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Preconcentration and Seperation of Indigo Carmine (E132) in Food Samples by Dispersive Solid Phase Microextraction Method

Figen EREK[1*],
[1] Department of Chemistry, Faculty of Science, Dicle University, Diyarbakir, Türkiye
[*1] figen.erek@dicle.edu.tr

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Abstract: In this study, a microextraction technique was produced using Fe_3O_4@XAD-7 nanoparticle for the preconcentration and separation of Indigo Carmine (E132) in some food samples prior to spectrophotometric analysis. Firstly, optimum conditions of parameters of developed method such as pH, amount of Fe_3O_4@XAD-7 nanoparticle, type of desorption solvent, vortex time, sample volume were determined. Under the optimum conditions, the obtained results, developed solid phase microextraction method possessed excellent linearity with a correlation coefficient (R^2) of 0.999 in the concentration range of 8–250 µgL̀$^{-1}$ and detection limit are 2.1 µgL^{-1}. Relative standard deviation % (RSD%) of the method was calculated as 2.3%. Enhancement factor (EF) and preconcentration factor (PF) of the developed method were calculated to be 85 and 71, respectively. The developed method was applied to real food samples and high recoveries were obtained in the range of 98-105%.

Key words: Dispersive solid phase microextraction method, preconcentration, spectrophotometric analysis, indigo carmine.

Gıda Örneklerindeki İndigo Karminin (E132) Dispersif Katı Faz Mikroekstraksiyon Yöntemiyle Önderiştirilmesi ve Ayrılması

Öz: Bu çalışmada, Fe_3O_4@XAD-7 nanopartikülü kullanılarak spektrofotometrik analiz öncesinde bazı gıda örneklerindeki İndigo Karminin (E132) önderiştirilmesi ve ayrılması için bir mikroekstraksiyon tekniği geliştirilmiştir. Öncelikle geliştirilen yöntemin pH, Fe_3O_4@XAD-7 nanopartikül miktarı, desorpsiyon çözücüsü türü, vorteks süresi, numune hacmi gibi parametrelerinin optimum koşulları belirlenmiştir. Optimum koşullar altında elde edilen sonuçlar, geliştirilen katı faz mikroekstraksiyon yönteminin 8–250 µgL̀$^{-1}$ konsantrasyon aralığında 0,999'luk bir korelasyon katsayısı (R^2) ve 2,1 µgL^{-1}'lik bir tayin sınırı ile iyi doğrusallığa sahip olduğu bulunmuştur. Yöntemin % bağıl standart sapması (% RSD) % 2,3 olarak bulunmuştur. Geliştirilen yöntemin zenginleştirme faktörü (EF) ve önderiştirme faktörü (PF) sırasıyla 85 ve 71 olarak hesaplanmıştır. Geliştirilen yöntem gerçek gıda örneklerine uygulanmış ve %98-105 aralığında yüksek gerikazanımlar elde edilmiştir.

Anahtar kelimeler: Dispersif katı faz mikroekstraksiyon metodu, önderiştirme, spektrofotometrik analiz, indigo karmin.

1.Introduction

Indigo Carmine (E132, CI: 73015) is an organic dye known as 5,5'-indigo disulfonic acid sodium salt. It is a dye widely used in food, pharmaceutical, textile, paper, leather, plastic and cosmetic industries. It has many chemical and biological applications [1].

Some studies in the literature have reported that Indigo Carmine (IC) causes immunological, respiratory, reproductive, eye, and skin diseases, and some tumors. Due to these harmful effects on humans, its use in food is restricted. Accordingly, the accepted daily intake is 0–5 mg kg-1 [1-4].

Many different studies for analysis of Indigo Carmine in food samples have been carried out such as UV-visible spectrophotometry [2-3,5], high-performance liquid chromatography (HPLC) [6], multiple pulse amperometric [7], differential pulse voltammetry [8]. Since the use of IC in food is limited, its quantitative analysis in food is very important. In particular, the development of easy, sensitive and economical methods is of utmost importance. Spectrophotometric methods are the most preferred techniques in this respect [1-3,5]. In addition, some studies have been carried out to remove this dye, which is dispersed into the environment with industrial waste, from wastewater [9-10].

