

Simultaneous Determination of Brilliant Blue FCF and Carmoisine in Food Samples by Aqueous Two-Phase System and Spectrophotometric Detection

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## Abstract

**Introduction:** A novel, simple, and more sensitive spectrophotometric procedure has been developed for the determination of Brilliant blue FCF and Carmoisine in food samples by an aqueous two-phase system (ATPS).

**Materials and methods:** After extraction, the absorbance values were measured at 527 and 632 nm for Carmoisine and Brilliant blue FCF, respectively. The effects of different parameters such as, polyethylene glycol (type and amount), pH, salt (type and amount), and temperature were investigated and optimum conditions were established.

**Results:** Linear calibration curves were obtained in the range of 0.1-120 ng mL<sup>-1</sup> and 0.2-260 ng mL<sup>-1</sup> for Carmoisine and Brilliant blue FCF, respectively. Under optimum conditions, detection limit based on three times the standard deviation of the blank (3S<sub>b</sub>) was 0.014 ng mL<sup>-1</sup> for Carmoisine and 0.017 ng mL<sup>-1</sup> for Brilliant blue FCF. At concentration level of 30 ng mL<sup>-1</sup>, the relative standard deviation (RSD) was 3.17% and 1.87% for Carmoisine and Brilliant blue FCF, respectively.

**Conclusion:** The method was successfully applied to the determination of target analytes in spiked samples with satisfactory results.

**Keywords:** Spectrophotometric, Carmoisine, Brilliant blue FCF, food samples, ATPS

## Introduction

Carmoisine (disodium 4-hydroxy-3-(4-sulfonato-1-naphthylazo)-naphthalene-1-sulfonate) and Brilliant Blue FCF (bis {4-(*N*-ethyl-*N*-3-sulfophenylmethyl)aminophenyl}-2-sulfophenyl methylum disodium salt) find a wide range of applications in the textile, paper, leather,

food, cosmetics, agrochemical, and pharmaceutical industries (1-6). These food colors are used for the maintenance and improvement of color appearance in foods such as beverages, dairy products, powders, jellies, confections, icings, syrups and so on (7-9). Carmoisine and Brilliant blue FCF

impart red and blue color to foods, respectively (10-11). However, some synthetic colorants such as Carmoisine may be toxic and dangerous for human health, especially if consumed in large amounts (12,13). Therefore, the amount of many dyes in foods must be controlled.

Numerous procedures were applied for the determination of Carmoisine and Brilliant blue FCF in different matrix, such as ion polarography (14), capillary electrophoresis (15), chromatography (16), and stripping voltammetry (17). However, most of these methods are time-consuming, polluting, needing a great deal of hazardous organic solvents, require a highly qualified operator and costly instrumentation.

In this study, spectrophotometric determination using an aqueous two-phase system (ATPS) was applied for the determination and measurement of Carmoisine and Brilliant Blue FCF in food samples. ATPS is usually composed of two or more polymers, a polymer and a salt [18]. Polyethylene glycol (PEG) -potassium phosphate and PEG-magnesium sulfate are among the most frequently used polymer-salt systems (9). ATPS has advantages over the conventional extraction systems using organic solvents (e.g., short processing time, low energy consumption, relative reliability in scale up, and a biocompatible environment) (11).

## Materials and methods

**Reagents and instruments:** PEG with a molecular weight of 4000, 6000, 8000 and 10000, sodium sulfate, sodium carbonate, potassium phosphate and ammonium sulfate were obtained from Merck (Darmstadt, Germany). Carmoisine, Brilliant blue FCF and other reagents were of analytical grade and were purchased from Merck (Darmstadt,

Germany). A stock standard solution of Carmoisine and Brilliant Blue FCF at a concentration of 50 mg L<sup>-1</sup> was prepared. The working solutions of Carmoisine and Brilliant Blue FCF were prepared by appropriate dilutions of the stock solution immediately prior to their use. All aqueous solutions were prepared with double distilled water.

The UV-Vis spectrophotometer (model 6705, Jenway, England) was used for all the absorbance measurements with a 10 mm quartz cell. pH measurements were made with a 827 pH meter (Metrohm, Switzerland) equipped with a combined glass electrode. The centrifuge (K280R, UK) was used for centrifuging.

