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Deep eutectic solvent (DES) based dispersive Liquid-Phase microextraction of Sunset yellow FCF in food and pharmaceutical products

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ABSTRACT

In this study, a new, sensitive, rapid, simple, cheap, readily available, and rather safe dispersive liquid–liquid microextraction method (DLLME) utilizing a deep eutectic solvent (DES) as the green extraction solvent was developed for the extraction and determination of sunset yellow for Coloring Food (FCF), in drugs, vitamins, beverage, foods, and environmental samples. The method is based on the deep eutectic solvent system containing a kind of cationic and anionic (hydrogen bond donor/ hydrogen bond acceptor) species. For this purpose, decanoic acid and tetrabutylammonium bromide (molar ratio of 2:1) were used to obtain a deep eutectic solvent. Sunset yellow FCF is analyzed with a UV–Vis spectrophotometer after ultrasonication-assisted dispersive liquid-phase microextraction procedure. The essential operational parameters such as pH, DES volume, THF volume and sample volume were found 2.0, 200, 400 μL and 20 mL, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were found at pH 2.0 as 0.05 μg L^{-1} and 0.17 μg L^{-1}, respectively. The enrichment technique was validated by using addition/recovery studies and applied to the determination of analyte content of various drugs, vitamins, beverages, foods, and environmental samples.

1. Introduction

Food additives, which have more than 2500 types in the global market and with or without nutritional value, are added to several food products for coloring, sweetening, stabilizing, obtaining charming, nutritional enrichment, texture improvement, durability, and increasing food safety [1,2]. According to the purpose of utilization, food additives are classified as nutritive value enhancers, thickeners, emulators and stabilizers, surface conditioners, sweeteners, flavor enhancers, flavorings, antioxidants, dyes, chemical preservatives.

Food dyes using to make food stand out, are one of the most popular food additives. The food dyes are classified into three groups of natural, synthetic, and semisynthetic. The natural food dyes which are used to color many foods are derived from a variety of plants such as grapes, saffron, turmeric, paprika, carrots, beets, and algae [3]. On the other hand, synthetic dyes are produced chemically. Because of their low cost and increasing commercial need, synthetically produced ones are commonly opted. Synthetic dyes are manufactured and used increasingly in various industries such as textiles, pharmaceutical, plastics, food, printing, paper, leather, and cosmetics [4–8].

Some food additive (dyes) materials excessive use has toxic effects owing to the existence of functional groups in their structures such as the aryl and azo (– N = N–) [4,9,10]. Over intake of synthetic colorants into the body causes food intoxication and shows toxic effects [11]. Due to the toxic effect of synthetic dyes, they have various negative health effects such as cancer, genetic diseases, asthma, anaphylactic reactions, cardiovascular complications, weakening of the immune system, and allergic reactions [12–16]. It is also thought that these dyes contribute to some adverse effects on several behavioral developmental periods such as hyperactivity and some aggressive behaviors among children [17–20]. In addition, they cause structural and functional deterioration by directly binding to DNA [21,22].

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Sunset yellow, which is from the azo dye group, has been also entitled as disodium salt and disulfonates (chemical formula, $C_{16}H_{10}N_2Na_2O_7S_2$). In other words, it contains two sulfonate groups connected to both ends of the molecule (two central aryl groups: naphthyl and phenyl rings) which are bonded by an azo bridge (N = N)) (Fig. 1.). Sunset yellow is a reddish-orange that is commonly produced synthetic dye. It is utilized to improve the appearance and texture of some substances such as fruit juices, cereals, candies, jelly candy, chocolates, cakes ice cream, ready-made soups, drinks, powdered juices, pastry, desserts, snacks, dairy products, yogurts, drugs, vitamins, fillings, liqueurs, puffs, pastilles, jams [23–25]. According to the Food and Agriculture Organisation (FAO), and World Health Organization (WHO) admissible daily intake (Average Daily Intake) dose range of Sunset yellow FCF is 0 to 2.5 mg kg^{-1} [4,14,16,24–31].

Some studies illustrated that sunset yellow is caused the allergic reaction in people who have an intolerance to benzoic acid and aspirin. In many studies, it was found that overuse of sunset yellow FCF (SY-FCF) cause some illness such as asthma, migraines, eczema, urticaria, angioedema, anxiety, diarrhea, gastric problem, asthmatic illness, and stomach upset [24–27,32–35]. In addition, SY- FCF may lead to potential liver injuries due to some disruptive effects on the body's total lipid storage [29–39]. Because of these hazardous effects, some European countries such as Finland, and Sweden have banned this dye use [40].

