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Spectrophotometric determination of carmoisine after cloud point extraction using Triton X-114

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Abstract: Carmoisine (E122) is a synthetic azo dye that is generally used to give a red color to food products. In this work, a sensitive and simple method was developed for the determination of carmoisine based on cloud point extraction. To obtain the optimum conditions for the extraction efficiency of carmoisine, the effects of different analytical parameters such as H_2SO_4 concentration, concentration of Triton X-114, and equilibrium temperature were studied. The calibration graph was linear between 0.05 and 5.0 μg/mL and the detection limit based on three times the standard deviation of the blank was 7.2 μg/L. The method was applied under the optimum experimental conditions for the analysis of carmoisine in several drink samples and a medicine. The relative standard deviations of the determinations were less than 5%. The proposed method could be used as a rapid screening method for carmoisine detection in food samples.

Key words: Carmoisine, Triton X-114, cloud point extraction

1. Introduction

Carmoisine, also named Azorubine, Food red 3, or E122 in Europe, is a synthetic dye belonging to the azo dye class. It is added to food, beverages, medicine, and cosmetics to give a red color. Synthetic dyes can give more stable color to food and because of this reason they have a wide range of applications. At present, in many developed countries some dyes are permitted for use as food coloring agents, while many others have been banned in the last two decades due to their toxicity and carcinogenicity. One of these dyes is carmoisine and it can cause potential toxicity during its transmission and metabolism in the human body and it can affect adversely and alter biochemical markers in vital organs, e.g., the liver and kidneys, not only at higher doses but also at low doses.[1–6] Accurate and reliable methods for the determination of carmoisine is a requirement to ensure and control food safety.

The structure of carmoisine is given in Figure 1.[7] Determination of food dyes in various food and in high consumption products such as beverages is an important subject in analytical chemistry and therefore various separation and preconcentration methods, including cloud point extraction (CPE),[7–10] solid phase extraction (SPE),[11,12] dispersive liquid–liquid microextraction (DLLME),[13] and determination techniques such as spectrophotometry,[2,4,14–16] differential pulse polarography,[17,18] and chromatographic methods[13,19–21] have been developed. In recent years, CPE has been used as a preconcentration and determination method in analytical chemistry, owing to several advantages such as low cost, safety, and speed. Moreover, it is a simple

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procedure with high recoveries and high preconcentration factors. Surfactants are less toxic and cheaper than organic extractants used in liquid–liquid extraction.[22,23]

In the present work CPE using Triton X-114 was developed and applied to the preconcentration and spectrophotometric determination of carmoisine in food samples. The effect of concentrations of H_2SO_4 and Triton X-114, equilibration temperature, and incubation time on the extraction of dye was studied. The presented method is comparable with the methods for carmoisine in the literature,[1,15,24–26] with short analysis time, lower use of organic solvents, and lower detection limit.

2. Results and discussion

In order to obtain quantitative recoveries of carmoisine by CPE the enrichment/separation procedure was optimized for various analytical parameters such as H_2SO_4 concentration, sample volume, Triton X-114 concentration, effects of the equilibration temperature, and incubation time. Maximum absorbance of carmoisine was obtained after CPE and found to be 520 nm.

2.1. Influences of H_2SO_4 concentration

The influences of H_2SO_4 concentration on the recoveries of carmoisine were investigated in the range of 0.2–0.7 mol L^{-1} H_2SO_4. The results are shown in Figure 2. Carmoisine was quantitatively recovered between 0.2 and 0.5 mol L^{-1} H_2SO_4. Thus, an acid concentration of 0.3 mol L^{-1} H_2SO_4 in the solution was chosen as the optimum for subsequent experiments.

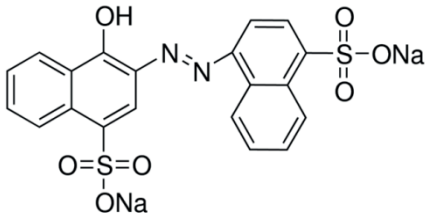

Figure 1. Chemical structure of carmoisine.