Solid phase microextraction methods are sample preparation techniques that can provide easy, fast and accurate answers and are used in many applications in the food, environmental, bioanalytical and clinical fields [11-12]. In solid phase microextraction methods, a solid adsorbent is contacted with the substance to be adsorbed

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[*] Corresponding author: figen.erek@dicle.edu.tr.[1] ORCID:0000-0002-2861-5504

in a matrix. Then, the analyte adhering to the solid adsorbent is analyzed by removing it from the solid with another suitable solvent. These suitable solvents are used in very low volumes [12-13]. Dispersive solid phase microextraction (DSPME) is a technique in which extraction is performed by dispersing the solid phase. In this method, the sorbent is dispersed to increase the adsorption of the analyte from the matrix in which it is located or to increase the contact surface with the solvent to be desorbed and to significantly reduce the time [14].

In this study, a solid phase microextraction method was developed for the preconcentration and separation of IC using magnetic amberlite XAD-7 nanoparticle, which was synthesized and characterized in our previous study [15]. The developed method was optimized by investigating several parameters such as pH, amount of magnetic XAD-7 naparticle, desorption solvent, vortex time, sample volume. The developed microextraction technique was successfully applied to industrial food samples.

2. Materials and Method

2.1 Apparatus

The spectra were recorded by a Carry 100 Bio UV-visible model double beam spectrophotometer, attached with 10.0 mm quartz cells was used for the absorbance measurements. A Toledo (Mettler) brand pH meter was used for pH measurements. A Isolab brand vortex was used to facilitate the dispersion of nanoparticles in the sample solution.

2.2 Reagents and Solutions

Indigo Carmine (>99%), Allura Red (>98%), and Tartrazin (>98%) were taken from Sigma Aldrich (St. Louis, MO, USA). 100 µg/mL of Indigo Carmine (Sigma Aldrich) prepared by dissolving distilled water. Acetonitrile, acetone, ethanol, and methanol used as desorption solvent were taken from Sigma-Aldrich (St. Louis, MO, USA). All solvents used are of HPLC grade purity (>99%). Ammonia, acetate, and phosphate buffer solutions were used for pH adjustments.

2.3 Synthesize of Fe_3O_4@XAD-7

Fe_3O_4@XAD-7 nanoparticles were synthesized as reported in the study by Erek et al [15]. 2 g of Amberlite XAD-7 was added to the solution containing $FeCl_3$ and $FeSO_4$ prepared in 2 molL^{-1} HCl solution at a ratio of 2:1. Then, 50 mL of 1 mol L^{-1} ammonia solution was added dropwise to this mixture. Magnetic nanoparticles in the mixed solution after 5 min were collected with a magnet and seperated from aqueous solution. The particles collected with magnet were washed with water and then dried.

2.4 Real sample procedure

The real samples, blue candy and blue drage chocolate, were taken from markets in Diyarbakir, Türkiye. 1.154 g and 2.323g of candy and drage chocolate samples were weighed, respectively. Then, the weighed parts were transferred to the Erlenmeyer. They were dissolved in 50 mL of water and the turbid solutions were filtered. Then, the solutions were diluted to 100 mL with pure water in volumetric flasks. Afterwards, 2mL of these prepared solutions was taken for the application of the developed method.

2.5 Analytical procedure

50 mL of 100 µgL^{-1} IC model solutions were prepared at pH 4. Then, 70 mg of magnetic XAD-7 was added to the model solutions. The solutions were then vortexed for 10 min. Immediately afterwards, the magnetic nanoparticles were collected with a strong neodymium-doped magnet and the water phase in the tubes was removed. 700 µL of desorption solvent (acetonitrile) was added to the tubes and then these mixtures were vortexed for 15 min. After the magnetic particles were collected with the magnet, the absorbance of the solutions was measured.

