate and the means of the experiments results were used for optimization.

Selection of IL

In ISILF–LLME, when a water-miscible IL and KPF_6 which was an ion-pairing agent were reaction, a water-immiscible IL should form. Some properties of the hydrophobic IL should be taken into account, such as good extraction capability of the analysis, water-immiscible, higher density than water, to enable the hydrophobic IL to settle at the bottom of the plastic centrifugal tube.

In the current study, four water-miscible ILs, namely, 1-hexyl-3-methylimidazolium chloride ([C_6MIM][Cl]), 1-hexyl-3-methylimidazolium bromide ([C_6MIM][Br]), 1-octyl-3-methylimidazolium chloride ([C_8MIM][Cl]), and 1-octyl-3-methylimidazolium bromide ([C_8MIM][Br]) were investigated. The cloudy solution formed easily for these ILs. The experimental results showed that [C_8MIM][Br] was the best extraction solvent among the four ILs used. This IL was employed as the water-miscible IL in the subsequent experiments.

The amount of [C_8MIM][Br] used in the pre-concentration procedure was a main factor in obtaining high extraction efficiency. Thus, the extraction process was researched to define the lowest volume of the IL-phase necessary to achieve the highest extraction efficiency. The effect of IL concentrations on the recovery rate of ERY was investigated by increasing the amount of [C_8MIM][Br] from 50 μL to 350 μL. The highest fluorescence was obtained in the solution containing 100 μL of [C_8MIM][Br] as shown in Figure 1. Increasing the amount of [C_8MIM][Br] led to decreased concentrations of ERY which could be attributed to the formation of a higher volume of [C_8MIM][PF_6] formed. Lower amounts of [C_8MIM][Br] resulted in decreased recovery. Which could be due to the incomplete extraction into small [C_8MIM][PF_6] drops. Thus, 100 μL of [C_8MIM][Br] was selected for further experiments.

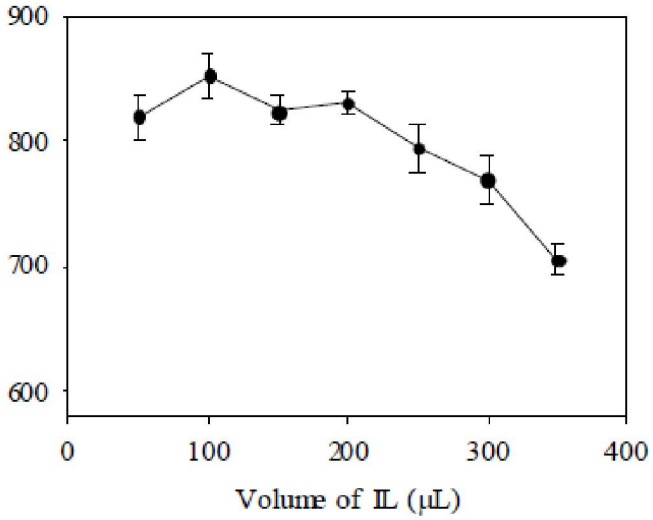

Figure 1 : Effect of volume of Ionic liquids. Extraction conditions: concentration of ERY, 70 ng mL^{-1}; KPF_6, 40 mg; pH, 11.0; standing, 3 min; centrifuged, 10 min; rate, 3500 rpm (1685×g); volume of 200 μL

Effect of KPF_6

The amount of KPF_6 was investigated from 20 to 80 mg containing 100 μL [C_8MIM][Br]. The results are shown in Figure 2. Adding KPF_6 to [C_8MIM][Br] caused the formation of [C_8MIM][PF_6]. Increasing the amount of KPF_6 increased the volume of [C_8MIM][PF_6] that led to an increase in ER. When the quantity of KPF_6 was raised to 40 mg, the ER was at its maximum and then leveled off with further increase of KPF_6. Thus, the final quantity of KPF_6 used in the subsequent experiments was 40 mg.

