# A Simple Method for Simultaneous Spectrophotometric Determination of Brilliant Blue FCF and Sunset Yellow FCF in Food Samples after Cloud Point Extraction

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**Summary:** In this study, a simple and low-cost method for extraction and pre-concentration of brilliant blue FCF and sunset yellow FCF in food samples using cloud point extraction (CPE) and spectrophotometric detection was developed. The effects of main factors such as solution pH, surfactant concentration, salt and its concentration, incubation time and temperature on the CPE of both dyes were investigated and optimized. Linear range of calibration graphs were obtained in the range of 16.0–1300 ng $mL^{-1}$ for brilliant blue FCF and 25.0–1300 ng $mL^{-1}$ for sunset yellow FCFunder the optimum conditions. Limit of detection values for brilliant blue FCF and sunset yellow FCFwere 3 and 6 ng $mL^{-1}$, respectively. The relative standard deviation (RSD) values of both dyes for repeated measurements (n=6) were less than 4.57 %. The obtained results were demonstrated the proposed method can be applied satisfactory to determine these dyes in different food samples.

Keywords: Brilliant blue FCF, Sunset yellow, Triton X-100, Cloud point extraction, Spectrophotometric, Food samples.

## Introduction

Food additive is a general term for compounds which use in order to sustain or improve the appearance of food, or protection of edible products from microbial spoilage [1, 2]. Synthetic dyes are included in food additive compounds and have adverse effects on human health. In general, food colors to enhance the attractiveness of the appearance of food products [3, 4]. Durability and brightness are two main advantages of synthetic dyes than natural dyes. Synthetic colors in high concentrations may cause toxic effects on human health. Thus, in order to control and monitoring of concentration levels of synthetic dyes in food products, continuous measurement of the amount of these additives is essential [5, 6]. Brilliant blue FCF and sunset yellow FCFare used as food dyes in many different products including juices, ice cream, yogurt, jelly and candy [7]. These dyes are the synthetic food additives which authorized in very countries. The acceptable daily intake (ADI) values of brilliant blue FCF and sunset yellow FCFbased on milligram per kilogram of body weight per day are 10 and 2.5, respectively [8]. Many methods such as capillary electrophoresis (CE) [9] differential pulse polarography (DPP) [10] high-performance ion chromatography (HPIC) [11] high-performance liquid chromatography (HPLC) [12, 13] mass spectrometry (MS) [14] spectrophotometry [15] and spectrofluorimetry [16] were suggested for determination of various synthetic dyes in food products. Some of these methods, e.g. polarography and chromatography techniques due to use of organic solvents in chromatography and mercury in polarography can not be classified as environmentally friendly methods. On the other hand, HPLC and CE techniques are interpreted as more impressive alternative methods. These methods are costly, time-consuming and generate wastes with a high amount of organic solvents. In spite of high sensitivity of electroanalytical methods, the selectivity of these methods is low. Disadvantages of stripping voltammetry (SV) than spectrophtometric method are including longer analysis time and existence of interferences which can lead to restrictions in real samples analysis.

Cloud point extraction (CPE) is an alternative solvent extraction technique which used to extract of different analytes from various matrixes. As compared with traditional solvent extraction methods, CPE uses surfactants as extracting solvent which are non-toxic and compatible with different analytical instruments [17]. CPE method has been widely applied for extraction, pre-concentration and clean-up of target analytes in food and drug samples [18-21].

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The purpose of this work was to develop a simple and sensitive CPE method for determination of brilliant blue FCF and sunset yellow FCFin food samples using spectrophotometric detection. Extraction, clean-up and pre-concentration of brilliant blue FCF and sunset yellow FCFfrom aqueous samples were performed simultaneously using Triton X-100 as extracting solvent. The effects of main factors on the extraction yield of brilliant blue FCF and sunset yellow FCFwere optimized. Figure of merits of the proposed method were compared with several reported methods in literature.