Since SY-FCF is progressively used in industrial areas like food and pharmaceutical and damaging to living beings, it is considered to improve a rapid, simple, cost-effective procedure for the analysis and separation of it in some pharmaceutical, beverages and ecological samples [20]. For this purpose, there are many analytical methods high-performance liquid chromatography (HPLC), electrochemical sensors, UV–vis spectrophotometry, mass spectrometry, capillary electrophoresis, fluorescence, planar chromatography, and immunological assay have been utilized for the detection of SY-FCF [9,40–43].

Preconcentration methods are used to prevent the matrix effects and bring a measurable level of the trace amount of dye in drug, environmental, cosmetic, toy and food samples. Some of -these methods divided into liquid–liquid extraction (LLE) and solid-phase extraction (SPE) [9,40–43]. Some microextraction methods were hollow-fibre membrane liquid-phase microextraction (HF-LPME), solidified floating organic drop microextraction (SFODME), solid-phase microextraction (SPME), headspace liquid-phase microextraction (HS-LPME), solvent bar microextraction, single-drop microextraction (SDME), ultrasonic assisted microextraction, dispersive liquid–liquid microextraction (DLLME) [4,44–48]. Deep Eutectic Solvent (DES), a new eco-friendly solvent has some advantages that used instead of conventional solvents (i.e. carbon tetrachloride, dichloromethane etc.) and DES's are also used at very low volumes in extraction studies [49–50]. In Addition, phase separation carries out at extraction studies very fast and simple. DES has been developed for extracting SY-FCF in real samples. DES is a kind of dispersive liquid-phase microextraction procedure, consisting of a hydrogen bonding acceptor and hydrogen bonding donors.

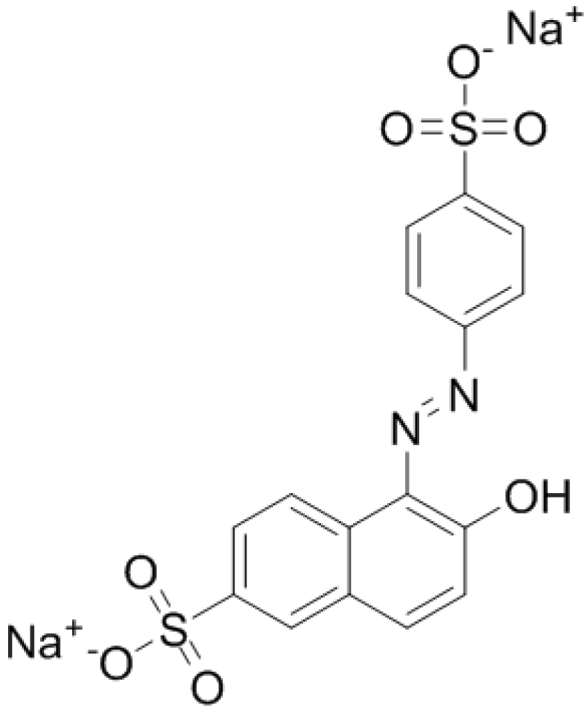

Fig. 1. Sunset Yellow FCF.

In this work, a new, simple, and rapid ultrasonic-assisted deep eutectic solvent-based dispersive liquid-phase microextraction (UA-DLLME-DES) integrated with UV– Vis Spectrophotometry for the determination of some drugs, foods, tablets of vitamins samples. The method was applied to the determination of analyte content of several drugs, vitamins, beverages, foods, and environmental samples.

2. Experimental

2.1. Instrumentation

An ultraviolet–visible spectrometer (UV–Vis spectrometer) from Perkin-Elmer (Lambda 25; Norwalk, CT, USA) was utilized to measure SY-FCF concentrations. HANNA Instruments HI 2211 pH/ORP Meter (Woonsocket, Rhode Island, USA) made with glass electrode was used to measure pH values. Distilled water using throughout the entire study was obtained from Nüve Water Distiller ND-4 (Ankara, Türkiye). In addition, a model Nüve NF400 (Ankara, Türkiye) centrifuge was utilized. [51].

2.2. Reagents

The SY-FCF dye stock solution (1.0×10^{-3} M) had been prepared in water to optimize experimental parameters. Also, working and standard solutions were arranged by diluting the SY-FCF solution. The range of pH 2–3, 4–5, 6–7 and 8 buffer solutions were formulated from phosphate ($H_3PO_4/H_2PO_4^-$), acetate (CH_3COO^-/CH_3COOH) and ammonium (NH_4^+/NH_3), respectively. Deionized water was utilized for preparing of all chemicals reagents and analytical grade solutions.