Extraction conditions: concentration of ERY, 70 ng mL^{-1}; IL, 100 μL; pH, 11.0; standing, 3 min; centrifuged, 10 min; rate, 3500 rpm (1685×g); volume of 200 μL.

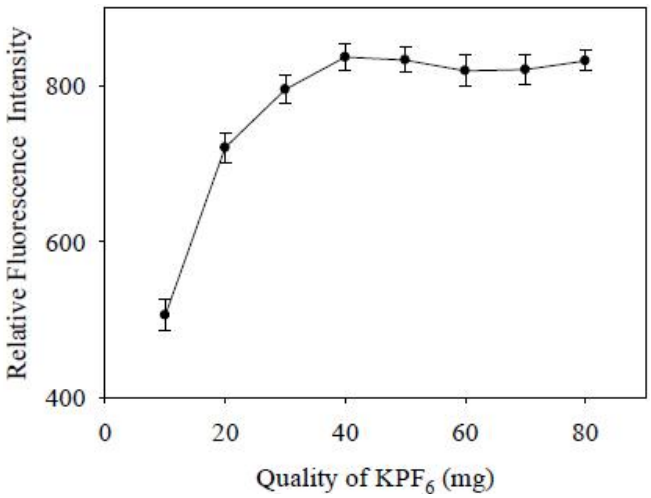

Figure 2 : Effect of the amount of KPF_6

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Influence of sample pH

Sample pH was an important factor for in extracting the analytes by affecting the existing forms of the target compounds, which should be adjusted to ensure that the analyte is electrically neutral so that it can be efficiently extraction. Influence of sample pH on the recovery was studied pH range from 1.0 to 12.0 using HCl and NaOH. The result is presented in Figure 3. The best relative fluorescence intensity was obtained at pH 11.0. Thus, the pH value 11.0 was selected for further experiments.

Extraction conditions: concentration of ERY, 70 ng mL^{-1}; IL,100 µL; KPF_6, 40 mg; standing, 3 min; centrifuged, 10 min; rate, 3500 rpm (1685×g); volume of 200 µL.

Influence of extraction time

The cloudy solution easily formed at room temperature; thus, the temperature of 25ºC was adequate for the extraction process. In this study, extraction time refers to the time from that time the ion-paring agent was added to solution to the time before centrifugation was initiated. The Influence of extraction time were investigated in detail, extraction time was examined from 0 to 10 min at 25ºC. The results indicate that extraction time had no significant effect on the relative fluorescence intensity. Relative fluorescence intensity was basically unchanged within 3 10 min. Thus, the extraction time of 3 min was used in the subsequent experiments.

Influence of centrifuge time

Centrifugation is one of the prime steps that influence the extraction of the target compounds. After a centrifugation time, the IL separate well from the aqueous phase. Centrifugation times varying from 5 min to 15 min were applied to determine the optimum centrifugation time that could achieve the best extraction efficiency as shown in Figure 4. At 10 min, ER became basically unchanged, indicating the IL phase complete transfer to the bottom of the centrifugation tube. Thus, a centrifugation time of 10 min was selected.

Extraction conditions: concentration of ERY,70 ng.mL^{-1}; IL,100 µL; pH, 11.0; KPF_6, 40 mg; standing, 3 min; rate, 3500 rpm (1685×g); volume of 200 µL.

Excitation and emission spectra

Excitation and emission spectra of the ERY were obtained as shown in Figure 5. Excitation and emission wavelengths of ERY were observed at 540 and 560 nm, respectively. In addition, reagent blanks have no effect on the determining ERY; thus, wavelengths of 540 and 560 nm were selected as the excitation and emission wavelengths.

Excitation and emission spectra of ERY after extraction (1); excitation and emission spectra of ERY in solution (2), concentration of ERY, 70 ng. mL^{-1}.