## Experimental

### *Reagents and Materials*

All chemicals in this work were analytical reagent grade and deionized water was used for sample preparation. Brilliant blue FCF, sunset yellow FCFand Triton X-100 were purchased from Merck Chemicals Company (Darmstadt, Germany). A solution of nonionic Triton X-100 surfactant (40% w/v) was prepared by dissolving accurately 40 g of Triton X-100 in water and diluting to 100 mL in a volumetric flask. Buffer solution (pH 6) was prepared by adding 1.0 mol $L^{-1}$ of sodium hydroxide solution to acetic acid solution (0.1 mol $L^{-1}$). Food samples were purchased from local supermarkets in Khorramabad (Lorestan, Iran).

### *Apparatus*

Absorption data were acquired using a Jenway spectrophotometer (model 6715, UK) equipped with a 1 cm glass cell. A Metrohm digital pH meter (model 632, Switzerland) with a combined glass electrode was used to measure pH values. A centrifuge (Behsan, Iran) was used to accelerate the phase separation process. A thermostatic water bath (Memmert, Germany) was used to keep the temperature in desired values.

### *Standard and Sample Preparation*

Stock solutions of 1000 μg $mL^{-1}$ of brilliant blue FCF and sunset yellow FCFwere prepared by dissolving 0.1 g of each dye in deionized water and diluting to 100 mL in a volumetric flask. Fresh working standard solutions were obtained by appropriate dilution of the stock solutions daily.

Appropriate amounts of food samples were dissolved in deionized water. In order to remove of suspension particles, sample solutions were filtered using a PTFE membrane filter (0.45 μm). 1 mL of the filtrated sample solutions were diluted to 10 mL in a volumetric flask using acetate buffer (pH 6) as the diluent. Finally, 8 mL of these solutions were treated under the optimized CPE procedure for the determination of brilliant blue FCF and sunset yellow.

### *Cloud Point Extraction Procedure*

8.5 mL of the acetate buffer solution (pH 6) containing of brilliant blue FCF and sunset yellow FCFwas transferred to a 15 mL centrifuge tube. Then 1.5 mL of Triton X-100 (40% w/v) and 1.5 g of NaCl salt were added to this solution. After the dissolving of salt, the mixture was placed in a thermostat water bath at 55 °C for 15 min. The phase separation was accelerated by centrifuging the test tube for 5 min at 4000 rpm. The surfactant-rich phase was separated and collected at the upper of the tube (Fig. 1). Hence, the aqueous phase was removed using a syringe with a long needle that passed through the surfactant-rich phase. The surfactant-rich phase was diluted with water. The absorbance of the solution was measured at 631 and 486 nm for brilliant blue FCF and sunset yellow, respectively. A blank solution (without brilliant blue FCF and sunset yellow) was also prepared according to the same procedure and used for baseline correction.

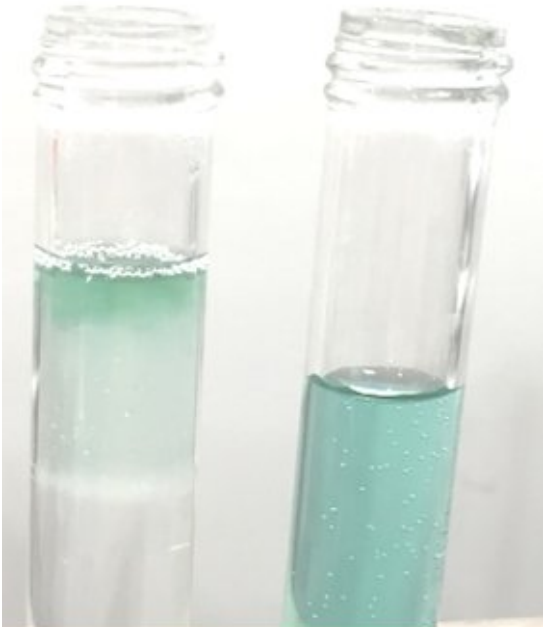
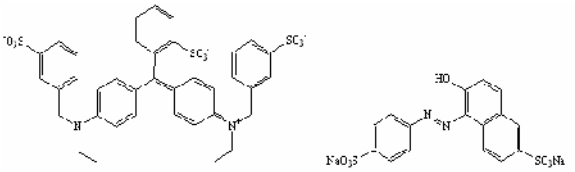


Fig. 1: Aqueous solution of sunset yellow FCFand brilliant blue FCF. Before extraction process (right) and after CPE method (left). Two dyes are transferred to surfactant-rich phase.