3. Results and Discussions

3.1. Effect of pH

One of the most important parameters affecting the adsorption and desorption behaviors of an adsorbent is pH [16]. Because pH affects surface charge distribution and the overall stability of the nanocomposite, the pH of the sample solution must be adjusted [17]. In order to determine the optimum pH at which IC is best adsorbed onto the nanoparticle, the pH was investigated in the range of 2-7. As shown in Figure 1, at pH 2 and 3, the presence of excessive H^+ ions in the environment prevents the adsorption of IC onto the magnetic XAD-7. pH 4 is the acidic environment where the optimum balance is established. After pH 4, the decrease in the stability of the nanoparticle and IC towards the neutralization of the pH of the solution may have led to a decrease in adsorption. pH 4 was selected as the optimum pH since the absorbance obtained was the highest.

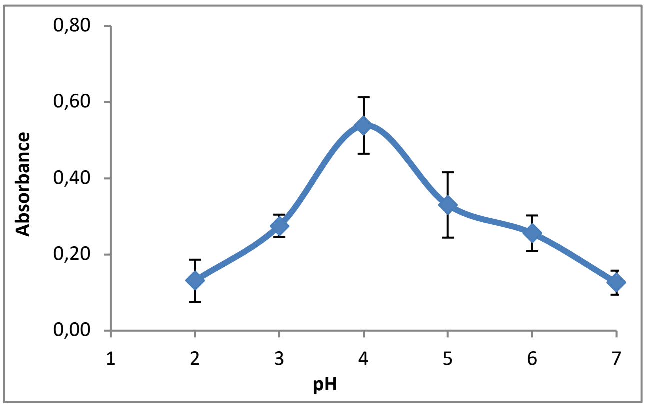

Figure 1. The effect of pH.

3.2. Effect of amount of Fe_3O_4@XAD-7

There may be π-π interactions and electrostatic interactions between the analyte and the adsorbent. In order for these interactions to reach equilibrium at optimum pH, the effect of the amount of adsorbent on the adsorption of the analyte should be investigated [16]. To investigate the effect of the amount of nanoparticles on the recovery of IC, the range of 30-100 mg was examined. As seen in Figure 2, the optimum amount of nanoparticles was determined to be 70 mg.

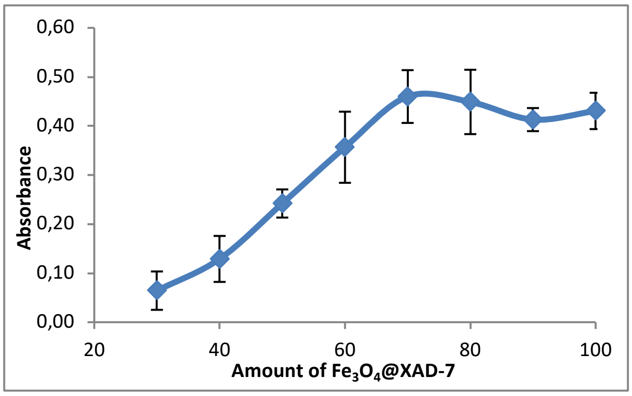

Figure 2. The effect of amount of Fe_3O_4@XAD-7.

3.3. Effect of solvent type

A suitable solvent must be determined to completely elute the adsorbed analyte from the surface of the adsorbent [16]. In order to determine the solvent that provides the highest recovery of IC in desorption, the effects of methanol, ethanol, acetonitrile and acetone were investigated. As seen in Figure 3, the best solvent was determined to be acetonitrile.

