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Spectrophotometric Determination of Sunset Yellow (E-110) in Powdered Beverages and Pharmaceutical Preparations after Cloud Point Extraction Method

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Abstract: In this study, Brij 58 was used for the spectrophotometric determination of sunset yellow (SY) (E-110) in pharmaceutical preparations and powdered beverages after cloud point extraction (CPE). Certain parameters such as pH, surfactant concentration, extraction time and temperature, speed of centrifugation, and salt concentration were optimized. Linear range in the optimum conditions was 0.01 – 4.00 μg mL^{-1} and the correlation coefficient was 0.9995. The limit of detection (LOD) and the limit of quantification (LOQ) of this method were 0.0078 μg mL^{-1} and 0.0261 μg mL^{-1}, respectively.

Keywords: Brij 58; Sunset Yellow (E-110); Cloud Point Extraction (CPE); Spectrophotometric Determination; Surfactant

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INTRODUCTION

Food dyes are used to provide more attractive, appetizing appearances to enhance the taste, flavor, and color of foods (1). Food coloring Sunset Yellow (6-hydroxy-5- [(4-sulfophenyl) azo] -2-naphthalenesulfonic acid disodium salt) (SY) (E-110) is a food additive commonly used in foods, pharmaceuticals, and cosmetics. Nevertheless, it is vital that SY is one of the synthetic dyes most likely present in widespread food products that may lead to allergies, diarrhea, anxiety migraines and also could harm to the kidney and the liver if it is consumed to the extreme (2-4). It is also known as Orange Yellow S, INS No.110 and CI Food Yellow 3. Acceptable daily dose of SY, an azo dye, is 2.5 mg kg^{-1} (5). Nowadays, the use of such additives is taking much attention owing to a series of health risks induced by food dyes, since it plays an indispensable part in human public health (6). For this reason, it is imperative to make and monitor the identification of SY and similar azo dyes in various samples.

Several methods such as high performance liquid chromatography (HPLC) (7), capillary electrophoresis (CE) (8), thin layer chromatography (TLC) (9), voltammetry (10), and spectrophotometry (11) have been given in the literature for the determination of SY. These analytical methods require the use of harmful solvents especially for laboratory workers and the environment (12).

The cloud point extraction (CPE) method is also an alternative to other enrichment and separation methods as simple, sensitive, inexpensive, non-polluting, and environmentally sensitive (13). The aqueous solutions of nonionic surfactant materials become cloudy when their temperature reaches the cloud point temperature and analyte collapses with a small volume by a surfactant. The CPE procedure can also be termed as temperature induced phase separation or micelle-mediated extraction (14-16). Thus, CPE can lead to high recovery efficiency and a large preconcentration factor due to a very small volume of micellar phase binding the analyte that has been dispersed in the original matrix (17). CPE is not only preconcentration procedure but also a separation method.

Non-ionic surfactants such as Triton X-100 (18), Triton X-114 and Brij 56 (19) have been used for the determination of SY. To the best of our knowledge, there is no enrichment of the SY with CPE method using Brij 58, another non-ionic surfactant, in the literature.

In this study, it was aimed to develop a new method of CPE using Brij (polyethylene glycol hexadecyl ether) 58 for the first time the spectrophotometric determination of SY.

EXPERIMENTAL

Instrumentation

Absorption spectra and absorbance measurements have been made by an Agilent (Stevens Creek Boulevard, Santa Clara, CA, USA) Transport 60 UV spectrophotometer. 1.00 cm light-beam quartz cells have been used in these measurements. To measure the pH of the solutions, a ThermoOrion 720A model pH / ion counter (Beverly, MA, USA) with Orion 71-03 brand glass electrode has been used. Centrifugations were performed using a Sigma 1-6P (Osterode am Harz, Germany) centrifuge.

