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Cloud Point Extraction and Spectrophotometric Determination of Allura Red (E129) in Foodstuffs

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ABSTRACT: A new cloud point extraction method was developed for preconcentration of allura red food dye as a prior step to its spectrophotometric determination by UV-Vis spectrometry. Allura red was determined at 506 nm. Extraction parameters such as H_2SO_4 and surfactant concentrations, equilibration time and temperature were investigated and optimized. Interference efects of matrix components were investigated. Preconcentration factor of the method was obtained as 25. The relative standard deviations of the method were lower than 6%. Detection limit and quantitation limit of the method were determined as 3.0 and 8.5 ng mL^{-1}, respectively. Linear calibration curve was plotted in the range of 0-6 µg mL^{-1}. Finally, the method was successfully applied to foodstuffs to determine the allura red contents of energy drink, candy, drink powder, syrup and jelly samples. Allura red concentrations of foodstuffs were determined between 9-499 µg g^{-1} and 47-231 µg mL^{-1} for solid and liquid samples, respectively.

Keywords: Allura red, cloud point extraction, foodstuffs, spectrophotometry.

Gıda Maddelerindeki Allura Kırmızısının (E129) Bulutlanma Noktası Ekstraksiyonu ve Spektrofotometrik Tayini

ÖZET: Allura kırmızısı gıda boyasının UV-Vis spektrometresi ile spektrofotometrik tayini öncesi ön deriştirme basamağı olarak yeni bir bulutlanma noktası ekstraksiyon metodu geliştirilmiştir. Allura red 506 nm'de tayin edilmiştir. H_2SO_4 ve yüzey aktif madde konsantrasyonu, denge zamanı ve sıcaklığı gibi ekstraksiyon parametreleri incelenmiş ve optimize edilmiştir. Matris bileşenlerinin analize girişim etkisi incelendi. Metodun zenginleştirme faktörü 25 olarak saptandı. Metodun bağıl standart sapması 6% değerinin altında bulunmuştur. Metodun gözlenebilme ve tayin sınır değerleri sırası ile 3.0 ve 8.5 ng mL^{-1} olarak tayin edilmiştir. 0 ile 6 µg mL^{-1} değerleri arasında doğrusal kalibrasyon eğrisi çizilmiştir. Son olarak, metot, allura kırmızı boyası içeriğini tayin etmek amacıyla allura kırmızısı boyası içeren enerji içeceği, şeker, meşrubat tozu, şurup ve jöle örneklerine uygulanmıştır. Katı ve sıvı gıda maddeleri örneklerinin allura kırmızısı konsantrasyonları sırası ile 9-499 µg g^{-1} ve 47-231 µg mL^{-1} değerleri arasında tayin edilmiştir.

Anahtar Kelimeler: Allura kırmızısı, bulutlanma noktası ekstraksiyonu, gıdalar, spektrofotometri.

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INTRODUCTION

Allura red (AR) is an azo synthetic food dye which is manufactured from petroleum and its derivatives. It has an azo group (-N=N-) and three aromatic benzene rings. Allura red has a food additive number which is E 129. It can be used in foodstuffs to give them flavor, nice appearance, stability, color, taste and smell (Bişgin et al., 2015; Rovina et al., 2016). Although allura red is not acute toxic, it has been permitted to use in foodstuffs in limited quantities and usage of AR has also been forbidden some countries (Misaghpour and Nooshabadi, 2018). Consumption of excessive amounts foodstuffs containing AR dye can lead to allergic reactions, eczema and asthma in adults. On the other hand hyperactive behavior disorder was observed in children (Bişgin et al., 2016; Yu et al., 2016). Therefore, developing accurate and reliable extraction and determination methods for the food dyes is of great importance to control and ensure the quality and safety of the foodstuffs.

Spectrophotometry is the most used and attractive technique because it is simplistic and has lower operational cost than other instrumental techniques. Spectrophotometric determination of dyes could be problem because of low levels of dyes and interference effect of matrix in real samples (Bişgin et al., 2015). Therefore, various analytical methods combined with UV-Vis spectrometry such as cloud point extraction (CPE) (Karatepe et al., 2017; Pourreza and Zareian, 2009), dispersive liquid-liquid micro extraction (DLLME) (Bazregar et al., 2018) and solid-phase extraction (SPE) (Yu and Fan, 2016) have been developed for determination of food dyes. CPE is a separation, purification and enrichment procedure which has been widely applied for detection and determination of trace harmful substance in different matrixes such as heavy metal ions (Li et al., 2017) and dyes (Heidarizadi and Tabaraki, 2016; Nambiar et al., 2017).