**Experimental method:** A 3 mL portion of the standard solutions containing Carmoisine and Brilliant blue FCF at concentration level of 250 ng mL<sup>-1</sup> was placed into a 15 mL glass tube and adjusted pH to 9 with 7.5 mL of borate buffer solution at concentration level of 0.05 mol/L. Then, 3.5 g (23.33% w/w) of PEG 6000 and 3 g (20% w/w) of sodium carbonate was added to the glass tube and diluted to the mark with double distilled water. The final concentration of analytes was 50 ng mL<sup>-1</sup> at this volume. The mixture was shaken at 45 °C in bath water for a few minutes. Then, the mixture was centrifuged at 3500 rpm for 10 min. The upper phase was completely transferred to a quartz cell using a syringe. Finally, the absorbance values of dyes extracted into the upper PEG-rich phase were measured at 527 and 632 nm for Carmoisine and Brilliant blue FCF versus reagent blank, respectively. Figure 1, shows the absorption spectra of Carmoisine and Brilliant Blue FCF.

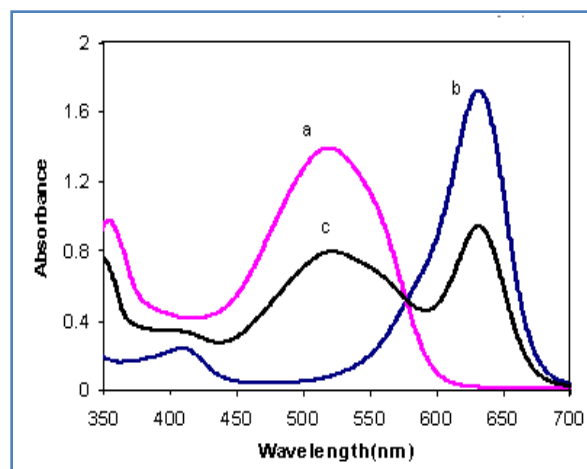


Figure 1. The absorption spectra of (a) Carmoisine, (b) Brilliant blue FCF, and (c) Carmoisine and Brilliant blue FCF in a mixture.

**Preparation of real samples:** Appropriate amounts of strawberry jelly (Tehran, Iran), fruity candy (Ben Ben, Iran), Smarties (morvarid, Iran), gummy candies (Yupi-Indonesia), noshmak (Tehran, Iran) and jell gum with fruit taste (Shiba Co) (PASTIL) samples were dissolved in warm water, filtered if necessary, and diluted in a volumetric flask. An aliquot of the above sample solutions was treated under the general procedure for ATPS and subsequent determination of Brilliant blue FCF and Carmoisine.

## Results

To achieve high extraction efficiency and recovery, the effect of several parameters such as salt (type and amount), polyethylene glycol (type and amount), temperature, and pH of sample solutions were evaluated and optimized. The optimization process was carried out using one variable at a time method for simplifying the optimization procedure.

**Effect of type and amount of PEG:** Carmoisine and Brilliant blue FCF showed a decrease in their partition coefficient value as the PEG molecular weight increased due to an increase in the polymer hydrophobic character which agrees with a general rule

observed for most ATPS. Fig.2 showed the effect of PEG with different molecular weight (4000, 6000, 8000 and 10000). The result demonstrated that higher absorption signal was obtained for Carmoisine and Brilliant Blue FCF by using PEG-6000.

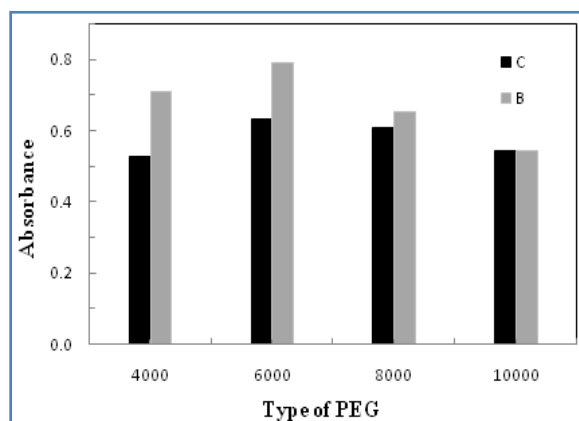


Figure 2. Effect of type of PEG on the extraction efficiency; C (Carmoisine), B (Brilliant blue FCF), Experimental conditions: volume of buffer; 7.5 mL, pH of sample; 9, amount of PEG; 3.5 g, amount of sodium carbonate; 3 g, temperature; 45 °C, 50 ng/mL of Carmoisine and Brilliant blue FCF.