The deep eutectic solvents used in our developed method were prepared by mixing investigated amounts of two chemicals at a certain temperature. In this paper, decanoic acid (DA) and tetrabutylammonium bromide (TBA-Br) were used for formulating DES at different ratios (1:2, 1:3 and 1:4). (TBA-Br: DA).

2.3. Ultrasonic assisted dispersive Liquid-Liquid microextraction method based on deep eutectic solvent procedure (UA-DLLME-DES)

Ten mL model solution was prepared adding 1.0×10^{-5} M SY-FCF and DES at pH 2.0 for optimizing the UA-DLLME-DES method. Initially, 1 mL buffer solution and 1.0×10^{-5} M SY-FCF were added to 50 mL standard solution. All solutions were adjusted to pH 2 in order to DES and analyte interaction. After pH regulation, 200 μL DES was added to the sample solution and then it was vortexed for 30 s to get a homogeneous solution. After that the solution, adding -400 μL THF, was dispersed in an ultrasonic bath for 3 min. to make a cloudy solution. Owing to the ultrasonication procedure, DES aggregates slowly came apart to nano-sized. After this step the solution, was ultrasonicated, was centrifuged for 5 min at 4100 rpm. Thus, the mixture was separated into two phases with the aid of centrifugation procedure. These phases consist of water and DES. Further, the DES phase contains an analyte (SY-FCF). Then the phases were separated by injection syringe and the water phase was discarded. Eventually, ethanol was added to the last

solution remaining in the tube, and measurements of absorbance were made by UV– Vis Spectrophotometer at 485 nm.

2.4. Preparation of samples for UA-DLLME-DES

The UA-DLLME-DES method was applied to some food samples and some drugs used in certain treatments to the feasibility and accuracy of the developed method assessment. In addition, the UA-DLLME-DES for addition/recovery has been carried out to drinking water under the optimum conditions.

Firstly, antirheumatics/anti-inflammatory syrup orange-colored, orange-scented, flowing homogeneous suspension, pain reliever, antipyretic and anti-inflammatory syrup, and also anti-inflammatory syrup were analyzed. For this reason, 5 mL of each of these syrup samples were taken into different tubes and ethyl alcohol was added to them. This mixture was stirred in the shaker for 1 h then the centrifugation process was applied to them. Then, 100 μL were added to each tube in three duplicate and the proposed UA-DLLME-DES microextraction procedure was applied to them.

On the other hand, two different vitamin C tablets were dissolved by using distilled water and the proposed method was applied by taking 100 μL into tubes for SY-FCF analysis. UA-DLLME-DES procedure was applied to the drinking water sample for testing the accuracy of the developed method. SY-FCF was added increasing concentrations in these water samples. Then UA-DLLME-DES microextraction method was applied. Consequently, SY-FCF concentrations in these drugs and tablets samples were analyzed by UV–Vis spectrophotometer.

3. Results and discussion

3.1. Effect of pH

In extraction methods for dyes species, pH of an aqueous solution is a critical step for quantitative recovery of analytes. The pH of model solutions containing SY-FCF was adjusted at the range of 1.0 – 8.0 to investigate the effects of pH on the recoveries of analyte. The recoveries of SY-FCF at the pH 2.0 was found quantitative (greater than95%). The experimental results illustrated that recovery values were decreased before pH 2.0 and also after pH 6.0. Because of the transition of sunset yellow FCF to a less charged form as the pH rises the recovery values were decreased. Also, analyte and DES interaction may decrease due to increased solubility of decanoic acid in strongly basic solutions. The results were illustrated in Fig. 2.

The recovery of SY-FCF was quantitative at pH 2.0. So, all following parameters were arranged at pH 2.0 by means of acetate buffer [11]. The mechanism of DES, which consists of TBA-Br and DA, is explained by the hydrogen bond formed between them. As can be seen from the results tabulated in the table, as the pH values increases, DA loses hydrogen in the carboxyl group. This causes the structure of DES to deteriorate. Therefore, subsequent experimental studies were done at pH 2.0.