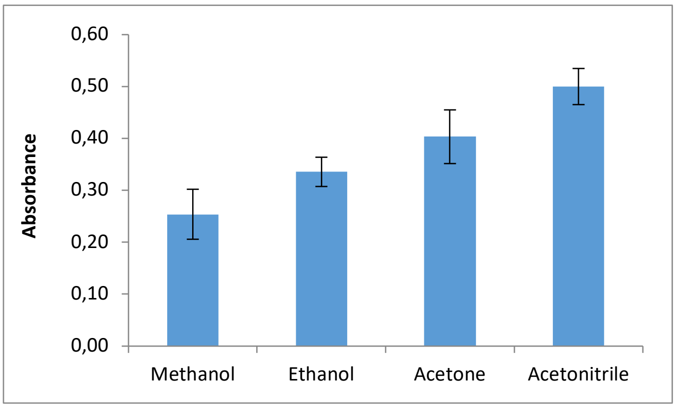

Figure 3. The effect of solvent type.

3.4. Effect of vortex time

The duration of the vortexing process was investigated as a parameter that increases the interaction of the analyte with the nanoparticle in its adsorption and with the extraction solvent in its desorption. For the adsorption of IC to the nanoparticle, it was investigated between 1-25 min and for the desorption into the organic solvent, it was investigated between 1-10 min. It was determined that the most effective vortexing time in increasing the interaction of the nanoparticle and the analyte was 10 min, and the most effective vortexing time in transferring the analyte into the organic solvent was 15 min.

3.5. Sample volume

To obtain a high preconcentration factor, the sample volume was investigated in the range of 10-50 mL [15]. After the analyte-loaded nanoparticle was separated with a magnet, it was mixed with 0.7 mL of acetonitrile, which is a desorption solvent. The optimum sample volume was found to be 50 mL. In this case, the preconcentration factor was found to be 71. All the results obtained are shown in Table 1.

Table 1. The effect of sample volume on the recovery % of IC.

Sample Volume (mL)	Recovery %
10	101
20	99
30	98
40	101
50	100

3.6. Reusability of Fe_3O_4@XAD-7 as a sorbent

To investigate the re-usability of Fe_3O_4@XAD-7 as a sorbent in magnetic solid phase extraction method, 5 mL of acetonitrile was washed three times and reused. The obtained results showed that Fe_3O_4@XAD-7 could be re-used 4 times consecutively without a decrease in the recovery of IC [16].

3.7. Matrix Effect

Some inorganic and organic species are frequently present in water and may have a signal enhancing or decreasing effect on the determination of the analyte [16]. For this purpose, the effects of Fe^{3+}, Mg^{+2}, K^{+}, and Cl^{-} ions and Allura red, eosin dyes on the recovery of IC were investigated as matrix effect. For the interference study, K^{+} and Cl^{-} ions were used at 2000 mgL^{-1}; Mg^{+2} ions at 100 mgL^{-1}; Fe^{3+} at 2.0 µgL^{-1}; Allura Red and Eosin at 1.0 mgL^{-1}, respectively. As seen in Table 2, it was observed that the examined ions and dyes did not interfere.

Table 2. Interfering species and their effects on recovery % of IC.

Interfering Species	Concentration (mg L^{-1})	Recovery %
K^{+}	2000	95.6
Mg^{2+}	100	97.8
Fe^{3+}	2	101.6
Cl^{-}	2000	95.3
Eosin	1	94.2
Allura Red	1	103.2

3.8. Analytical merits

The linear equation of the developed method was found A= 0.0034C -0.0113 with correlation coefficient R^2= 0.9993 (C: Concentration of IC (µgL^{-1}); A: Absorbance of IC). LOD and LOQ were calculated from 3s/m and 10s/m, respectively (s: standard deviation, m: slope of the calibration curve) [15]. In the method, LOD and LOQ were calculated 2.12 µgL^{-1} and 7.0 µgL^{-1}, respectively. As presented in Table 3, the linear range, preconcentration factor (PF), and enhancement factor (EF) of the developed method were found to be 8-250 µgL^{-1}, 71, and 85, respectively.

Table 3. Summary of calibration parameters obtained using the developed microextraction approach.