The effect of the amount of PEG-6000 on the absorption was studied using various amounts of the reagent ranging from 2.5 g to 5 g (16.66% to 33.33% w/w), and the results were shown in Fig. 3. When 2.5 (16.66% w/w) g of PEG-6000 was used, the two-phase system was not found. As can be seen, by increasing the amount of PEG-6000, the analytical signal increased, reaching a maximum value of 3.5 g (23.33% w/w), and then decreased. When the PEG-6000 content increased, the phase ratio also rose up and enlarged the volume of PEG phase. With 23.33% w/w PEG-6000, the transformations yielding from aqueous sample to PEG-rich phase was the highest. At this point, the largest contact area between the two phases could be observed when the system was mixed. According to this result, all further experiments were carried out at the amount of 3.5g (23.33% w/w) of PEG 6000.

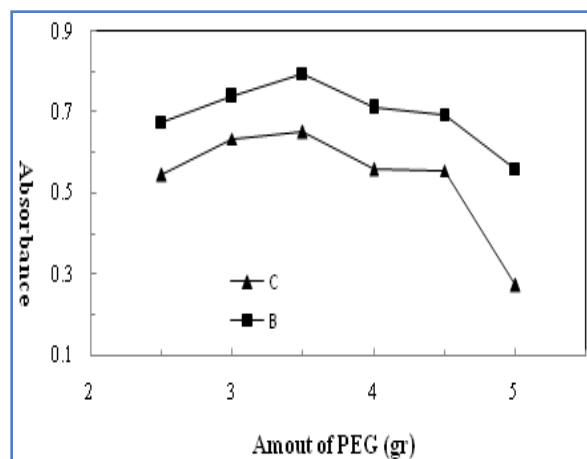


Figure 3. Effect of amount of PEG 6000 on the extraction efficiency; C (Carmoisine), B (Brilliant blue FCF), Experimental conditions: volume of buffer; 7.5 mL, pH of sample; 9, amount of sodium carbonate; 3 g, temperature; 45°C, 50 ng/mL Carmoisine and Brilliant blue FCF.

**Effect of pH and volume of buffer:** The pH is evaluated as a critical parameter for regulating the partitioning of the analytes in the PEG-rich phase which seems to control the extraction efficiency. Therefore, the effect of pH on ATPS of Brilliant blue FCF and Carmoisine was investigated in the range of 2.95-11.2 by the addition of hydrochloric acid and sodium hydroxide. As can be seen in Fig. 4, the signal increased with the raise in pH from 2.95 to 9, and then declined with pH higher than 9. Therefore, pH 9 was selected for further study. Borate buffer was used to achieve pH 9 in all further experiments. The effect of the buffer's volume in the extraction process was examined in the range of 1.5-12 mL. The result of Fig.5 verified that an optimum volume of buffer with pH 9 has obtained 7.5mL.

**Effect of type of salt and amount of salt:** Salts have been added to PEG-salt systems to increase the selectivity of molecule partitioning in the aqueous two-phase methodology application for extraction and separations. In many techniques of liquid-liquid extraction, the ionic strength of aqueous solution affects the extraction

efficiency. In this research, the increase of ionic strength was performed by adding different salts such as sodium sulfate, sodium carbonate, potassium phosphate, and ammonium sulfate into the sample solutions.

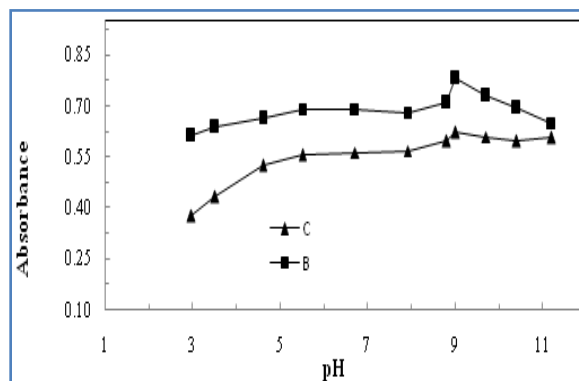


Figure 4. Effect of pH on the extraction efficiency; C (Carmoisine), B (Brilliant blue FCF), Experimental conditions: volume of buffer; 7.5 mL, amount of PEG 6000; 3.5 g, amount of sodium carbonate; 3 g, temperature; 45°C, 50 ng/mL of Carmoisine and Brilliant blue FCF.