Chemicals

All the reagents are of analytical grade. Brij 58 (polyethylene glycol hexadecyl ether), Sunset Yellow (6-hydroxy-5- [(4-sulfophenyl) azo] -2-naphthalenesulfonic acid disodium salt) (SY), CH_3COOH, H_3PO_4, NaOH, HCl, NaCl, Na_2CO_3, NaH_2PO_4, Na_2HPO_4, and anhydrous Na_2SO_4 were purchased from Sigma-Aldrich. All reagents were diluted using deionized water.

For the routine calibration and monitoring of our pH instruments, a ready-to-use buffer solutions with exact pH reference values (pH 4.01= citric acid/sodium hydroxide/hydrogen chloride, pH 7.00= di-sodium hydrogen phosphate/potassium dihydrogen phosphate, pH 10.04= boric acid/potassium chloride/sodium hydroxide in 20 °C, Merck) have been used. Buffer solutions (pH 2-11) were prepared by using 0.04 M H_3PO_4, 0.04 M CH_3COOH, 0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4, and 0.04 M boric acid and the pH was adjusted with 0.2 mol L^{-1} NaOH solution. The pH 2 solution was prepared with 0.1 mol L^{-1} HCl.

Pharmaceutical preparations (Bemiks® tablets, *Zentiva Health Products Industry and Trade Co., Kırklareli, Turkey;* Magnorm® tablets, *NEUTEC Ilac Sanayi ve Ticaret A.Ş., Istanbul, Turkey)* and powdered beverage drinks have been purchased by pharmacies and local grocery stores.

Preparation of Standard and Sample Solutions

To prepare 100 µg mL^{-1} stock solution of SY, 0.1 g of the dye was dissolved in deionized water and diluted to 1000 mL. with deionize water. The working standard solutions are prepared daily by diluting the desired concentrations from the stock solution. These solutions were stored in the dark at 4 °C. To prepare Brij 58 solution of 20% (w/v), twenty grams of the Brij 58 is weighed and dissolved in 100 mL of deionized water in an ultrasonic bath.

Bemiks® tablet contains vitamin B1, vitamin B2, vitamin B6, vitamin B12, folic acid, niacinamide, Ca-pantothenate and certain inactive ingredients, such as quinoline sarcine (E104), sunset yellow FCF (E110), titanium dioxide (E171), Ponceau 4R (E124). Ten Bemiks® tablets have been weighed and powdered. Accurately weighed 0.9496 ± 0.0005 g (amount of 5 tablets) has been transferred to a 100 mL volumetric flask. The required volume with deionized water has been completed. Subsequently, an appropriate amount of this solution has been taken by filtration and used for the analysis.

Magnorm® tablet contains magnesium oxide and certain inactive ingredients, such as citric acid anhydrides, citric acid anhydrides, sodium hydrogen carbonate, povidone K30, sodium saccharin, sorbitol (E420), polyethylene glycol, aerosil 200, sunset yellow (E110), orange flavor, and apricot flavor. Ten Magnorm® tablets were powdered and mixed. An amount of 3.7885 g (1 tablet) was powdered and taken into a 500 mL volumetric flask. Then, the solution was diluted to 500 mL with deionized water. The 5 mL of this solution was taken and filtered, then the solution was diluted to 100 mL with deionized water.

The powdered beverage drink of 5.0000 ± 0.0005 g has been weighed and taken into a 250 mL volumetric flask and the required volume was diluted with deionized water. Subsequently, appropriate amounts of this solution have been taken by filtration and they have been used for the analysis.

Proposed CPE Method

The method has applied to the standard solution of SY: A solution containing 8 mL of the buffered SY in NaH_2PO_4 and Na_2HPO_4 (pH 6) has been transferred to a 15 mL screw-capped centrifuge tube (Figure 1a). 1 mL of 20% (w/v) Brij 58, 2.0050 ± 0.0005 g Na_2SO_4 were added and the solution has been diluted 10 mL with deionized water (Figure 1b). This mixture was immediately stirred until it was dissolved. The mixture left at 70 °C for 15 minutes until a cloudy phenomenon occurred. Then it has been cooled down to the room temperature (Figure 1c). The mixture has been centrifuged at 4000 rpm for 5 minutes for phase separation (Figure 1d). The surfactant-rich phase has been separated and it has been collected on the top of the tube (Figure 1 e). Finally, the aqueous phase has been removed by means of a syringe and the surfactant-rich phase has been diluted with 1 mL of water and the absorbance of the solution was measured by UV-Vis. against blank at 482 nm.