In CPE method, two phases are observed with phase separation (Shi et al., 2004). Above the critical temperature which is known cloud point temperature, surfactant containing solution turbids and separates two phases. First is surfactant rich phase which has small volume containing target analyte. Second is diluted aqueous phase which has large volume (Lemos et al., 2007). Due to this phenomenon analyte can be preconcentrated in a small volume and separated from complex matrix as pure (Sürme et al., 2007; Candir et al., 2008).

Aim of this paper was to develop new cloud point extraction method for detection and determination of AR food dye in different foodstuffs. For this purpose Tergitol NP-7 surfactant was used in experimental works and analytical parameters of the method were investigated and optimized. In order to validate the method, purchased foodstuffs containing AR dye from Turkish markets were analysed.

MATERIALS AND METHOD

Chemicals

The chemicals (surfactant Tergitol NP-7, H_2SO_4, NaCl, stock dye solutions, metal salts and ethanol) which have high purities were used in the experiments and purchased from Sigma (Germany) and Merck (Germany). 1000 µg mL^{-1} stock AR solution was prepared with distilled water and stored in refrigerator at 4 ºC. More diluted solutions were prepared daily with using the stock dye solution. Chemical structure of Allura red is given in Fig. 1.

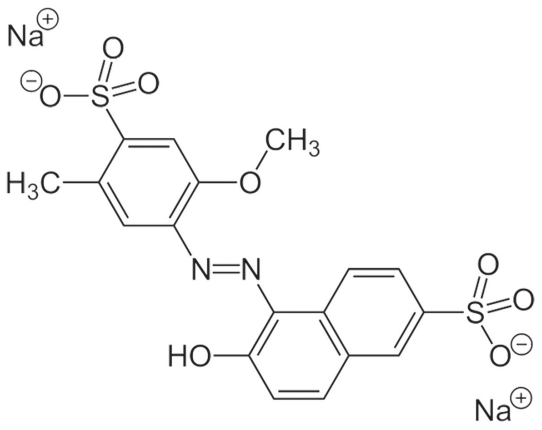

Figure 1. Chemical structure of Allura red food dye

Instruments

A Shimadzu UV-160A double beam UV–vis spectrophotometer (Japan) was used for spectrum and absorbance measurements of the AR. A Nuve BM-402 (Turkey) digital temperature and time controlled thermostatic water bath was used to achieve desired temperature. Separation of micelle and aqueous phase was performed with using Nuve NF-400 (Turkey) centrifuge. A Velp RX3 (Italy) model vortex mixture was used to dissolve surfactant rich phase.

Extraction Procedure

5 µg of AR, 5 mL of 2 mol L^{-1} H_2SO_4 solution, 12.5 ml of 4% (w/v) Tergitol NP-7 stock surfactant solution and 1.5 mL of 2 mol L^{-1} of NaCl solution were added in a 50 mL volumetric centrifuge tube. Prepared solution was placed in a thermostatic water bath at 30 ºC for 20 minutes. After then hot turbid solution was immediately centrifuged at 3500 rpm for 5 minutes. After the phase separation dilute phase was decanted. Surfactant rich phase was diluted to 2 mL of final volume with ethanol. Absorbance measurement of the solution was performed at 506 nm with spectrometer.

Preparation of Real Samples

Analyzed food samples were applied to the method after being filtered through a PTFE membrane filter (0.45 µm) and necessary dilutions with distilled water. Preparation steps of foodstuffs were given in Table 1.

Table 1. Sample preparation steps

Sample	Amount (g)	Dillution (mL)	Applied volume (mL)
Red candy	4.2014	250	25
Red jelly	3.5285	250	10
Rose hip-flavored drink powder	0.2487	250	25
Pomegranate-flavored drink powder	0.4116	250	25
Cherry-flavored drink powder	7.2338	250	25

Red energy drink and strawberry flavored syrup samples containing AR were directly analyzed using the given method after being filtered through a PTFE membrane filter (0.45 µm) and necessary dilutions.