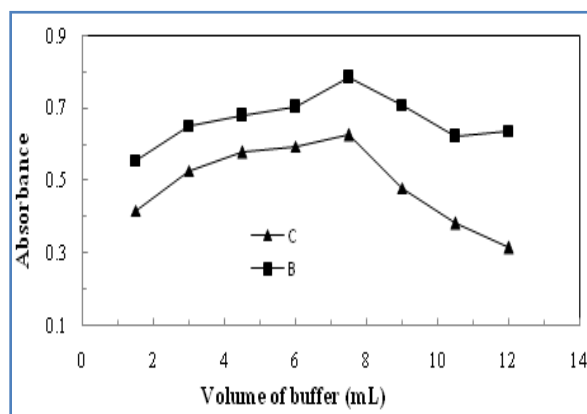


Figure 5. Effect of volume of buffer on the extraction efficiency; C (Carmoisine), B (Brilliant blue FCF), Experimental conditions: pH of sample; 9, amount of PEG 6000; 3.5 g, amount of sodium carbonate; 3 g, temperature; 45 °C, 50 ng/mL of Carmoisine and Brilliant blue FCF.

As can be seen in Figure 6, sodium carbonate provided better extraction efficiency than other salts. Hence, sodium carbonate was chosen as the most appropriate salt for subsequent experiments. Effect of sodium carbonate's amount on the extraction of Brilliant blue FCF and

Carmoisine was investigated in the range of 1-3.5g (6.66%-23.33% w/w).

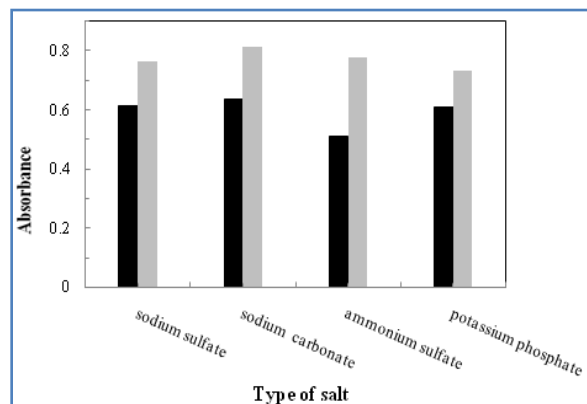


Figure 6. Effect of type of salt on the extraction efficiency; C (Carmoisine), B (Brilliant blue FCF), Experimental conditions: volume of buffer; 7.5 mL, pH of sample; 9, amount of PEG 6000; 3.5 g, amount of salt; 3 g, temperature; 45°C, 50 ng/mL of Carmoisine and Brilliant blue FCF.

When sodium carbonate amount was not sufficient (less than 1 g), only one phase existed. Figure 7 shows the differences observed in the signals at various amount of sodium sulfate. According to this result, all further experiments were carried out at the optimum amount of 3 g (20% w/w) of sodium carbonate.

**Effect of temperature:** Temperature affects the mass transfer process and the extraction efficiency. To determine the influence of the extraction temperature, 15 mL aqueous solution containing 50ng mL<sup>-1</sup> of target analytes was extracted at different temperatures ranging from 35 °C to 60 °C. Figure 8 shows the absorbance values of Carmoisine and Brilliant Blue FCF against the temperature. Increasing the temperature is beneficial for decreasing the viscosity and increasing the diffusion of analytes into the PEG-rich phase. According to these results, the temperature that gave the best results for the majority of the target analytes was 45 °C

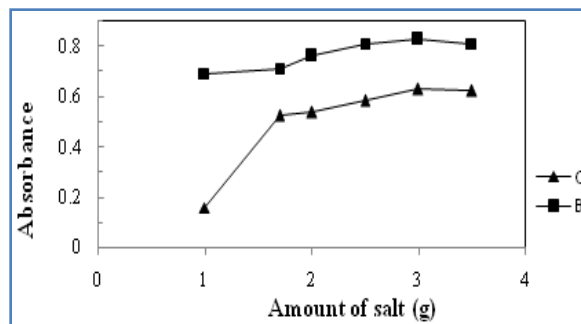


Figure 7. Effect of amount of sodium carbonate on the extraction efficiency; C (Carmoisine), B (Brilliant blue FCF), Experimental conditions: volume of buffer; 7.5 mL, pH of sample; 9, amount of PEG 6000; 3.5 g, temperature; 45°C, 50 ng/mL Carmoisine and Brilliant blue FCF

Fig. 8 shows the absorbance values of Carmoisine and Brilliant Blue FCF against to temperature. Increasing temperature is beneficial for decreasing the viscosity and increasing the diffusion of analytes into the PEG-rich phase. Accordingly with these results, the temperature that gave the best results for the majority of the target analytes was 45 °C.

