

New Ionic Liquid Modified Polymeric Microspheres for Solid-Phase Extraction of Four Sudan Dyes in Foodstuff Samples

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ABSTRACT: The new ionic liquid modified polymeric microspheres (ILPM) were synthesized by precipitation polymerization using 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF₆]) as the functional monomer, divinylbenzene (DVB) as the cross-linker, and a mixture of toluene and acetonitrile as the dispersant solvent. The obtained ILPM showed good adsorbability to the four kinds of Sudan dyes and were successfully applied as a special sorbent of solid-phase extraction (SPE) coupled with high-performance liquid chromatography (HPLC) for the simultaneous isolation and determination of four Sudan dyes from foodstuff samples. Compared with the traditional SPE adsorbents such as neutral alumina and C₁₈, the ILPM sorbents provided higher recovery with a significant purification effect. Good linearity was obtained in a range of 0.05–4.0 μg/g ($r^2 \geq 0.9996$) for the four kinds of Sudan dyes, and the average recoveries at three spiked levels ranged from 92.5% to 106.1% with the relative standard deviations $\leq 5.7\%$. The proposed ILPM-SPE-HPLC method could be potentially applied to rapid isolation and determination of Sudan dyes in complicated food samples.

KEYWORDS: ionic liquid modified polymeric microspheres, solid-phase extraction, Sudan dyes, foodstuff, high-performance liquid chromatography

INTRODUCTION

In food industries, color additives are often added to food to enhance its visual aesthetics and promote sales. Sudan dyes are synthetic organic colorants which are extensively used for a variety of industrial and scientific applications (coloring of fuel, staining for microscopy, etc.) due to their colorfastness and low cost.¹ However, the use of Sudan dyes in foodstuffs is forbidden by global food regulations because the metabolites of Sudan dyes represent a potential risk to public health especially associated with increased occurrence of bladder cancer.² Sudan dyes are known as possible human carcinogens and mutagens and are classified as category 3 carcinogen by the International Agency for Research on Cancer (IARC). Unfortunately, a variety of foodstuffs such as chilli, tomato sauces, salami, and olive oil contaminated with Sudan dyes (particularly Sudan I) have been found recently throughout Europe and Asia.³ Also, in 2006, Sudan IV was found in some red-yolk duck eggs and related products in China. The 2006 RASFF (Rapid Alert System for Food and Feed) annual report showed contamination with either Sudan I or Sudan IV or a combination of both, in spices in the region of India and Pakistan. In order to protect the public health, a rapid, accurate, and reliable method for isolation and determination of Sudan dyes in complex foodstuffs is desired.

Currently, several analytical methods have been utilized for determination of Sudan dyes in different foodstuffs, such as liquid chromatography,⁴ gas chromatography,⁵ enzyme-linked immunosorbent assay,^{6–8} pressurized capillary electrochromatography,⁹ UV–visible spectroscopy, and ¹H nuclear magnetic resonance (¹H NMR).¹⁰ Among them, HPLC combined with mass spectrometry,^{11,12} diode array detector (DAD),¹³ and UV–vis detector^{14,15} are commonly used. No matter which determination process is selected, a simple and effective sample

pretreatment procedure is necessary in order to remove the matrix interference and reach the required low determination limits. In recent years, several new pretreatment methods including dispersive liquid–liquid extraction,^{16,17} liquid-phase microextraction,^{18,19} solid-phase dispersion extraction,²⁰ cloud point extraction,²¹ and dual solvent–stir bar microextraction²² have been reported for the pretreatment of food samples. However, solid-phase extraction (SPE)^{23,24} is still recognized as the most common pretreatment method to purify the analytes from biological and food samples, in which the adsorbent plays an important role due to its ability of isolation and purification. However, because the common adsorbents (C₁₈, C₈, silica, florisil, alumina, etc.) lack special selectivity for analytes, SPE is confronted with difficulty of extracting trace analytes from complex samples. In order to improve the extraction efficiency and purification effect, further development of new adsorbent materials is an urgent work.