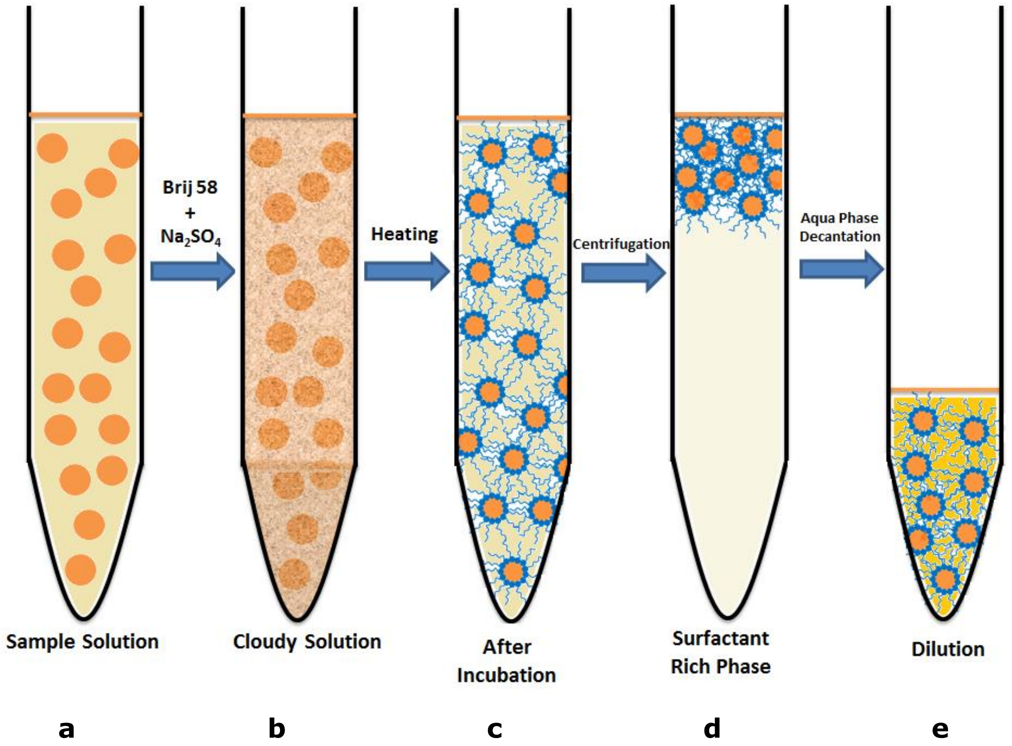

Figure 1: **a-** Sample solution, **b-** Mixture after Brij 58 + Na_2SO_4 added, **c-** After incubation, **d-** After centrifugation, **e-**After decantation of 1 mL of water-soluble surfactant-rich phase.

The developed method has applied to two drugs and two powdered beverages as detailed in 'The preparation of standard and sample solutions' section in a preceding page.

RESULT AND DISCUSSION

The maximum absorbance of 0.01-4.00 μg mL^{-1} the SY standard solution is 482 nm (Figure 2a). The surfactant (Brij 58) has been added to the medium. It did not make a significant change in the maximum wavelength of the dye by comparing the SY solution (Figure 2b). For this reason, all absorbance measurements have been made at this wavelength. The effects of the main variables in the CPE method such as the medium pH value, surfactant concentration, salt concentration, temperature, and time, have been optimized to achieve maximum sensitivity and recovery.