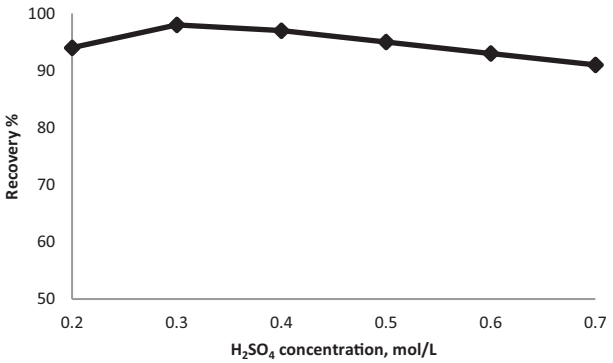

Figure 2. Effects of H_2SO_4 concentration on the recovery of carmoisine.

2.2. Effects of amount of Triton X-114

The effect of the surfactant concentration was investigated in order to ensure maximum extraction efficiency. Triton X-114 concentration was studied in the range of 0.02–0.14 mol L^{-1}. According to the results illustrated in Figure 3, the absorbance increased with increasing surfactant concentration and quantitative extraction was observed when the Triton X-114 concentration was between 0.1 and 0.14 mol L^{-1}, and then 0.12 mol L^{-1} was selected for the further works.

2.3. Effect of salt concentration

When an electrolyte is added to the system the phases separate more easily and the extraction efficiency of both the dye and surfactant increases with the salt concentration.[27] Moreover, cloud point temperature decreases,

resulting in more efficient extraction.[28] To see the effect of NaCl concentration on the recovery values of carmoisine, it was studied in a concentration range of 0.01–0.1 mol L^{-1} and the results shown in Figure 4 were achieved. Quantitative recoveries were in the range of 0.04–0.06 mol L^{-1} and 0.05 mol L^{-1} NaCl was found to be optimum.

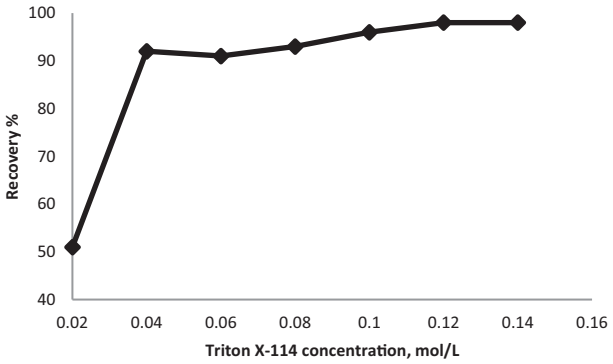

Figure 3. Effects of Triton X-114 concentration on the recovery of carmoisine.

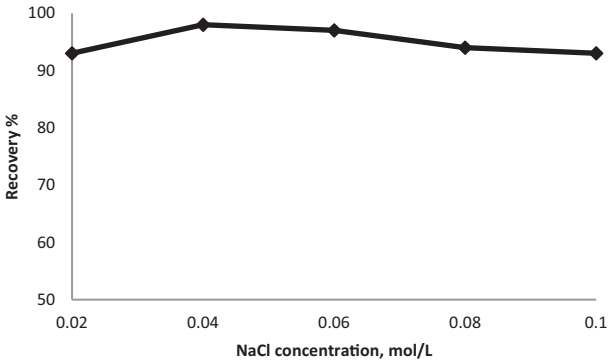

Figure 4. Effects of NaCl concentration on the recovery of carmoisine.

2.4. Effect of the equilibration temperature and incubation time

The equilibration temperature and incubation time should be investigated to achieve successful phase separation and efficient preconcentration values. To find the optimum equilibration temperature it was studied in the temperature range 30–80 °C as shown in Figure 5. The extraction efficiency of dye increases with temperature.

The recovery values were quantitative between 60 and 80 °C. Therefore, the equilibration temperature was set to 70 °C for quantitative analysis. Then to find the optimum incubation time the model solutions were heated at 70 °C in the time range of 5–45 min. The results shown in Figure 6 indicate that when the incubation time was between 25 and 45 min carmoisine was recovered quantitatively.