**Results and discussion**

The structures of brilliant blue FCF and sunset yellow FCF were shown in Fig. 2. The absorption spectra of brilliant blue FCF and sunset yellow FCFindicate that maximum absorbances occur at 631 and 486 nm, respectively (Fig 3a and 3b). Also the presence of surfactant (Triton X-100) does not have considerable effect on the maximum wavelengths of these dyes (Fig. 3d). Therefore, all the absorbance measurements were accomplished at these two wavelengths. The effects of main factors in CPE method such as pH of the medium, surfactant concentration, salt and its concentration, incubation time and temperature were optimized in order to acquire the maximum sensitivity and recovery.



Brilliant Blue FCF Sunset yellow FCF

Fig. 2: The structures of sunset yellow FCF and brilliant blue FCF.

*Effect of pH*

The pH is an influential factor in CPE method which can affect the partition coefficient of the analytes between aqueous and surfactant-rich phases. In CPE method with nonionic surfactants as extracting solvent, the neutral analytes more extracted into the surfactant phase. Therefore, the effect of pH on extraction efficiencies of brilliant blue FCF and sunset yellow FCF was examined in the pH range of 2–8. The absorbance of surfactant-rich phase containing both dyes was recorded at 631 and 486 nm for brilliant blue FCF and sunset yellow FCF, respectively. As it was observed from the results in Fig. 4, absorbance value of surfactant phase for both dyes at pH 6 is higher than other pHs. The reason for this behavior can be attributed to sulfonate groups ($-SO_3^-$) which neutralized in acidic medium. Consequently, pH 6 was chosen as the optimum pH for subsequent experiments.