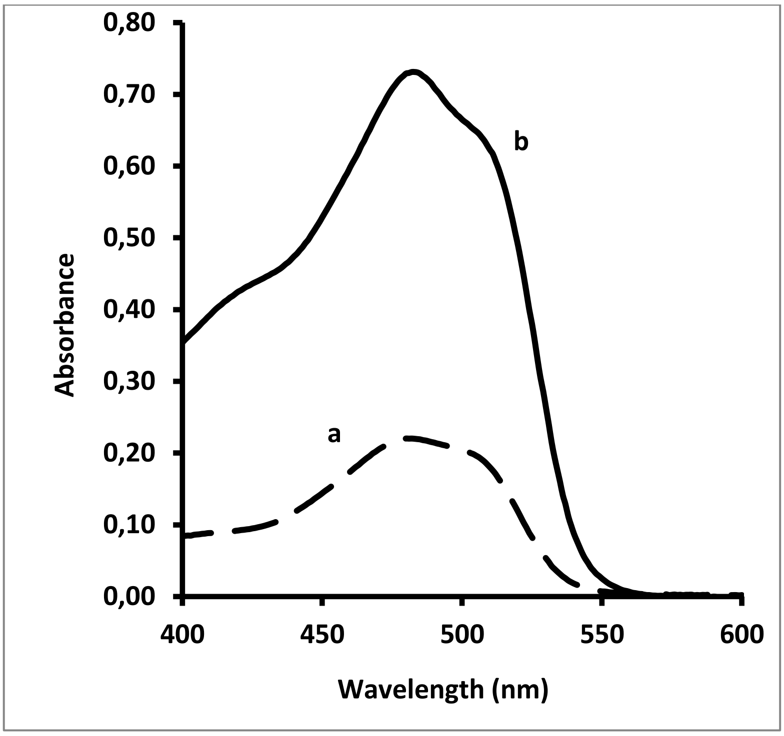

Figure 2: Absorption spectra for 4 μg mL^{-1} SY, **a-** without extraction, **b-** after CPE.

The Effect of pH

In the CPE procedure, the pH is an important parameter that increases the coefficient of dispersion of the analyte between the aqueous phase and the surfactant-rich phase. For this reason, the pH effecting on SY extraction efficiency has been examined between pH 2 and 11. The pH of the solution was adjusted to the desired value by the addition of hydrochloric acid and sodium hydroxide and using a pH meter. The SY solution was prepared in detail in the section of '*Proposed CPE Method*'. The absorbance of the SY has been measured at 482 nm (Figure 3). The increase has been observed in absorbance up to pH 6 and, then the absorbance did not change too much up to pH 8. The results indicate the range of pH 6-8 is most suitable for dye in the viewpoint of CPE efficiency and the pH range was selected as an optimum pH. All the studies have been carried out at pH 6-8.

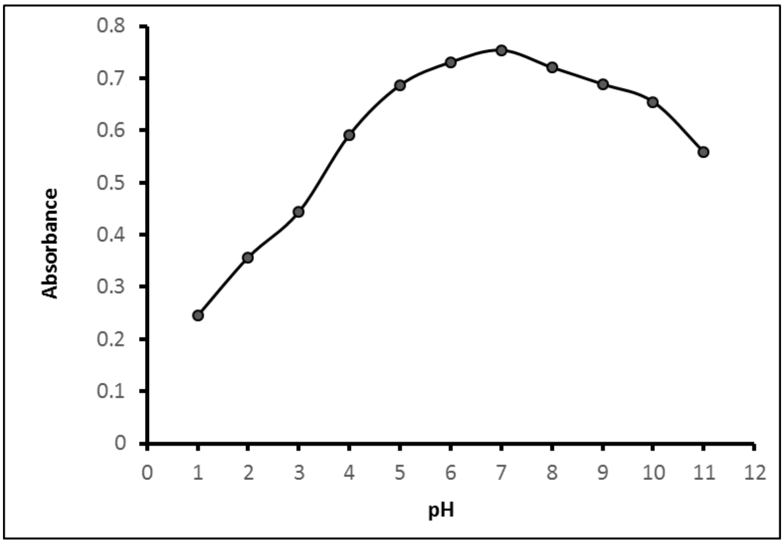

Figure 3: Effect of pH on CPE efficiency. 4 μg mL^{-1} SY, 2% Brij 58, 2.00 g Na_2SO_4, incubation at 70 ° C for 15 minutes.