RESULTS AND DISCUSSIONS

AR is an anionic dye in aqueous solution. Performance of CPE with using non-ionic surfactant depends on neutral form of dye and variable parameters. Therefore the optimum extraction parameters were investigated in order to provide quantitative extraction of AR dye. Determination of AR was performed at 506 nm throughout the experiments.

Effect of H_2SO_4 Concentration

Acid concentration is important to ensure enough proton for anionic AR dye molecules. Effect of H_2SO_4 concentration on the extraction of AR dye was investigated between 0.1 and 0.6 mol L^{-1}. The results are given in Fig. 2 with standard deviations.

Quantitative extraction of AR was observed in the range of 0.1 and 0.3 mol L^{-1} H_2SO_4. Recovery values decreased with increasing acid concentration higher than 0.2 mol L^{-1}. 0.2 mol L^{-1} acid concentration was chosen optimum and applied to solutions in all subsequent experiments.

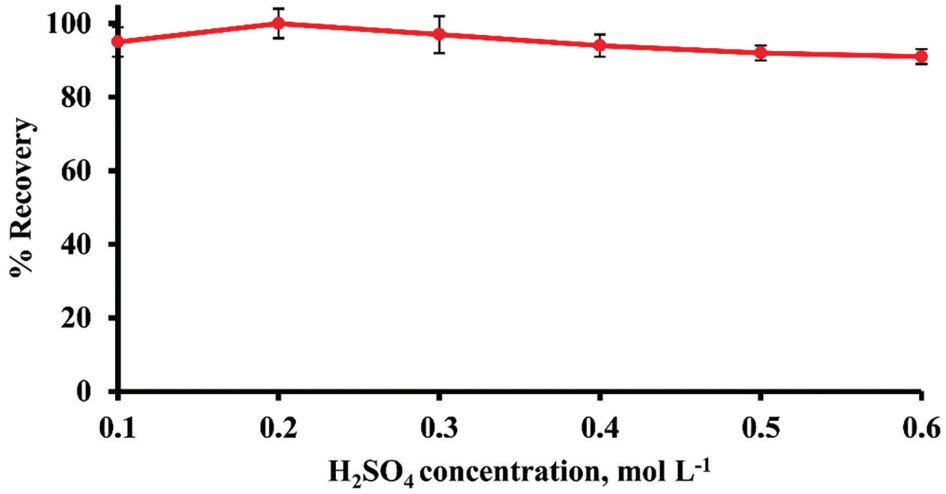

Figure 2. Effect of H_2SO_4 concentration on the extraction of AR, (1.0% Tergitol NP-7, 0.06 mol L^{-1} NaCl, 30 ºC, 20 minutes), (N=4)

Effect of Surfactant Concentration

Surfactant concentration is important factor for quantitative extraction and micelle formation. Effect of surfactant concentration was examined between 0.0% and 1.0% to provide maximum and quantitative extraction. Results are given in Fig. 3 with standard deviations. The obtained results showed that the recoveries increased up to surfactant concentration of 0.8% and remained constant above this value. This result showed that there were enough micelles in the solution for quantitative extraction. Therefore, concentration of 1.0% Tergitol NP-7 was selected as optimum.

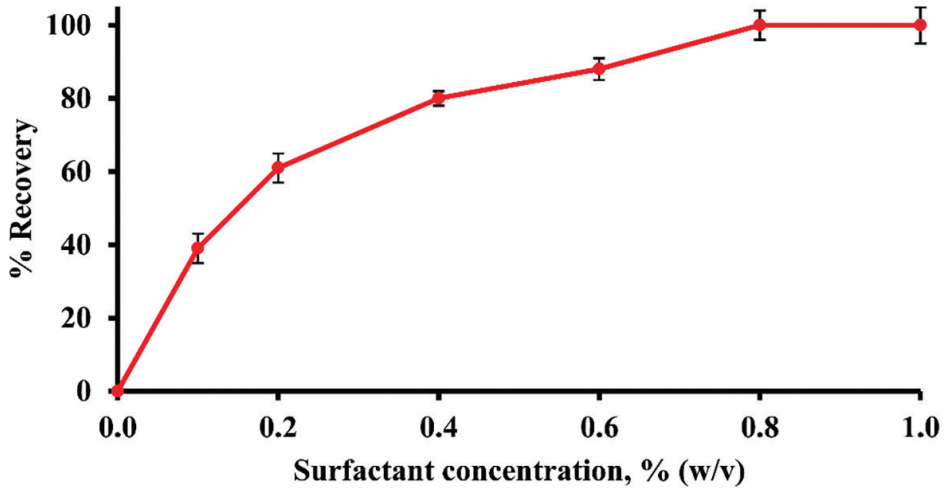

Figure 3. Effect of TNP-7 concentration on the extraction of AR, (0.2 mol L^{-1} H_2SO_4, 0.06 mol L^{-1} NaCl, 30 ºC, 20 minutes), (N=4)