Ionic liquids (ILs), which are ionic media resulting from the combination of organic cations and various anions, have attracted attention taking into account their special features, such as high electric conductivity, thermal stability, nonvolatility, a variety of distinct condensed phases, and interesting dynamical and transport phenomena.²⁵ They are amphiphilic molecules with a hydrophilic polar headgroup (ionic in nature) and a hydrophobic carbon chain (the tail) giving it an affinity for both polar and nonpolar molecules.²⁶ Although the liquid state of ILs is lost when they are immobilized on a silica surface or polymerized, the other unique properties, such as polarity

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and low volatility associated with nonpolar and ionic interactions, are maintained.²⁷ Over the past few years, ILs had been widely applied to various fields such as reaction solvents,²⁸ separation–extraction solvents,^{29,30} and electrolyte materials.³¹ In addition, as a result of their strong ion hydrogen bonding interaction with dissolved molecules, ILs had been employed to dissolve and stabilize protein.^{32,33} Recently, ILs have been used as polymeric materials for SPE,³⁴ organic surface modifiers of polymers,^{35,36} or functional monomers to prepare molecularly imprinted polymers.³⁷

The objective of this study is to synthesize the new ionic liquid modified polymeric microspheres (ILPM) by precipitation polymerization using [Bmim][PF₆] as a unique monomer and apply it as a special sorbent of SPE to simultaneous extraction of four Sudan dyes from foodstuff products. The obtained ILPM showed higher affinity to four Sudan dyes than the common SPE sorbents such as C₁₈ and alumina. The presented ILPM-SPE-HPLC method combined the advantages of ILs, porous polymer, and SPE and could be potentially applied for the rapid screening and determination of Sudan dyes in food samples.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents. Sudan I, II, III, and IV were obtained from Fuchen Chemical Co. Ltd. (Tianjin, China), and its molecular

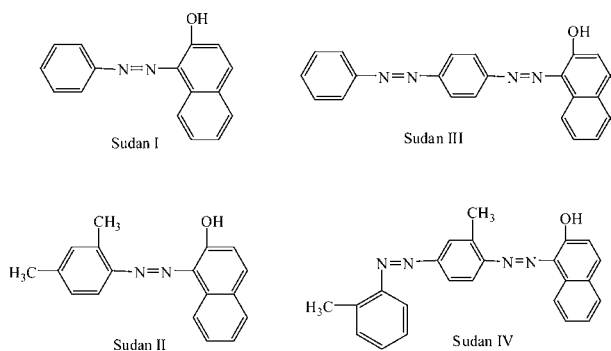


Figure 1. The chemical structures of the four Sudan dyes.

structures are shown in Figure 1. Chloroform, ethyl acetate, dichloromethane, and hexane were obtained from Huaxin Chemical Reagent Co. (Baoding, China). Acetone, methanol, acetic acid, and acetonitrile were purchased from Huadong Chemical Reagent Co. (Tianjin, China). 2,2-Azobisisobutyronitrile (AIBN) was obtained from Kermel Chemical Reagents Development Center (Tianjin, China). Divinylbenzene (DVB) and C₁₈ were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-Butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF₆]) was obtained from Chengjie Chemical Co. Ltd. (Shanghai, China). Double deionized water was filtered with a 0.45 μ m filter membrane before use.

Instrumentation and Experimental Conditions. HPLC analysis was performed using a Shimadzu HPLC system equipped with two LC-20AT solvent delivery units, an SUS-20A gradient controller, and an SPD-20A Detector (Shimadzu, Kyoto, Japan). An N-2000 chromatographic workstation (Zheda Zhineng Co. Ltd., Hangzhou, China) was used as a data acquisition system. The C₁₈ column (5 μ m, 150 mm \times 4.6 mm) was purchased from Agilent Co. Ltd. (Santa Clara, CA, USA). The mobile phase was methanol–methanoic acid (99.9:0.1, v/v), and its flow rate was set at 1.0 mL/min. The detection wavelength of the detector was set at 475 nm. The HSE-12D SPE apparatus was obtained from Hengao Tech. Co. (Tianjin, China), and a KQ3200E ultrasonic oscillator (Kunshan Instrument Co. Ltd., Jiangsu, China) was set at 40 kHz and 25 $^{\circ}$ C.

Synthesis of the Ionic Liquid Polymeric Microspheres. 1-Butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF₆]) (2.5 mmol) was dissolved in a mixed solvent of 4.0 mL of toluene and 26.0 mL of acetonitrile in a flask. DVB (12.5 mmol) and AIBN (0.15 g) were added to the above solution and then degassed ultrasonically and purged with nitrogen for 10.0 min. The flask was placed in a water bath at 60 $^{\circ}$ C under a nitrogen stream to perform the polymerization. After 24 h, the obtained particles were dried and washed by Soxhlet extraction with methanol–acetic acid (9:1, v/v) for 24 h, and then dried under vacuum at 60 $^{\circ}$ C for 24 h. The morphology of ILPM was characterized by scanning electron microscope (SEM; Hitachi S-4200 field emission SEM) and Fourier transform infrared spectrometer (FTIR; Shimadzu FTIR-8400S).