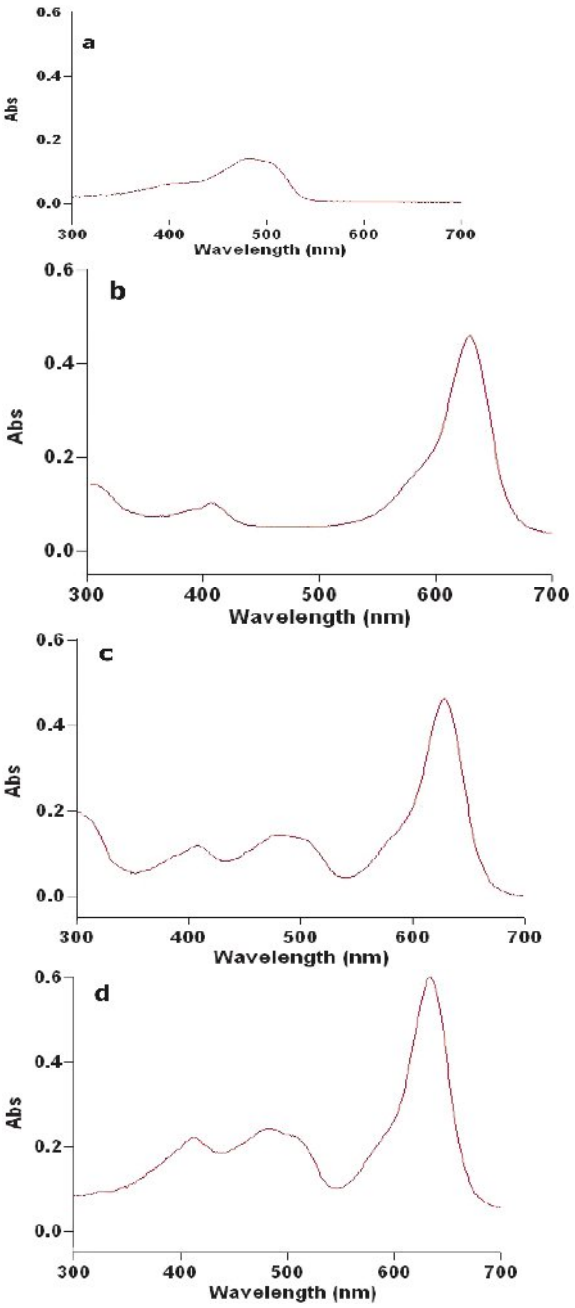



Fig. 3: Visible spectra of analytes before and after extraction. a) sunset yellow FCF in aqueous solution (2 µg $mL^{-1}$), b) brilliant blue FCF in aqueous solution (2 µg $mL^{-1}$), c) mixture of both dyes in aqueous solution (2 µg $mL^{-1}$) and, d) mixture of both dyes in surfactant-rich phase (0.2 µg $mL^{-1}$).

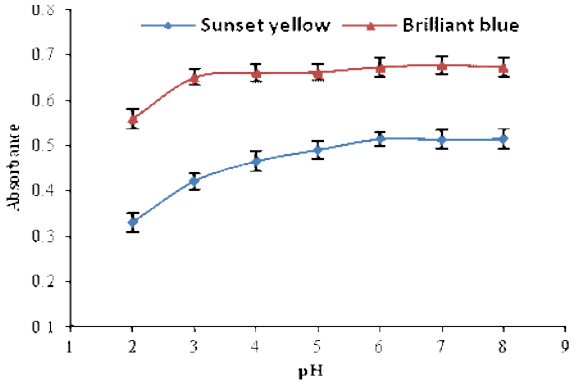



Fig. 4: The effect of pH on the extraction efficiency of brilliant blue and sunset yellow. Extraction conditions: Triton X-100 concentration, 6 %w/v; incubation temperature, 55 °C; incubation time, 15 min; salt concentration, 15 %w/v.

*Effect of Triton X-100 Concentration*

Surfactant concentration can affect the extraction efficiency of analytes and their enrichment factors, hence the concentration of surfactant should be optimize. The effect of Triton X-100 concentration on the absorbance of brilliant blue FCF and sunset yellow FCF was investigated in the range of 2–12% w/v. The results in Fig. 5 illustrate that the absorbance of extracted both dyes increase up to 6% w/v and then reduce. In order to obtain the maximum pre-concentration, 6% w/v was chosen as the suitable surfactant concentration in this study.

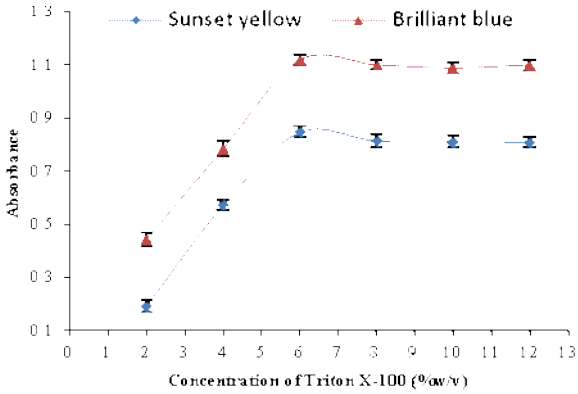



Fig. 5: The influence of Triton X-100 concentration on the extraction efficiency of brilliant blue and sunset yellow. Extraction conditions: incubation temperature, 55 °C; incubation time, 15 min; sample pH, 6; salt concentration, 15 %w/v.

*Effect of Equilibrium Temperature and Incubation Time*

Equilibrium temperature and incubation time are two very important factors in CPE technique. The effect of equilibrium temperature on the extraction efficiency of brilliant blue FCF and sunset yellow FCF was optimized in the range of 50–75 °C. The results in Fig. 6 were shown the extraction efficiencies of two analytes were enhanced with an increase in temperature from 50 to 55 °C and then decreased. Usually, critical micelle concentration (CMC) changes with temperature [28]. Therefore, it can be assumed that an increase in temperature leads to change the CMC and reduces the micelle formation. Therefore, 55 °C was chosen as the optimum temperature.