Parameters	Values
Linear equation	*A= 0.0034C -0.0113
R^2	0.9993
Linear range (µg L^{-1})	8-250
LOD (3 s/m) (µg L^{-1})	2.12
LOQ (10 s/m) (µgL^{-1})	7.0
RSD %	2.3
EF	85
PF	71

*C:Concantration of IC (µgL^{-1}), A: Absorbance of IC

3.9. IC content of real samples

In this study, the amount of IC used in two different industrial foods was determined. All the obtained results are shown in Table 4. According to the Turkish Food Codex, the maximum dose of IC in foodstuffs and beverages can be 500 mgkg^{-1} and 200 mgL^{-1}, respectively [18]. Total IC content of industrial foods used in the study is presented in Table 5. As seen in Table 5, the total content of IC in the analyzed foods is suitable for the Turkish Food Codex.

Table 4. Study of the accuracy of the method with real samples.

Sample	Added, µgL^{-1}	Found, µgL^{-1}	Recovery %
Candy	30	51.2	105
	60	82.3	104
	120	139.2	99.6
Drage chocolate	30	73.6	101
	60	102.1	98
	120	166.3	103

Tablo 5. The total content of the IC in the food samples.

Sample	$mgkg^{-1}$
Candy	42.68
Drage chocolate	46.60

3.10. Comparison of analytical parameters

In Table 6, the extraction or eluent solvents, linear ranges, LODs and RSDs % of some other methods in the literature are compared with those of the developed method. The RSD% of the proposed method is considerably lower than the origami microfluidic for separation, coupled by colorimetric method. The LOD of the developed method is lower than all other methods in Table 6 and is the best result. The working range (linear range) is wide. The developed method is a much more economical and easy method compared to other methods.

Table 6. Comparison of analytical parameters.

Method	Enstrument	Real samples	Extraction or eluent solvent	The linear range	LOD	RSD%	Ref.
UA-HDES-DLLME	UV-Vis	beverage, colored chocolate, chewing gum and nail polish	Ethanol	-	2.79 μgL^{-1}	0.8-2.7	[2]
cloud-point extraction (CPE) method coupled with first-derivative spectrophotometry (FDS)	UV-Vis	Edible drink and smarties samples	Triton X-100 and cetyltrimethylammonium bromide	300–1800 μgL^{-1}	48 μgL^{-1}	1.2	[19]
origami microfluidic for separation, coupled by colorimetric	three-dimensional origami microfluidic device for paper chromatography	solid jelly powder, candy, and soft drinks	-	0.07-10.0gL^{-1}	0.06 gL^{-1}	3.05-11.86	[20]
vortex-assisted magnetic dispersive solid phase microextraction	UV-Vis	Candy and drage chocolate	Acetonitrile	8-250μgL^{-1}	2.1μgL^{-1}	2.3	This work

- UA-HDES-DLLME: ultrasonic-assisted hydrophobic deep eutectic solvent based dispersive liquid liquid microextraction

4. Conclusions

A microextraction method was produced using Fe_3O_4@XAD-7 nanoparticle for the preconcentration and separation of IC in food samples. In the optimization of the method, the effects of some parameters such as pH, nanoparticle amount, vortexing time, desorption solvent type, sample volume on the recovery of IC were investigated. Under optimum conditions, the solid-phase microextraction method developed for the separation and determination of IC has a detection limit of 2.1 μgL^{-1} in the concentration range of 8–250 μgL^{-1}. The relative standard deviation (RSD%), enrichment factor (EF) and preconcentration factor (PF) of the developed method were calculated as 2.3 %, 85 and 71, respectively. This study has many advantages such as high accuracy and sensitivity for IC analysis, easy application without requiring intervention, low cost and less solvent usage. This study, with its advantages, has enabled the easy determination of IC in complex matrices. In this method, the reason for choosing Amberlite XAD-7, which is a good adsorbent in magnetic nanocomposite, is to increase the interaction with hydrophilic dye. The use of magnetic nanocomposite in the method facilitated the removal of the adsorbent from the solutions by magnets. The developed method was successfully applied to 2 different industrial foods (candy and dragee chocolate) as real samples.

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