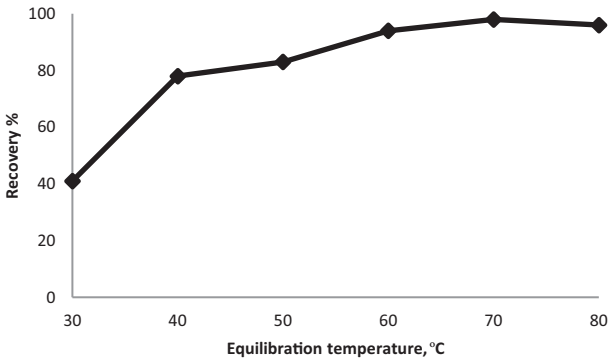

Figure 5. Effects of the equilibration temperature on the recovery of carmoisine.

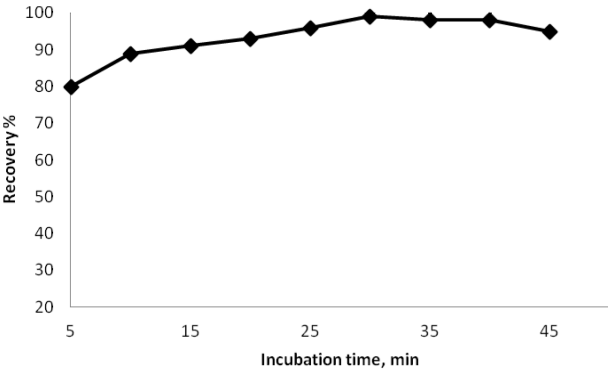

Figure 6. Effects of incubation time on the recovery of carmoisine.

2.5. Effect of sample volume

Sample volume is another important experimental parameter to be studied. In this preconcentration procedure the effect of sample volume was studied in the range of 20–50 mL. The results were quantitative between 30 and 50 mL of sample volume.

2.6. Influences of matrix ions and some other dyes on recoveries

The effect of common coexisting ions on the recoveries of carmoisine was also investigated. The proposed preconcentration method was applied to model solutions containing various ions and dyes under the optimum conditions found from the earlier experiments. The results of this study are given in Tables 1 and 2 for matrix ions and some other dyes, respectively. As can be seen from Tables 1 and 2, common existing ions and some food dyes do not adversely affect the recovery of carmoisine. It is clear that the proposed method is selective and can be used in various samples for the determination of carmoisine dye without interference.

Table 1. Effects of matrix ions on the recovery of carmoisine.

Ion	Concentration (μg/mL)	Added as	Recovery %
Na^+	2500	NaCl	98
K^+	2000	KCl	97
Mg^{2+}	20	$Mg(NO_3)_2$	96
Cu^{2+}	10	$Cu(NO_3)_2.3H_2O$	99
Fe^{3+}	10	$Fe(NO_3)_3.9H_2O$	98
CO_3^{2-}	1000	Na_2CO_3	94
NO_3^-	5000	KNO_3	99
SO_4^{2-}	2500	Na_2SO_4	98
PO_4^{3-}	1000	Na_3PO_4	97
Cl^-	4000	NaCl	98

Table 2. Effects of some other dyes on the recovery of carmoisine.

Added Dye	Concentration (μg/mL)	Recovery %
Tartrazine	0.5	97
Sunset Yellow	0.5	96
Brilliant Blue	1	101

2.7. Analytical performance of the presented method

In order to evaluate the analytical applicability of the proposed method, it was applied to the determination of carmoisine in a soft drink sample from Kayseri, Turkey. The accuracy of the presented method was tested by adding different amounts of carmoisine to the soft drink sample. The presented method given in the Experimental section was applied to the resulting solutions. The results are shown in Table 3. There was good agreement between the added and measured carmoisine amounts. Due to these quantitative values it can be said that carmoisine in real samples can be determined by the presented method. The relative standard deviations of the determinations were less than 5% and the detection limits, defined as the concentration equivalent to three times the standard deviation (N = 20) of the reagent blank, was 7.2 μg L^{-1}. The preconcentration factor of the method was 10, found by dividing the maximum quantitative sample volume, which was 50 mL, by the final solution volume, which was 5 mL. The regression equation of calibration curve was linear in the range of 0.05–5 mg L^{-1}, A = 0.03208 C + 6.434 $\times$ 10^{-3} (A: absorbance, C: concentration), r = 0.999.

Table 3. Test of addition/recovery for the application of method (N = 3).