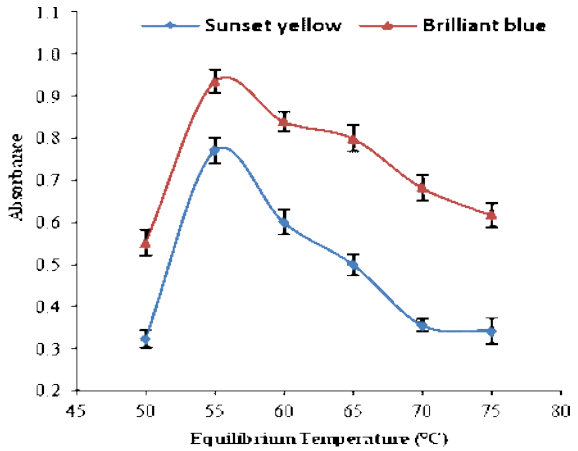



Fig. 6: The effect of equilibrium temperature on the extraction efficiency of brilliant blue and sunset yellow. Extraction conditions: Triton X-100 concentration, 6 %w/v; incubation time, 15 min; sample pH, 6; salt concentration, 15 %w/v.

In order to acquire acceptable extraction efficiency, the incubation time of sample solution was studied in the range of 5–30 min. Fig. 7 indicates incubation time of 15 min is the convenient time for maximum analytes extraction.

*Effect of Salt Concentration*

Addition of salt to the mixture of aqueous sample and surfactant has several advantages including change of density the aqueous phase and facilitate the phase separation, decrease in the cloud point temperature and increase in analyte transfer from aqueous phase to surfactant phase due to salting-out phenomenon. Therefore, it is essential to investigate the effect of salt type and its concentration on extraction process. For these reasons various salts such as NaCl, $Na_2SO_4$ and $Na_2CO_3$ were selected and their influence on the

extraction process were studied. The results of tests were indicated NaCl has the greatest effect on the extraction efficiency of analytes. Thus, NaCl chosen as the suitable salt and different concentrations for this salt were tested. The results (Fig. 8) show that dyes absorbance signals were increased up to 15% w/v of NaCl and then leveled off. According to the results of Fig. 6, 15% w/v of NaCl was selected as the optimum salt concentration.

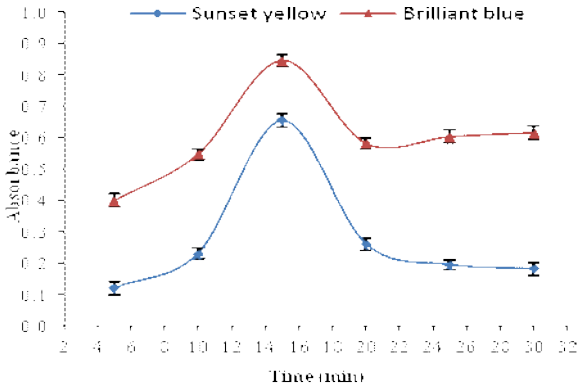



Fig. 7: The effect of incubation time on the extraction efficiency of brilliant blue and sunset yellow. Extraction conditions: Triton X-100 concentration, 6 %w/v; incubation temperature, 55 °C; sample pH, 6; salt concentration, 15 %w/v.

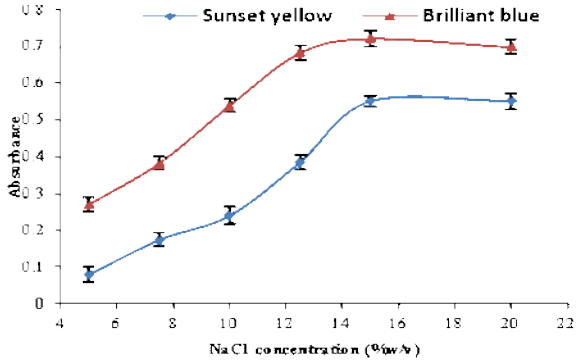



Fig. 8: The influence of NaCl concentration on the extraction efficiency of brilliant blue FCF and sunset yellow. Extraction conditions: Triton X-100 concentration, 6 %w/v; incubation temperature, 55 °C; incubation time, 15 min; sample pH, 6.