Effect of Salt Concentration

In CPE studies use of salt usually increases the extraction efficiency by providing effective separation between dye molecules and aqueous phase and decreasing the cloud point temperature of the surfactant (Pourreza et al., 2011). Recovery values of AR reached from 91% to 98 % and increased with increasing salt concentrations up to 0.04 mol L^{-1} of NaCl and decreased above 0.08 mol L^{-1} of NaCl concentration. Results are given in Fig. 4 with standard deviations. Therefore 0.06 mol L^{-1} of NaCl concentration was applied the subsequent experiments.

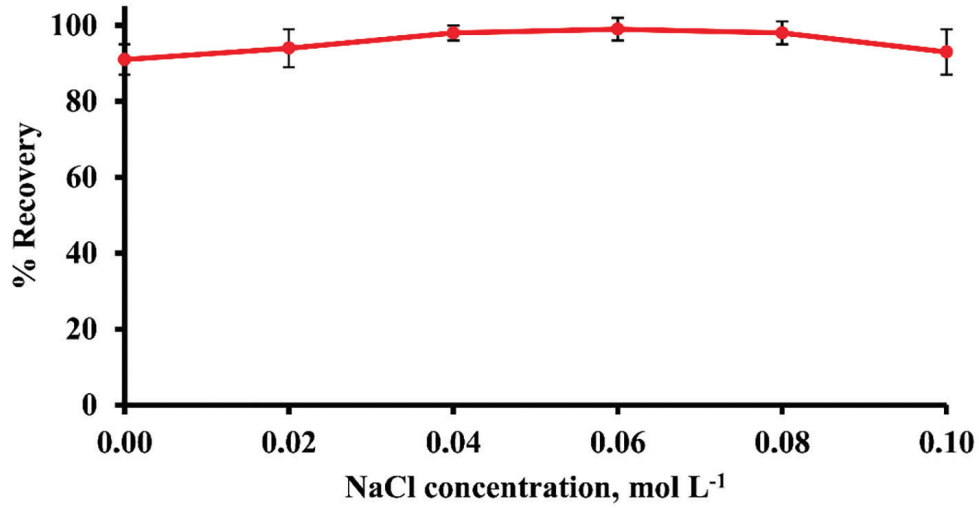

Figure 4. Effect of NaCl concentration on the extraction of AR, (0.2 mol L^{-1} H_2SO_4, 1.0% Tergitol NP-7, 30 ºC, 20 minutes), (N=4)

Effect of Incubation Time and Temperature

In CPE studies both incubation time and temperature is very important factor for formation of enough micelles in the aqueous solution. In order to find optimum temperature, incubation temperature was investigated between 25 and 50 °C. Quantitative extractions of AR were obtained between 25-35 ºC.

Recoveries of AR decreased at higher temperatures than 35 ºC. Because of this reason the cloud point temperature was kept at 30 ºC. Results are given in Fig. 5 with standard deviations.