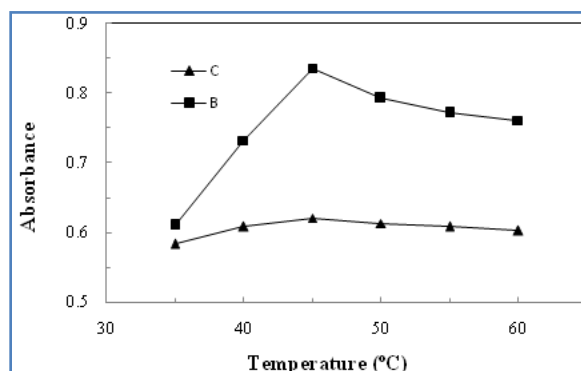


Figure 8. Effect of temperature on the extraction efficiency; C (Carmoisine), B (Brilliant blue FCF), Experimental conditions: volume of buffer; 7.5 mL, pH of sample; 9, amount of PEG 6000; 3.5 g, amount of sodium carbonate; 3 g, 50 ng/mL Carmoisine and Brilliant blue FCF.

**Effect of coexisting ions:** Interferences were studied in the presence of a constant concentration of Carmoisine and Brilliantblue FCF (1ng/mL) and different amounts of foreign analytes. Table 1 showed the tolerance limits of a foreign species. The

tolerance level was defined as the maximum concentration of the foreign ion causing a change in the analytical signal no higher

than  $\pm 5\%$ , when compared with the signal of 1ng/mL Carmoisine and Brilliant blue FCFalone.

Table 1. Effect of interference on extraction and determination of Carmoisine and Brilliant blue FCF.

Foreign species	Tolerance limit (ng/mL)
Glucose, Lactose, Sunset yellow	10000
Folic Acid, Vitamin A, Allure red	1000
Oxalate, Tartrate, Vitamin D, Citrate, Malic acid	500
$\text{NH}_4^+$ , $\text{Mg}^{2+}$ , $\text{F}^-$ , $\text{K}^+$ , $\text{Cu}^{2+}$ , $\text{Fe}^{2+}$ , $\text{Ca}^{2+}$ , $\text{Cl}^-$ , $\text{I}^-$	300
Ascorbic Acid, Vitamin B <sub>1</sub>	100
Vitamin B <sub>12</sub> , Vitamin B <sub>6</sub>	5

**Analytical figures of merit:** Important parameters such as the linear range (LR), precision, limit of detection (LOD), and recoveries were determined to evaluate the method performance. Under the above optimized conditions, the calibration graph was linear in the range of 0.1-120 ng mL<sup>-1</sup> with a correlation coefficient ( $R^2$ ) of 0.9996 (n=10) for Carmoisine and 0.2-260 ng/mL with a correlation coefficient of 0.9991 (n=10) for Brilliant blue FCF. The relative standard deviation (RSD) for eight replicates of 30 ng/mL was 3.17% and 1.87% for Carmoisine and Brilliant blue FCF, respectively. LOD was reached when the signal / noise (S/N) ratio was 3. The LOD for Carmoisine and Brilliant blue FCF were found to be 0.014ng mL<sup>-1</sup> and 0.017 ng/mL, respectively.

**Application of real samples:** To validate the proposed method, Carmoisine and Brilliant blue FCF contents, food samples were analyzed. Table 2 summarized the average recovery of analytes in the fortified samples. Food samples were spiked with 20 and 50ng mL<sup>-1</sup> of standard solution of Carmoisine and Brilliant blue FCF. As can be seen in Table 2, recoveries were more than 94.0%. The result indicated that the proposed method was applicable for quantitative determination of Carmoisine and Brilliant blue FCF in real samples. Table 3 showed the content of Carmoisine and Brilliant Blue FCF in strawberry jelly, fruity candy, Smarties, gummy candies, noshmak and jell gum with fruit taste samples.

Table 2. Determination of Brilliant blue FCF and Carmoisine in food samples by the proposed aqueous two-phase system (n=5).