*Analytical Performance*

Under the optimized conditions, the correlation between the analytical signal and the dyes concentration was obtained by calibration curves. Linear calibration graphs were obtained in the range of 16.0–1300 ng $mL^{-1}$ for brilliant blue FCF and 25.0–1300 ng $mL^{-1}$ for sunset yellow FCF. Equations were A= 0.975C + 0.034 and A= 0.505C + 0.014 for brilliant blue FCF and sunset yellow FCF, respectively. The regression coefficients were 0.9992 for brilliant blue FCF and 0.9990 for sunset yellow FCF. Detection limits based on theoretical calculations ($DL=3S_b/m$) were 3 and 6 ng $mL^{-1}$ for brilliant blue FCF and sunset yellow FCF, respectively. The relative standard deviation (RSD) values at the lowest concentration of linear ranges were 4.57 for brilliant blue FCF and 3.35% for sunset yellow FCF.

*Interferences*

In this paper, the effect of foreign ions on the determination of brilliant blue FCF and sunset yellow FCF were studied and ±5% in the absorbance values was considered as tolerable error. The results are shown in Table-1. Standard solutions containing 160 ng $mL^{-1}$ of both dyes and different concentration of other ions were prepared and subjected to the CPE procedure. As it is observed from these results most of the tested ions do not have significant effect on the dyes signals.

Table-1: The effect of several ions on the signals of brilliant blue FCF and sunset yellow FCF.

| Foreign ions | Tolerance limit (µg $mL^{-1}$) |
|---|---|
| $Na^+$, $Cl^-$, $CH_3COO^-$, $NH_4^+$ | 5000 |
| $K^+$ | 1000 |
| $CO_3^{2-}$, $SO_4^{2-}$, $Cr^{3+}$, $Ni^{2+}$, $Co^{2+}$, $Al^{3+}$, $Cd^{2+}$, $Pb^{2+}$ $Cu^{2+}$, $Mn^{2+}$, $Ag^+$, $Zn^{2+}$, $Fe^{3+}$, $Mg^{2+}$, $I^-$, $H_2PO_4^-$ | 100 |

*Applications*

The proposed method has been applied to determine of brilliant blue FCF and sunset yellow FCF in different food matrixes. The results are given in Table-2. According to these results from Table-3, the spiked concentration of brilliant blue FCF and sunset yellow FCF can be quantitatively recovered from the food samples by the proposed method. These results demonstrate ability of the CPE procedure to determine the both dyes in food samples. The analytical factors of the proposed method were compared with various reported methods in the literature (Table-4). The results show the LOD, LOQ and recovery values were enhanced by using the proposed CPE method. On the other hand, analysis time for this method was shorter than other methods particularly chromatographic methods. In this method, no organic solvent was used. The recommended method can be successfully applied to extract and quantify brilliant blue FCF and sunset yellow FCF in food samples.

Table-2: Results of analyzed food samples for brilliant blue FCF and sunset yellow FCF.

| Sample (n=5) | Sunset yellow FCF (ng mL$^{-1}$) ±SD[a] | Brilliant blue FCF (ng mL$^{-1}$) ±SD[a] |
|---|---|---|
| Soft beverage | 55±0.007 | 70±0.003 |
| Soft beer | 336±0.01 | 134±0.01 |
| Smarties | 220±0.02 | 102±0.03 |
| Custard | 192±0.005 | 25±0.01 |
| Snack | 293±0.02 | 28±0.02 |
| Jelly | 340±0.07 | 130±0.02 |
| Pastille | 100±0.03 | 16±0.02 |

[a]Standard Deviation

Table-3: Results of accuracy test using spike samples with different concentrations of analytes.