Sample Preparation of Foodstuff Samples. The chilli powder, dry red pepper, ketchup, and sausage samples were obtained from the local markets. 1.0 g of each sample was spiked with 2.0 μ g of the four Sudan dyes in a centrifuge tube (25 mL), and then were ultrasonicated with 2 \times 3 mL methanol for 5.0 min at room temperature. The homogenates were centrifuged at 4000 rpm for 5.0 min, and the supernatant solution in the centrifuge tube was collected together and concentrated to 1.0 mL for the ILPM-SPE procedure.

The Procedure of ILPM-SPE. A polyethylene frit was preplaced at the bottom of the empty cartridge (60 mm \times 10 mm), 150 mg of ILPM was packed into the cartridge, and then another frit was put on its top. The ILPM-SPE cartridge was pretreated with 5.0 mL of methanol, 1.0 mL of sample solution was loaded on, and the cartridge was washed with 4.0 mL of methanol and eluted with 5.0 mL of dichloromethane. The eluents were evaporated to dryness under vacuum and redissolved with 1.0 mL of mobile phase for further HPLC analysis.

RESULTS AND DISCUSSION

Preparation of the Ionic Liquid Polymeric Microspheres. Precipitation polymerization is an attractive, simple, and seemingly general method for producing high-quality polymer products, and it is a surfactant-free method that involves polymerization of monomers in dilute solution (<5%, w/v) in near θ -solvents. The diameters of obtained high-quality particles by precipitation polymerization are typically 1.0 μ m, which makes it potentially apply in analytical techniques such as competition assay and SPE. So a new ILPM is prepared by this procedure using [Bmim][PF₆] as functional monomer and acetonitrile–toluene (6.5:1, v/v) as dispersant solvent. Generally, the molar ratios between monomer and cross-linker play an important role in the formation of good morphology and high surface area. Therefore, the molar ratios between monomer and cross-linker of 1:3, 1:4, 1:5, and 1:6 were investigated, and the optimum molar ratio was selected at 1:5 to ensure that the shape of the particles changed from irregular to globular ones and the dispersibility increased. The ILPM particles prepared in acetonitrile–toluene (6.5:1, v/v) were nearly globular and homogeneous, while with a decrease in the percentage of acetonitrile, the shape of the particles changed from globular to irregular (Figure 2). Compared with the traditional SPE adsorbents such as neutral alumina and C₁₈, the ILPM using [Bmim][PF₆] as functional monomer provided the highest recovery with significant purification effect for isolation of Sudan dyes. Moreover, the ILPM synthesized using [Bmim][PF₆] as monomer revealed similar affinity to the four Sudan dyes as the polymer prepared by methacrylic acid as monomer. The FT-IR spectra revealed changes of the vibration peaks between the blank and ILPM (Figure 3). The spectra of the ILPM showed that there was an absorption peak at 1500 cm^{-1} –1600 cm^{-1} which corresponded to the skeletal vibration of an aromatic ring and a peak at 1550 cm^{-1} corresponding to the C–H vibration of an imidazole ring.

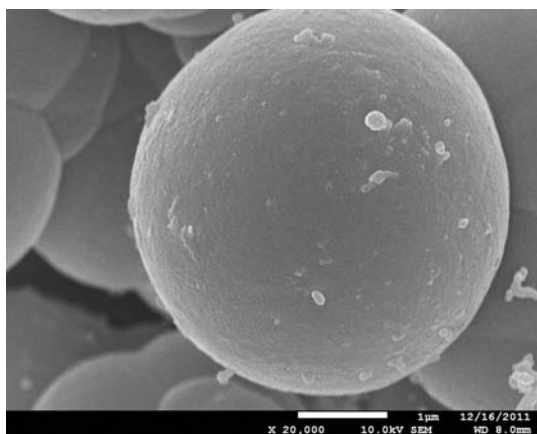


Figure 2. Scanning electron microscope (SEM) image of the ILPM.