Sample	Carmoisine				Brilliant blue FCF			
	Added (ng/mL)	Found±SD <sup>a</sup> (ng/mL)	RSD %	Recovery %	Added (ng/mL)	Found±SD <sup>a</sup> (ng/mL)	RSD %	Recovery %
Smarties	0	2.9±0.02	3.4	---	0	5.93±0.8	3.6	---
	20	23.6±0.7	3.9	103.0	20	26.28±1.4	2.7	101.8
	50	53.3±1.8	3.3	100.8	50	53.36±2.1	2.7	94.9
Jelly strawberry	0	36.7±1.2	4.1	----	0	32.79±1.6	3.3	----
	20	55.8±1.6	3.6	94.7	20	53.09±2.3	4.1	101.5
	60	96.5±2.7	4.7	99.6	60	84.09±3.2	4.2	102.7
Fruity candy	0	27.4±1.2	3.3	---	0	16.8±2.1	3.4	---
	20	46.7±1.1	2.9	96.5	20	37.1±1.6	4.1	101.2
	50	77.6±2.5	3.8	100.4	50	65.9±0.8	4.4	98.2
Gummy candies	0	34.6±2.4	2.6	----	0	23.1±1.7	3.4	----
	20	54.8±2.7	3.2	101.0	20	42.5±3.2	3.2	97.0
	50	83.2±2.8	3.9	97.2	50	74.2±3.4	2.6	102.2
Noshmak	0	45.5±1.3	3.4	---	0	0	3.7	---
	20	65.8±2.1	3.6	101.5	20	20.7±1.5	4.1	103.5
	50	95.8±2.3	3.4	100.6	50	49.28±2.1	3.6	98.6
Jell Gum with fruit taste	0	27.6±1.7	2.4	----	0	31.3±1.8	2.7	----
	20	46.8±2.2	2.6	96.0	20	50.5±2.3	3.8	96.0
	50	78.1±2.3	2.9	101.0	50	82.6±2.4	2.7	102.6

<sup>a</sup> Standard deviation

Characteristics of the proposed method were also compared to other methods used for the determination of Carmoisine and Brilliant Blue FCF. Table 4 compared the limit of detection, relative standard deviation, linear range, recovery and correlation coefficient ( $R^2$ ) of this study with differential pulse polarography (6), light-emitting diode based photocolourimeter (10), and high-

performance liquid chromatography (20). As can be seen, the proposed method provides similar quantification extraction efficiency, with advantages such as good linearity and correlation coefficient over many of the mentioned techniques. A detection limit of ATPS is lower than other mentioned method.

Table 3. Content of Carmoisine and Brilliant blue FCF in food samples.

Sample	Carmoisine ( $\mu\text{g/g}$ )	Brilliant blue FCF ( $\mu\text{g/g}$ )
Smarties	504.5 $\pm$ 8	370.7 $\pm$ 6
Jelly strawberry	2293.8 $\pm$ 13	2049.4 $\pm$ 12
Fruity candy	171.3 $\pm$ 4	105.0 $\pm$ 5
Gummy candies	1730.2 $\pm$ 11	1155.3 $\pm$ 9
Noshmak	1137.5 $\pm$ 10	-
Jell Gum with fruit taste	1380.6 $\pm$ 11	1565.0 $\pm$ 9

Table 4. Comparison of different methods for the determination of Brilliant blue FCF and Carmoisine.

Reference	Ref. [6]		Ref. [10]		Ref. [20]		This work	
	Brilliant blue	Carmoisine	Brilliant blue	Carmoisine	Brilliant blue	Carmoisine	Brilliant blue	Carmoisine
LR	-	0.02-4 (mg/L)	1-25 ( $\mu\text{g/mL}$ )	2-40 ( $\mu\text{g/mL}$ )	0.01-20 ( $\mu\text{g/mL}$ )	-	0.062-1.42 (ng/mL)	0.1-120 (ng/mL)
LOD	-	-	-	-	0.006 ( $\mu\text{g/mL}$ )	-	0.017 (ng/mL)	0.014 (ng/mL)
R <sup>2</sup>	-	0.999	0.995	0.999	0.999	-	0.999	0.999
Recovery %	-	>96	>97	>97	>90	-	>94	>94
RSD%	-	<1.5	2.83	2.4	<5.4	-	1.87	3.17

## Conclusion

In this study, aqueous two-phase system appeared to be a good choice for the determination of the Carmoisine and Brilliant blue FCF in food samples. The developed ATPS was successfully applied to the real food sample analysis, indicating that the proposed method was suitable for the extraction and determination of the effective component in food samples. The presented method had prominent advantages including instrumental simplicity, reduced reagents consumption, improved sensitivity, analytical efficiency as well as easy handling procedure.

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