The Effect of Surfactant Concentration

Optimizing the concentration of the surfactant is one of the important parameters to increase extraction efficiency. Thus, the effect of concentration of Brij 58 on the absorbance of the SY has been studied. The results are given in Figure 4. As can be seen, the absorbance does not change at concentrations greater than 2% (w/v) of Brij 58. For this reason, 2% (w/v) Brij 58 has been used throughout the study.

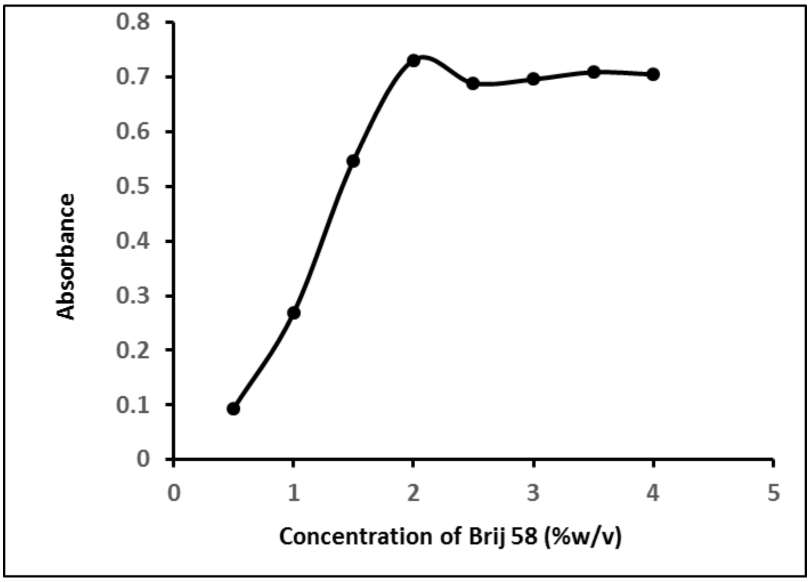

Figure 4: The effect of Brij 58 concentration on the CPE efficiency of SY. 4 μg mL^{-1} SY, pH 6, 2.00 g Na_2SO_4, 15 minutes of incubation time at 70 °C.

The Effect of Salt

A salt concentration is important in the CPE procedure because it helps the phase separation, to increase the mass transfer of the analyte from the aqueous phase to the surfactant-rich phase and to reduce the cloud point temperature. For these reasons, the commonly used salts such as NaCl, Na_2SO_4 and Na_2CO_3 have been tried and their effects on the extraction process have been investigated. The surfactant-rich phase has not been separated or the surfactant-rich phase was too poor to be separated when other salts were used. So, a 20% (w/v) concentration of Na_2SO_4, which maximizes the absorption of SY, has been selected as the optimum salt concentration. The results are given in Figure 5.