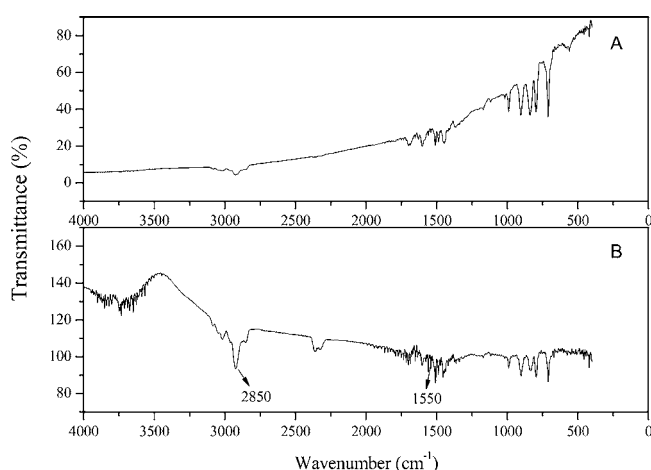


Figure 3. FT-IR spectra of the blank polymer (A) and ILPM (B).

Moreover, a strong peak at 2850 cm^{-1} meant that the $-\text{CH}_3$ groups of the $[\text{Bmim}][\text{PF}_6]$ interacted with the blank polymer.

Optimization of the ILPM-SPE Procedures. In order to measure the affinity of the ILPM to the four Sudan dyes, a comparison with neutral alumina and commercial C_{18} as SPE sorbents for pretreatment of the chilli powder samples was performed. The results (Figure 4) showed that the recovery using ILPM as SPE sorbent was much higher than that of

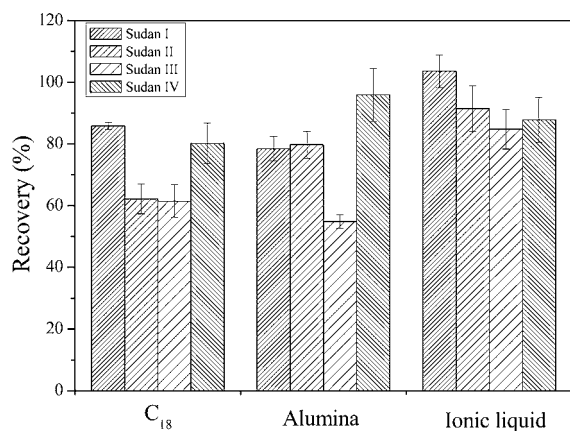


Figure 4. Effect of different SPE sorbents on the recovery of Sudan dyes.

neutral alumina and C_{18} except Sudan IV. In order to evaluate the applicability of the ILPM for the isolation of trace level of Sudan dyes in foodstuffs, general parameters of SPE (loading, washing, eluting) were optimized to achieve good selectivity and less interference. First, different extraction solvents (loading solvents) such as acetonitrile, chloroform, methanol, and hexane were evaluated, and the best extraction efficiency was provided by methanol as extraction solvent (Figure 5).

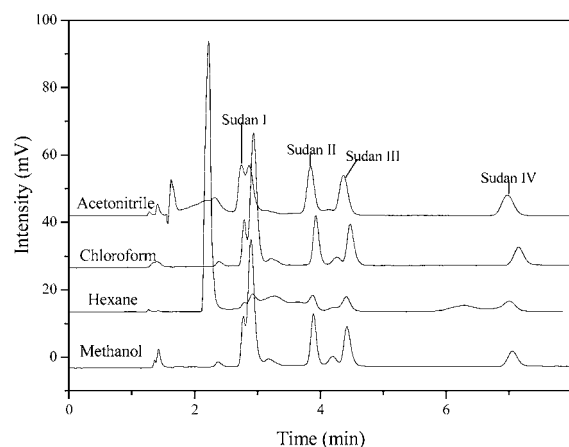


Figure 5. Chromatograms of supernatants by different extraction solvents.

Therefore, different loading volumes of methanol (1–7 mL) were investigated (Figure 6), and the best results were obtained when 1.0 mL of methanol was employed, in which all the loaded Sudan dyes were retained on ILPM cartridges.