| Sample (n=5) | Sunset yellow FCF | | | Brilliant blue FCF | | |
|---|---|---|---|---|---|---|
| | Added (ng mL$^{-1}$) | Founded (ng mL$^{-1}$)±SD | Recovery (%) | Added (ng mL$^{-1}$) | Founded (ng mL$^{-1}$)±SD[a] | Recovery (%) |
| | - | 55±0.007 | - | - | 70±0.003 | - |
| Soft beverage | 330 | 380±0.02 | 98.5 | 330 | 405±0.03 | 101.5 |
| | 660 | 711±0.01 | 99.4 | 660 | 718±0.04 | 98.18 |
| | - | 336±0.01 | - | - | 134±0.01 | - |
| Soft beer | 330 | 670±0.03 | 101.2 | 330 | 468±0.01 | 101.2 |
| | 660 | 982±0.01 | 97.88 | 660 | 788±0.02 | 99.09 |
| | - | 220±0.02 | - | - | 102±0.03 | - |
| Smarties | 330 | 530±0.03 | 93.94 | 330 | 425±0.02 | 97.88 |
| | 660 | 860±0.01 | 96.97 | 660 | 770±0.01 | 101.2 |
| | - | 192±0.005 | - | - | 25±0.01 | - |
| Custard | 330 | 527±0.03 | 101.5 | 330 | 350±0.01 | 98.48 |
| | 660 | 850±0.01 | 99.70 | 660 | 692±0.03 | 101.0 |

Table-4: Comparison of analytical parameters of the proposed CPE method with some of the methods reported in literature

| Sample preparation | Matrix | Analyte | Detection | Extraction solvent or adsorbent | Analysis Time (min) | LOD (ng mL$^{-1}$) | LR[a] (ng mL$^{-1}$) | RSD (%) | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Direct | Soft beverages | SY[b] | Commercial Scanner | -- | 30 | 2.5 | 7.8-39.7 | <10 | [22] |
| CPE | Foodstuffs | SY | Spectrophotometry | Triton X-100 | 26 | 5.0 | 20.0-452.0 | 1.49 | [23] |
| SPE[c] | Powdered beverages | SY | Spectrophotometry | DIAION Resin | 20 | 5.2 | 950.0-5400 | 3.90 | [24] |
| Direct | Soft drinks | SY | Ion-pair HPLC | -- | 20 | 24.0 | 60.0-1200 | <5 | [25] |
| Direct | Foodstuffs | BB[d] | SV[e] | -- | 1 | 1.53 | 8.0-80.0 | 2.20 | [26] |
| SPE | Foodstuffs | BB | Spectrophotometry | β-Cyclodextrin Polymer | 40 | 16.0 | 50.0-1200 | 3.40 | [27] |
| CPE | Candy and smarties | BB | Spectrophotometry | Triton X-100 | 35 | 16.0 | 50.0-3500 | 3.30 | [17] |
| Direct | Soft drinks | BB | Ion-pair HPLC[e] | -- | 20 | 3.0 | 7.5-300 | <5 | [26] |
| CPE | Drinks and smarties | BB | Spectrophotometry | Triton X-100 | 20 | 3.0 | 16.0-1300 | 4.57 | This work |
| CPE | Drinks and smarties | SY | Spectrophotometry | Triton X-100 | 20 | 6.0 | 25.0-1300 | 3.35 | This work |

[a] Linear range, [b] Sunset yellow, [c] Solid-phase extraction, [d] Brilliant blue, [e] Stripping voltammetry.

## Conclusions

A simultaneous cloud point extraction method has been introduced for the determination of brilliant blue FCF and sunset yellow FCF in food samples. Figure of merits the proposed method are comparable to the other previously reported methods for monitoring of both dyes in mixtures or alone. Moreover, the proposed method has several advantages including simplicity, environmentally friendly, low-cost, appropriate accuracy and precision. In this method no organic solvent was used in extraction process. Also, detection was achieved using spectrophotometer and sophisticated instruments such as chromatography or electroanalytical systems avoided. The obtained results demonstrate the proposed method can be applied satisfactory to determine brilliant blue FCF and sunset yellow FCF in different food samples.

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