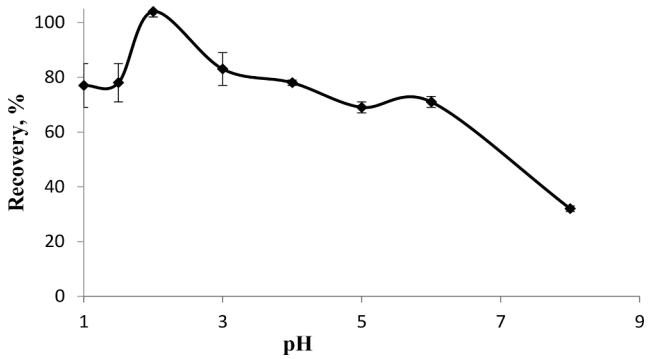

Fig. 2. Effects of pH on method on the recovery of Sunset Yellow FCF (N = 3).

3.2. Effect of the volume ratio of aprotic solvent

In this UA-DLLME-DES method, tetrahydrofuran (THF) was utilized as a aprotic solvent to separate DES from the aqueous phase and preconcentrate the DES phase. We studied the effect of THF volume on the recovery of the SY-FCF, which should be present in the DES, [51]. For this reason, the DLLME-DES procedure was done in which the volume of DES was fixed to 200 μL and the volume of THF was increasingly changed. Between 200 and 600 μL volume of THF was quantitative. The optimum volume of THF was chosen 400 μL. When dispersing agent THF was used less than 400 μL, this volume was being in sufficient for DES-analyte phase separation. In addition, the phase separation wasn't occurred in high level THF medium. All results for recovery of analyte were shown in Fig. 3. As can be understood from the results, subsequent experiments were studied using 400 μL THF and 200 μL DES.

3.3. Effect of DES ratio and volume

To obtain high recovery values for the SY-FCF ratio of DES components should be investigated. For this reason, DESs based on decanoic acid were studied [50]. DESs, which were in green chemistry approach, were prepared by mixing TBA-Br and DA at different ratios as 1:2 (DES_1), 1:3 (DES_2), and 1:4 (DES_3). The highest recovery values in DESs prepared with hydrogen bond acceptors and fatty acids as hydrogen bond donors mixed at 1:2, 1:3, and 1:4 ratios were obtained at the ratio of 1:2 (DES_1) as seen in Fig. 4.

Moreover, the effect of the volume of DES was prepared at this rate on the recovery values of SY-FCF was also investigated. For this aim, 100, 200, 300, 400, and 500 μL volumes of DES were added to the model solutions, and the developed procedure was applied. Fig. 5 was shown that quantitative recoveries were obtained when 200 μL DES was added to the aqueous sample solution. So optimum DES volume was chosen as 200 μL for further experiments.

3.4. Effect of sonication and centrifugation time

In many extraction methods, physical mixing procedures such as microwave, shaking by vortex, and sonication are applied to sample solutions for better-extracting analytes into an extractor [52–56]. We used sonication for mixing model solutions including analytes. So, the sonication time using to make up micelle in the ultrasonic bath and the centrifugation process time were examined in this section. The UA-DLLME-DES microextraction method was utilized at pH 2.0 for all model solutions.

Firstly, effect of sonication time was studied to determine the duration of stay in an ultrasonic bath on the recovery values of SY-FCF. The developed method was applied to various sample solutions to evaluate this parameter. Then, DA and THF were added to the samples were

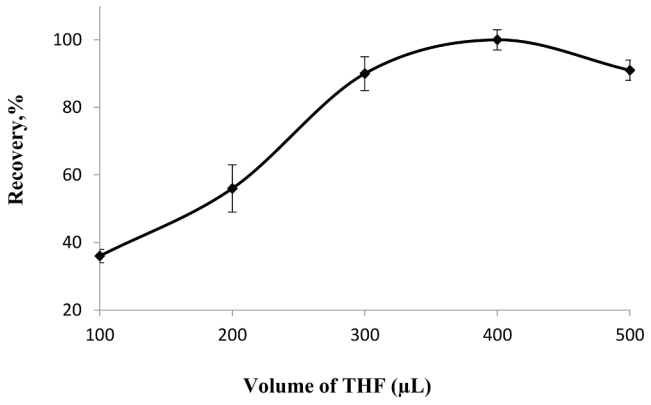

Fig. 3. The effect of THF on the recovery yield efficiency of the developed method (N = 3).