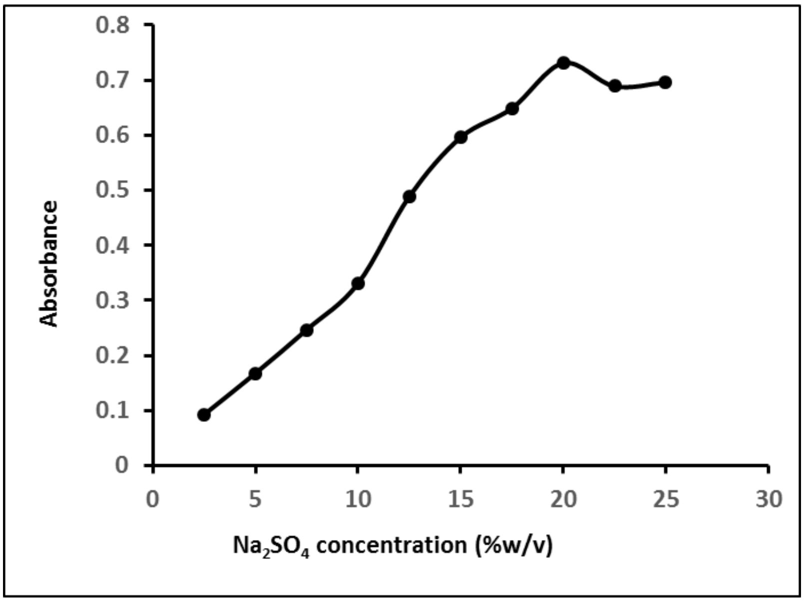

Figure 5: The effect of Na_2SO_4 concentration on the CPE efficiency of SY. 4 µg mL^{-1} SY, pH=6, 2% (w/v) Brij 58, 15 minutes of incubation time at 70 °C.

Effect of Equilibrium Temperature and Incubation Time

Two other important parameters examined in the CPE procedure are the equilibrium temperature and incubation time. The studied temperature ranges were 50-90 °C and the highest extraction efficiency was observed at 70°C. The incubation time in the range of 10-40 min has been studied. The maximum absorbance has been obtained for 15 min. In further studies, these optimum conditions have been carried out.

The Effect of Interference Ions

In this study, the effect of different anions and cations on the determination of 5 ppm SY has been studied by the proposed method. The tolerance limit has been examined taking into account the amount of foreign species causing the absorbance change of less than ± 5%. The results are given in Table 1.

Table 1: Comparison of the effect of interfering species on the determination of SY in the optimum conditions, C_{SY} = 4 mg L^{-1}.

Interfering species (I)	**Limiting mass ratio (SY: I)**
K^+, Mg^{2+}, Na^+	1:2000
Hg^{2+}, Mn^{2+}, Pb^{2+}, Be^{2+}, Fe^{3+}, Al^{3+}, Sr^{2+}, Sn^{2+} Co^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Si^{2+}	1:100
F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}	1:4000
AsO_4^-, $B_4O_7^{2-}$, HPO_4^{2-}, SCN^-	1:200

Analytical Performance

Correlation graphs with the proposed method have been obtained in the optimal conditions between increasing concentrations of SY versus the measured absorbance.

Absorbance of the SY obeys Beer's law between 0.01 μg mL^{-1} and 4.00 μg mL^{-1} in the optimum conditions. The correlation coefficient for the SY is 0.9995. The value of slope is 0.2105 and the intercept is + 0.0237. Thus, the calibration equation was A = 0.2105 C (μg mL^{-1}) + 0.0237.

The accuracy of this method was determined by performing five repetitions of 0.80 μg mL^{-1} SY. The RSD value is 1.44%. The limit of detection (LOD) of the method is 0.0078 μg mL^{-1} depending on the standard deviation of blank with repetition number of 3.

Sample Application of the Proposed CPE Method

This method has been applied to the spectrophotometric determination of SY in two different drug samples and two different powdered beverages. The analysis of the SY in the samples was realized as stated in the experimental section of '*Proposed CPE Method*'. Recovery experiments were performed by spiking the samples prior to the CPE with the addition of known amounts of SY. The recovery values obtained from the results in Table 2 are between 96.88% and 103.75%. According to these values, it can be said that the materials present in the samples do not interfere with the developed method. The acceptable Daily Intake for SY has been given as 0.0-2.5 mg/kg body weight by World Health Organization (WHO) (2). The results show that the developed CPE method can be used for the accurately determination of SY in these samples.

Table 2: The spectrophotometric determination of SY in powdered beverages and pharmaceutical preparations after the developed CPE.