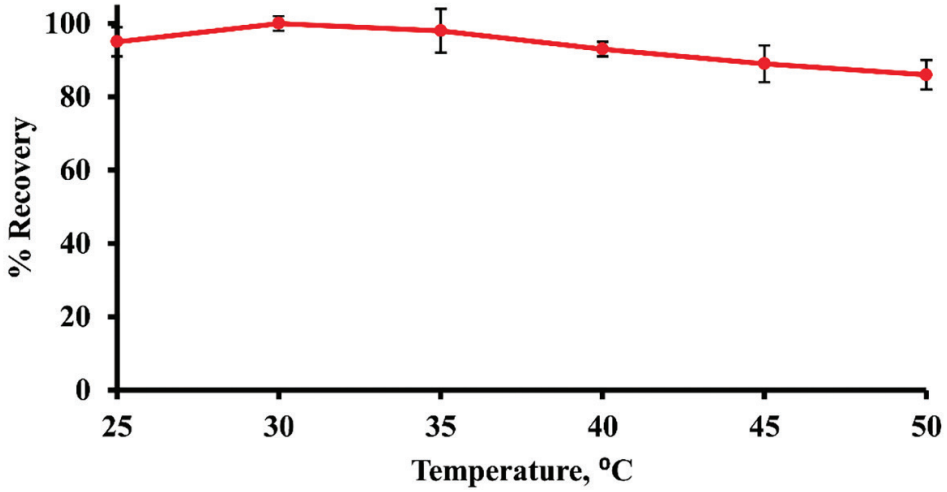

Figure 5. Effect of temperature on the extraction of AR, (0.2 mol L^{-1} H_2SO_4, 1.0% Tergitol NP-7, 0.06 mol L^{-1} NaCl, 20 minutes), (N=4)

Incubation time was investigated at 30 ºC between 5 and 30 minutes. Recovery values increased up to 15 minutes and remained constant above this value. As a result of this experiment optimum equilibration time of 20 minutes was applied in all further experiments. Results are given in Fig. 6 with standard deviations.

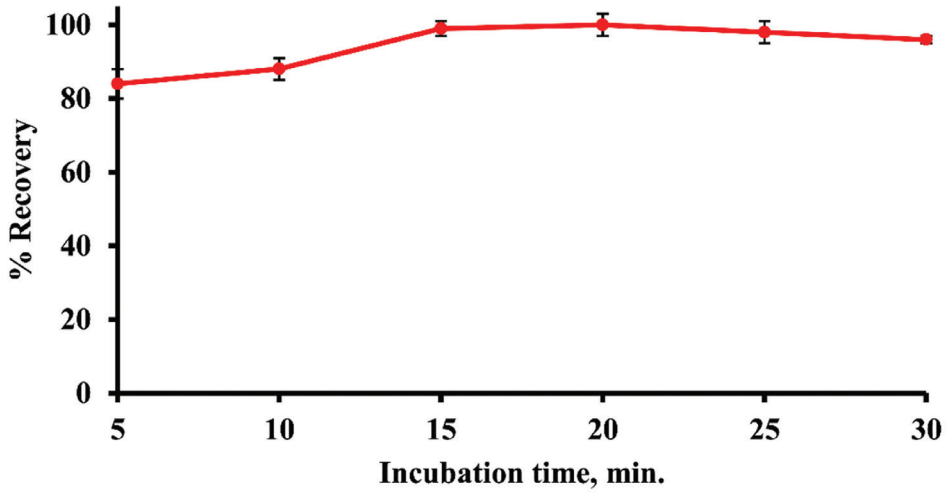

Figure 6. Effect of incubation time on the extraction of AR, (0.2 mol L^{-1} H_2SO_4, 1.0% Tergitol NP-7, 0.06 mol L^{-1} NaCl, 30 ºC), (N=4)

Effect of Sample Volume

Effect of sample volume on the extraction of AR was investigated between 10 and 50 mL. Quantitative extraction was obtained from all studied volume range by applying optimum factors of the method. According to highest sample volume, preconcentration factor was achieved as 25. Preconcentration factor was defined according to highest sample volume (50 mL) and 2 mL of final volume.

Interference Effect of Matrix Components

Interference effects of main anions, cations and some dyes which could be found together in foodstuffs were investigated.

The method exhibited good performance in presence of interfering species given concentrations in Table 2 and Table 3 for ions and organic dyes, respectively.

Table 2 Effect of matrix ions on the extraction of AR, (N=4)