Carmoisine added (μg)	Found (μg)	Recovery %
0	44.9 ± 0.3	-
20	64.7 ± 0.2	99 ± 1
40	82.7 ± 1.7	95 ± 4
80	120.9 ± 2.1	95 ± 2

2.8. Analyzing real samples by the presented method

The developed method was applied to the determination of carmoisine in various drink samples and a medicine sample collected from Kayseri, Turkey. The results are given in Table 4. For the preconcentration and determination of carmoisine, 0.1 g of the powder drinks was tared and dissolved in distilled water and 1 mL of soft liquid drink samples was analyzed by the recommended procedure. The medicine sample was tared and dissolved in distilled water before application of the proposed procedure.

Table 4. The levels of carmoisine in real samples (N = 5).

Samples	Concentration (μg/g)
Drink powder 1	355 ± 11
Drink powder 2	1079 ± 40
Medicine	2586 ± 124
Soft Drink Samples	Concentration (μg/mL)
Sample 1	24.0 ± 0.8
Sample 2	40.0 ± 1.5

3. Experimental

3.1. Reagents and solutions

Analytical reagent grade (Sigma, St. Louis, MO, USA) chemicals were used in this work without further purification. Triton X-114 (E. Merck, Darmstadt, Germany) was prepared by dissolving 1.0 g of Triton X-114 in 10 mL of methanol and dilution to 100 mL with distilled water in a volumetric flask. Standard stock solutions containing 1000 mg/L carmoisine (Sigma-Aldrich, Germany) were prepared daily in distilled water in calibrated flasks. The working standard solutions of carmoisine were prepared by diluting the stock solutions with water to get the desired concentration. High purity compounds were used for the preparation of stock solutions of diverse elements and dyes. High purity H_2SO_4 (E. Merck, Darmstadt, Germany) was used to prepare diluted H_2SO_4 solutions.

3.2. Instruments

A Hitachi-150-20 (Japan) spectrophotometer with a 10-mm quartz cell was used to measure the absorbance of carmoisine at 520 nm. All spectrophotometric measurements were carried out at room temperature. An Elga Elgastat Maxima (UK) water bath was used for heating the model solutions. A Human model RO 180 (Korea) was used for water purification, resulting in water with a conductivity of 1 μS/cm.

3.3. Test procedure

A cloud point experiment was carried out using model solutions prepared in centrifuge tubes. For this, 20 μg of carmoisine was added to these tubes by adding 200 μL 100 μg/mL carmoisine solution; then 1.2 mL of 0.12

mol L^{-1} Triton X-114, 1 mL of 0.2 mol L^{-1} H_2SO_4, and 200 μL of 0.01 mol L^{-1} NaCl in distilled water were added in a final volume of 25 mL. The concentration of carmoisine in the prepared model solutions was 0.8 μg/mL. The mixture was shaken and kept for 30 min in a thermostated bath at 70 °C. Phase separation of the aqueous and surfactant-rich phases was achieved by centrifugation for 10 min at 3500 rpm. After cooling the whole system in an ice bath the surfactant-rich phase and the bulk aqueous phase were easily removed with a pipette. The surfactant-rich phase was diluted to 5 mL with ethanol and then the analyte contents were determined by measuring the absorbance at 520 nm. The procedure was also applied to blank solutions and measured at the same time. Reference solutions containing 20 μg of carmoisine were prepared in 5 mL of ethanol and its absorbance was measured and recoveries were calculated by division of the sample absorbance by the reference absorbance value and by multiplying by 100; the results were given as recovery %.

3.4. Application to real samples

Fruit-flavored soft drinks and drink powders sold in markets in Kayseri, Turkey, and a medicine sample containing carmoisine were analyzed. Drink powder samples were prepared by taking 0.1 g of samples and dissolving in distilled water. Soft drink samples were prepared by taking directly 1 mL of sample and dissolving in distilled water. The medicine sample was dissolved in water after taring each pill. Then the preconcentration procedure was applied to the dissolved samples and the absorbance of the solutions was measured by UV-Vis spectrophotometer at 520 nm.

4. Conclusion

A new, simple, precise, and environmentally friendly preconcentration method for the determination of carmoisine has been established in the presented work. The method is less time consuming and inexpensive in comparison with the other preconcentration methods such as SPE and solvent extraction. The procedure offers a useful enrichment technique in various samples with acceptable accuracy and precision.

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