Sample	**Added, SY (μg mL^{-1})**	**Found, SY ± CL^{a} (μg mL^{-1})**	**Recovery^{b}, %**
Powdered beverage 1	-	0.95 ± 0.11	-
	0.80	1.74 ± 0.15	98.75
	1.60	2.51 ± 0.15	98.13
Powdered beverage 2	-	2.16 ± 0.01	-
	0.80	2.95 ± 0.07	99.24
	1.60	3.78 ± 0.06	100.99
Magnorm® (Pharmaceutical preparation)	-	1.46 ± 0.06	-
	0.80	2.26 ± 0.07	101.25
	1.60	3.06 ± 0.16	96.88
Bemiks® (Pharmaceutical preparation)	-	0.78 ± 0.07	-
	0.80	1.61 ± 0.10	103.75
	1.60	2.30 ± 0.07	98.13

[a] CL is confidence limits, $(\frac{ts}{\sqrt{n}})$; n=5

[b] Recovery= $\frac{(C_2 - C_1)}{C_3} \times 100$

C_1= Measured sample concentration (blank)
C_2= The measured "spiked sample" concentration (blank + spike)
C_3= Spike concentration

Comparison of the Proposed Method with Literature

An comparison of some apparent features of the developed method with other reported methods for the simultaneous determination of SY in various samples is shown in Table 3. All existing methods for SY are based on CPE. The LOD value obtained for SY in the present work is superior compared to some of these methods (12, 20). While the method is slightly less sensitive compared to the other two methods (18, 19), these methods have certain disadvantages. El-Shahawi MS, *et al*. used an extra chemical such as trioctylamine to increase the efficiency of their work (18) and the repeatability of study of Heydari R. *et al*. is low (19). Among the values in Table 3, our method has the widest linear range and almost the lowest LOD with a high recovery.

Table 3: Comparison of certain CPE methods for spectrophotometric determination of SY reported in the literature.

Surfactant	**Salt**	**pH**	**Inc.[a] Temp. (˚C)**	**Inc.[a] Time (min)**	**Centr.[b] Time (min.)**	**Solvent of Dilution**	**Linear Range μg mL^{-1}**	**LOD μg mL^{-1}**	**RSD %**	**Recovery %**	**Remarks**	**Ref.**
Triton X-100 0.03 M, 4 mL	Na_2SO_4 1 M, 1 mL	HCl 0.01 M	70	10	10	MeOH 0.1 mL	0.020-0.452	5.0 x 10^{-3}	1.49	96.79	Trioctylamine was used to increase efficiency	(18)
Triton X-100 200 g/L, 3 mL	Na_2SO_4 1 M, 5 mL	-	40	10	10	Water 5 mL	20-120	5.0	1.97	94.75	LOD is high	(12)
Triton X-114 200 g/L, 2 mL	Na_2SO_4 1 M, 5 mL	-	40	10	10	Water 5 mL	20-120	10.4	1.13	91.72	LOD is high	(12)
Brij 56 200 g/L, 3 mL	Na_2SO_4 1 M, 5 mL	-	60	15	10	Water 5 mL	20-120	2.8	1.06	89.13	LOD is high	(12)
Triton X-100 % 40, 1.5 mL	NaCl 1.5 g	6	55	15	5	Water 1 mL	0.025-1.300	6.0 x 10^{-3}	3.35	98.64	Repeatability is low	(19)
Triton X-100 0.5 M, 4.5 mL	KCl 1 M, 5 mL	4	80	40	-	Water 5 mL	0.020-4.000	9.0 x 10^{-3}	3.50	99.80	Repeatability is low	(20)
Brij 58 % 20, 1 mL	Na_2SO_4 2 g	6	70	15	5	Water 1 mL	0.010-4.000	7.8 x 10^{-3}	1.44	99.64[c]		This work

[a]Inc.: Incubation, [b]Centr.: Centrifugation, [c] $\overline{X}$: Recovery (%) average in Table 2.

CONCLUSION

The developed CPE technique has advantages such as low cost, sensitiveness, selectiveness, high extraction efficiency, and environmentally friendliness. Brij 58 was used, for the first time, as a surfactant for the determination of SY after CPE method. This new CPE spectrophotometric method can be used for the sensitive, selective and reproducible determination of SY in short time in routine analysis.

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