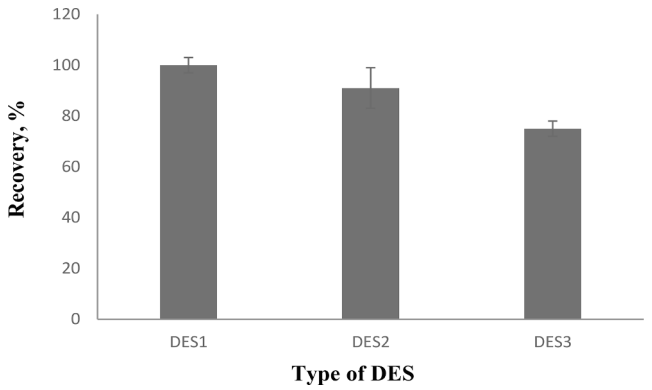

Fig. 4. Influences of DES type on the recovery value of the method (N = 3).

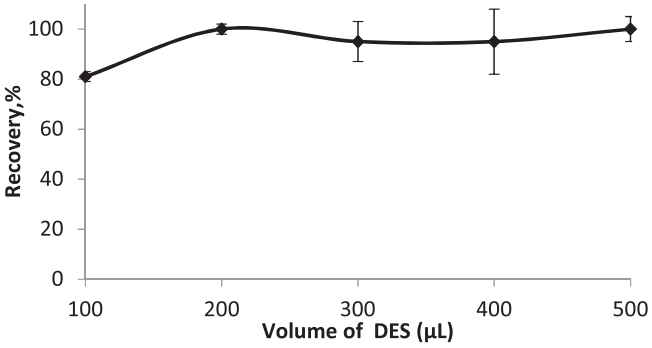

Fig. 5. Effects of DES volume on the recovery value of the method (N = 3).

sonicated for 2–8 min to obtain cloudy solutions. Quantitative recovery values were acquired after 3 min (Fig. 6).

We studied centrifugation time, effecting to separate phase was investigated in this part. For achieving quantitative recovery of SY-FCF centrifugation time was studied by setting in the range of 3–10 min. As shown in Fig S1. a centrifugation time and speed of 5 min and 4.100 rpm, respectively, were decided on optimum values for preconcentration of SY-FCF.

3.5. Effect of sample volume

To obtain high preconcentration factor sample volume is a critical parameter in extraction studies [57–61]. The effect of increasing sample volume on the preconcentration of SY-FCF was investigated between 5.0 and 30.0 mL of sample volume. Quantitative results of up to 20 mL have been found, as shown in Fig S2. Even though the recovery values of SY-FCF at a 20 mL sample volume are quantitative, they are getting to decrease when the sample volume is above this volume value. So, the maximum sample volume was chosen at 20 mL for the UA-DLLME-DES method for preconcentration of SY-FCF. The preconcentration factor was calculated on ratio of the maximum sample volume and the final volume. Due to the final volume being 0.5 mL preconcentration factor was calculated as 40.

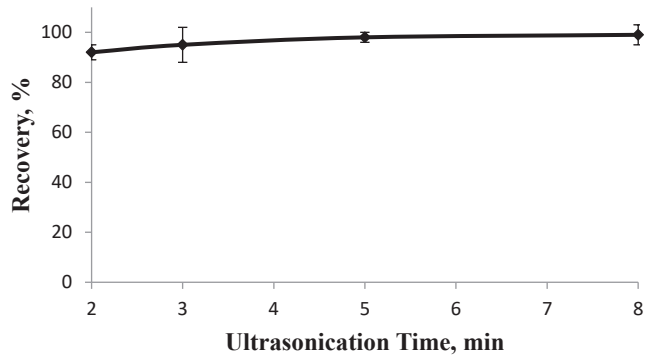

Fig. 6. Influences of ultrasonic bath time on the recovery value of the method (N = 3).

3.6. Matrix effects

The matrix effects of foreign ions and dyes is one of the most critical parameters in preconcentrating of organic or inorganic species [52–66]. Also, this section determines the selectivity of the developed method. That's why, some dyes such as Eosin B, CFB, Methylene blue, RBV-5, Sudan I, Chromotrope FB, Lissamine Green B, Chicago Sky Blue 6B and alkaline, earth alkaline, some cations such as Co^{2+}, Zn^{2+}, Al^{3+}, Na^{+}, Ca^{2+} causing matrix effect in the UV–Vis spectrophotometric determinations trace level of SY-FCF were studied. As a result, in this section, the tolerance concentrations of the organic and inorganic species mentioned. All the recovery results are illustrated in Table S1. The non-quantitative recovery of Sunset yellow with Methylene Blue and Co^{2+} at their concentration levels given in Table S1 was related with their extraction to presented DES media. In the lower levels of these matrix components, the quantitative recoveries were obtained.