Ion	Added as	Concentration (mg/L)	% Recovery
Cl^-	NaCl	1000	96±3
PO_4^{3-}	Na_3PO_4	1000	95±2
SO_4^{2-}	Na_2SO_4	100	98±4
NO_3^-	$NaNO_3$	1000	99±2
Mn^{2+}	$Mn(NO_3)_2.4H_2O$	100	97±3
Cd^{2+}	$Cd(NO_3)_2.6H_2O$	100	99±2
Co^{2+}	$Co(NO_3)_2.6H_2O$	100	99±3
Pb^{2+}	$Pb(NO_3)_2$	100	98±1
Mg^{2+}	$Mg(NO_3)_2.6H_2O$	1000	96±4
Ni^{2+}	$Ni(NO_3)_2.6H_2O$	100	100±1
Ca^{2+}	$CaCl_2$	1000	96±3
Cu^{2+}	$Cu(NO_3)_2.5H_2O$	100	96±2
Al^{3+}	$Al(NO3)_3.9H_2O$	40	97±3
Na^+	$NaNO_3$	1000	98±4
K^+	KNO_3	1000	99±2
Cr^{3+}	$Cr(NO_3)_3.3H_2O$	20	98±3

[a]Mean±standard deviation

Table 3 Interference effect of potentially interfering dyes, (N=4)

Dyes	Concentration (mg/L)	% Recovery
Sunset yellow, (λ_{max}=487 nm)	0.5	[a]102±4
Tartrazine, (λ_{max}=427 nm)	1.0	101±3
Brilliant blue, (λ_{max}=630 nm)	2.0	99±4

[a]Mean ± standard deviation

Analytical Features of the Method

Analytical features of the method were performed at the optimum conditions. The limit of detection (LOD) and quantitation limit (QOD) of the method for AR dye were determined as 3.0 and 8.5 ng mL^{-1}, respectively. Linear calibration curve was plotted in the range of 0-6 µg mL^{-1} with an equation of A=0.048C+0.0009 (R^2=0.9993) where A is absorbance unit, C is concentration of AR solution. Relative standard deviation (%RSD) values were lower than 6% throughout the experiments. Enhancement factor (EF) of the method was calculated as 25. Analytical features are given comparatively with reported CPE studies in Table 4.

Table 4. Previously reported studies on the CPE of AR with UV-Vis

Surfactant	Process	LOD, (ng mL^{-1})	EF	% RSD	Reference
TX-100, CTAB, TX-114	50 min. at 60 ºC	7.8	25	3.9	Pourreza et al., 2011
CTAB, TX-100	40 min. at 80 ºC	10.0	10	4.2	Heidarizadi and Tabaraki, 2016
Tergitol NP-7	20 min. at 30ºC	3.0	25	6.0	Present work

Real Sample Applications

Developed method was successfully applied foodstuff samples containing AR dye. AR contents of foodstuffs were determined. Analyte addition technique was also applied to real samples to prove reliability and applicability of the method. Analyze results are presented in Table 5.

Table 5 Analyte addition/recovery tests for determination of AR in foodstuffs, (N=4)

Sample	Energy drink		Strawberry-flavored syrup		Candy	
Added (µg)	Found (µg)	Recovery (%)	Found (µg)	Recovery (%)	Found (µg)	Recovery (%)
-	3.98±0.23[a]	-	5.18±0.13	-	5.23±0.06	-
2.00	5.93±0.07	98±4	7.13±0.05	98±2	7.20±0.05	99±3
4.00	7.91±0.05	98±1	9.20±0.14	100±4	9.21±0.13	100±3

[a]Mean±standard deviation

AR contents of energy drink, strawberry-flavored syrup, candy, jelly and drink powder samples were determined. The dye concentrations of the real samples are given in Table 6 as µg mL^{-1} and µg g^{-1} according to their physical (liquid or solid) form.

Table 6. AR contents of foodstuffs, (N=4)

Liquid sample	**Concentration (µg mL^{-1})**
Energy drink	47.14±1.26[a]
Strawberry-flavored syrup	231.64±2.47
Solid sample	**Concentration (µg g^{-1})**
Pomegranate-flavored drink powder	188.15±1.02
Rose hip-flavored drink powder	499.65±4.50
Cherry-flavored drink powder	241.78±1.31
Jelly	27.47±0.34
Candy	9.38±0.39

[a]Mean±standard deviation

CONCLUSION

LOD value of the method for AR dye was lower than previously reported studies. Preconcentration factor of the procedure was higher than other studies in Table 3. When the processes of the methods are compared, developed procedure has served shorter extraction time and lower operation temperature than others. Developed CPE is useful, simple, environment-friendly, reliable and has high extraction efficiency, lower cost, fast extraction, and low energy consumption.

Finally, the method may be used for routine applications in routine quality control laboratories which do not have skilled analyst and complicated instrumentation.

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