Effect of potential interfering species

Aliquots of aqueous solutions includeing 70 ng mL^{-1} ERY and certain numbers of other chemical species were obtained, and the proposed process was applied to study to selective separation and de-

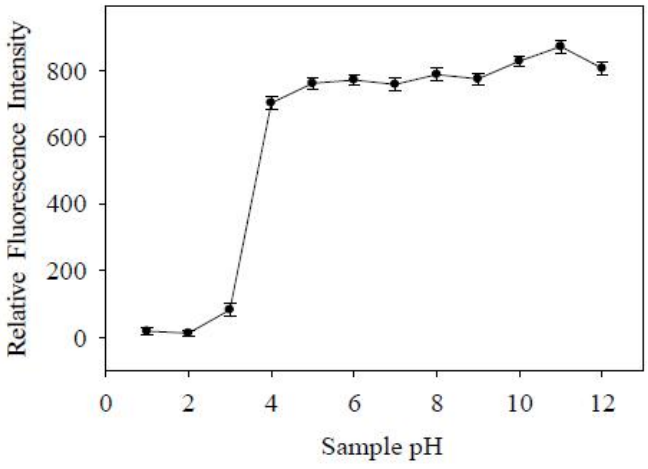

Figure 3 : Influence of sample pH

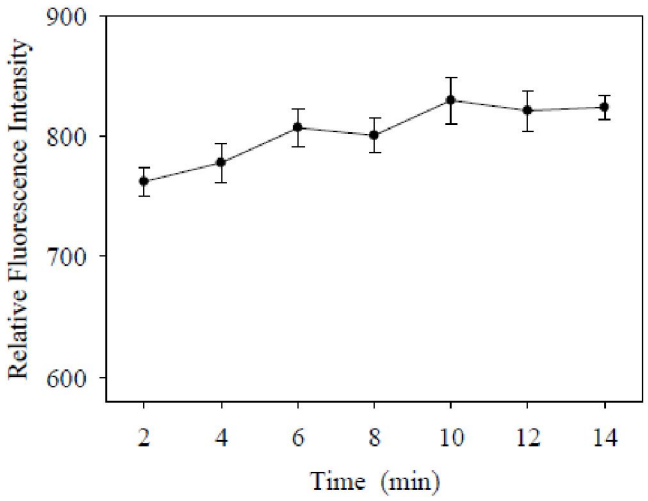

Figure 4 : Effect of centrifuge time

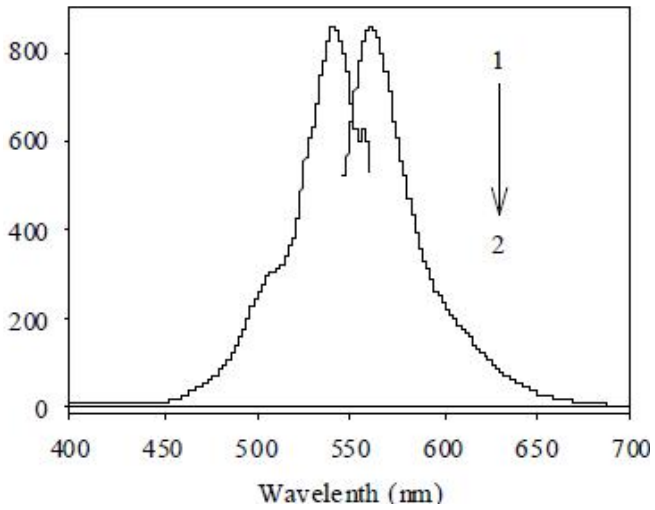

Figure 5 : Excitation and emission spectra

terminate for ERY in food samples containing various chemical species, particularly in other legitimate uses of synthetic food colors. The tolerance limit was defined as the concentration of the added interfering substance that caused less than ±5% relative error in the determination of the ERY. Several substances used, namely, Cu^{2+}, Cd^{2+}, Fe^{3+}, Co^{2+}, and F^- at 500-fold; Na^+, Zn^{2+}, K^+, Ca^{2+}, Mn^{2+}, Mg^{2+}, NH^{4+}, HPO_4^{2-}, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}, I^-, SCN^-, Br^-, Cl^-, glucose, starch, α-lactose, sucrose, citric acid, dextrin, and sodium cyclamate at 1000-fold, and common synthetic food colorants, namely, tartrazine and sunset yellow at 50-fold; amaranth, new red and brilliant blue at 10-fold, ponceau 4R at 5-fold and allura red at 1-fold, did not interfere with the determination of ERY, indicating the high selectivity of the method.