3.7. Analytical performance

In this part, the proposed UA-DLLME-DES method was applied for enrichment and extraction of Sunset Yellow at increasing concentrations. Analytical performance values such as limit of quantification (LOQ), limit of detection (LOD), preconcentration factor (PF), relative standard deviation (RSD), correlation coefficient (R^2), linear range (LR) for extraction of SY-FCF were calculated under the optimal conditions mentioned above.

Some criterions such as a limit of detection (LOD) ($3S_b$/m formula, Sb: standard deviation of ten blank solutions, m: the slope of the calibration curve) and a limit of quantification (LOQ) ($10S_b$/m formula) were calculated with a preconcentration factor as 0.05 µg L^{-1} and 0.17 µg L^{-1} for UA-DLLME-DES microextraction technique, respectively. In addition, the relative standard deviation (RSD), determined was (RSD = s/x) 4.1 %. The correlation coefficient for the good linearity was 0.9977 According to this value linear equation was found as A = 6.0313C + 0.0505 (A: Absorbance and C: Concentration of SY-FCF).

3.8. Application of real samples

The UA-DLLME-DES method was applied to some food samples and some drugs used in certain treatments to the feasibility and accuracy of the developed method assessment. In addition, the UA-DLLME-DES for addition/recovery has been carried out to drinking water under the optimum conditions. SY-FCF concentrations in these drugs and tablets samples were analyzed by UV–Vis Spectrophotometer Herein, all results were tabulated in Table 1, Table 2., Table S2 and Table S3.

Table 1
Addition/recovery studies of SY-FCF in water samples (pH: 2.0, N = 3).

Samples	Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery, %
Drinking water	0.0	N.D.[a]	-
	0.23	0.20 ± 0.05[b]	87
	0.68	0.69 ± 0.2	101
	1.15	1.16 ± 0.2	101

[a] N.D.: Not detected
[b] Mean ± standard deviations.

Table 2
Application of present method to drugs samples for determination of SY-FCF (pH: 2.0, N = 3).

Samples (Drugs)	Found (μg mL^{-1})
Syrup 1 (anti-inflammatory)	0.98 ± 0.02 [a]
Syrup 2 (anti-inflammatory)	0.79 ± 0.03 [a]
Syrup 3 (anti-inflammatory)	1.00 ± 0.02 [a]

[a] Mean ± standard deviations.

4. Conclusions

A simple and very fast UA-DLLME-DES microextraction method was studied and applied for the analysis of SY-FCF in beverage products, drugs, and vitamin tablets as well as drinking water. The applied method offers significant advantages in terms of selectivity, practicality simple application, and analytical precision, taking a short time from sample preparation to analysis, saving an organic solvent, and low toxicity. The DES used in this study consisted of tetrabutyl ammonium bromide and decanoic acid. DES's are less harmful to the environment than conventional organic solvents and is used in low volumes. DES's also has some superior properties such as low vapor pressure, high thermal stability, biodegradation and biocompatibility. Therefore, it is an environmentally sensitive method that is called green chemistry. In this preconcentration study, quantitative recovery values above 95 % were acquired at optimum conditions. In addition, the results were shown that the accomplished method can be correctly applied to several real-world samples (Table 2., Table S2 and Table S3) for both addition/recovery (Table 1) and direct analysis studies. That's why the procedure is suitable for the determination of SY-FCF in food, vitamin tablets, drugs, and water samples. On the other hand, the developed UA-DLLME-DES microextraction method for determination of trace level of SY-FCF was compared with other extraction procedures from a literature review. There are a few scientific papers of separation/preconcentration for this dyestuff, have been found in the literature.

According to the literature reviews as illustrated in Table S4, the UA-DLLME-DES microextraction method is the fastest among all the other procedures in the table [67–72]. Also, low limit of quantification (0.17 μg L^{-1}) and also limit of detection (0.05 μg L^{-1}) were obtained without spending much time.

CRediT authorship contribution statement

Nebiye Kizil: Methodology, Visualization, Supervision, Writing – review & editing. **Erkan Basaran:** Methodology, Visualization. **Duygu Erbilgin:** Methodology, Visualization. **Mehmet Lütfi Yola:** Methodology, Visualization. **Furkan Uzcan:** Methodology, Visualization. **Mustafa Soylak:** Methodology, Visualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2022.107734.

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