Method validation

Linear range, precision, and the limit of detection for ERY under the optimum extraction conditions, the linear ranges were investigated from 0.2 to 80 ng mL^{-1} for ERY. The calibration curve obtained was y = 6.97 + 51.83 c, where y is the fluorescence intensity and c is the concentration of ERY in ng.mL^{-1}. The calibration curves gave a high level of linearity, correlation coefficient of 0.9996. The ER% and EF of ERY were 91.3%, and 45.7, respectively. The relative standard deviation (RSD) of the method, which was determined by analyzing the standard solution at 100 ng mL^{-1} of ST (n=11), was 2.25%. The lower detection limit (LOD) was 0.055 ng mL^{-1} which was determined based on the IUPAC recommended formula LOD= KS_0/S, where K used is 3, n = 11, S_0 is the standard deviation of the blank, and S is the slope of the graph.

Comparison with other methods

The determination of ERY by ISILF–LLME combined with fluorescence was compared with other analytical methodologies are presented in TABLE 1. The proposed method exhibited relatively low LOD compared with previously reported techniques; Moreover, IL was the extraction phase instead of toxic organic solvents. All these results indicated that ISILF–LLME is a highly sensitive, environmentally friendly, and low cost technique that can be used in the pre-concentration of ERY from real food samples.

Sample analysis

To further validate the feasibility of the developed method, it was satisfactorily used for the determination of ERY in candy and tomato sauce obtained from a local market. The accuracy of the method was assessed through recovery studies, which were conducted by spiking the known amounts of ERY into the samples whch were five replicate and the results were given. TABLE 2 shows that the

TABLE 1 : Comparison with other microextraction techniques

Determination technique	Linear range (ng mL^{-1})	LOD (ng. mL^{-1})	Reference
RRS	19-10000	5.6	[20]
IL–DLLME–HPLC/UV	1.0-2000	0.32	[25]
CL	700-50000	30	[26]
Spectrophotometry	1800-4200	20	[27]
CE–LIF	0.4-450	0.4	[17]
SPE–Fluorescence	0.88-19.80	0.49	[31]
ISILF–LLME Fluorescence	0.2-80	0.055	This Work

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TABLE 2 : Determination of erythrosine in food samples. [a] Mean of five determinations ±standard deviation

Samples	Concentration (ng mL)	Spiked (ng mL)	Recovery[a] (%)	Content of ERY (mg/kg)
Gel candy	8.2	10	102.3±2.3	4.1
Fruit juice jelly	2.5	5	98.1±3.1	12.5
Cotton-candy	0.24	1	95.2±2.5	0.12
Tomato sauce	6.3	10	91.2±2.9	31.5

recovery of spiked samples is satisfactorily reasonable, indicating the capability of the method to determine ERY.

CONCLUSION

This research proved that the developed ISILF–LLME method exhibited an excellent green sample pre-concentration technique. The ISILF–LLME method combined with fluorescence was successfully applied for the pretreatment and determination of ERY in candy and tomato sauce. $[C_8MIM][PF_6]$ was chosen as the green IL which was instead of the traditional toxic organic solvent being used in this extraction system. The proposed method was a excellent enrichment performance, simple, stabile, easy operation, minimal cost and consumption of organic solvents technique. The LOD obtained for ERY was 0.055 ng mL^{-1}, which indicated that the developed method had high sensitivity in the analysis of real samples. Common synthetic food colorants did not interfere with the determination of ERY using the developed method. The excellent spiked recoveries of ERY in candy and tomato sauce indicated that ISILF–LLME combined with fluorescence would be a potential to be applied for the analysis of food samples in the future.

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