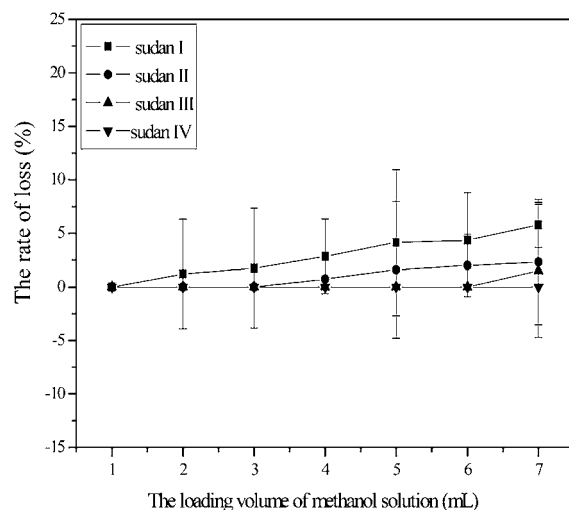


Figure 6. Effect of loading volume of methanol solution on loss rate of analytes.

The washing step was the crucial procedure to maximize the specific interactions between the analytes and ILPM, and to simultaneously decrease the interactions with the discard matrix components of the foodstuffs. Thus, a series of washing solvents including methanol, acetonitrile, hexane, dichloromethane, chloroform, acetone, and water were investigated (Figure 7). The results showed that the best recovery for the four Sudan dyes was obtained using methanol as washing solvent. Therefore, various volumes of methanol (1.0–7.0 mL)

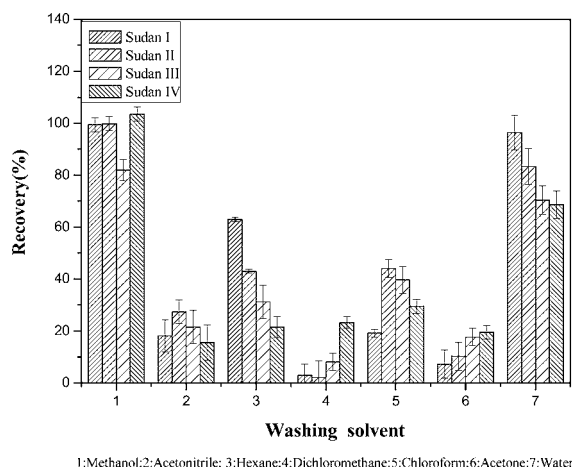


Figure 7. Effect of washing solvents on the extraction recovery of ILPM-SPE.

were tested, and the results showed that the peak areas of Sudan dyes were almost constant in a range of 1.0–4.0 mL and then decreased gradually with further increase in the volume from 5.0 to 7.0 mL. Considering the recoveries, purification efficiency, and volume of washing solvent, 4.0 mL of methanol was used as washing solution for further work.

Different elution solvents were evaluated including dichloromethane, acetonitrile, dichloromethane–acetic acid (85:15, v/v), acetonitrile–acetic acid (85:15, v/v), ethyl acetate–acetic acid (85:15, v/v), ethyl acetate–ammonia (95:5, v/v), etc. The results (Figure 8) indicated that dichloromethane allowed the

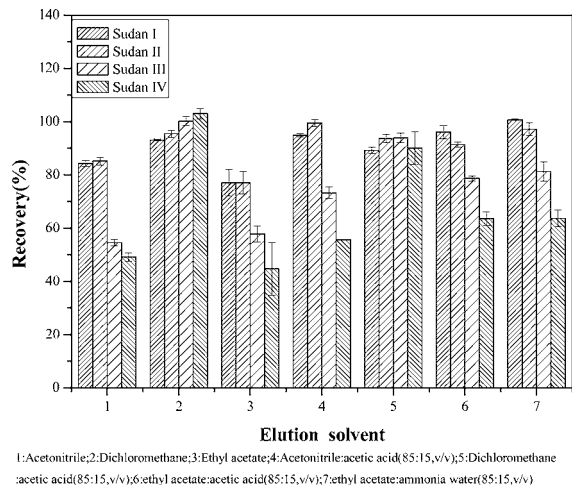


Figure 8. Effect of elution solvents on the extraction recovery of ILPM-SPE.

adsorbed Sudan dyes to be fully desorbed. For the optimization of the volume of eluent, various volumes of dichloromethane (1.0–7.0 mL) were tested in the SPE process. The results showed that the peak areas of the four Sudan dyes increased as the volume of eluent increased from 1.0 to 2.0 mL, then increasing slightly in the range of 2.0–5.0 mL, and finally leveled off in the range of 5.0–7.0 mL. To provide high and stable recovery, 5.0 mL of dichloromethane was used as elution solvent, by which Sudan dyes could be eluted from ILPM cartridges effectively.

Validation of the ILPM-SPE-HPLC Method. In order to validate the ILPM-SPE-HPLC method, linearity, correlation

coefficient, detection limits, and repeatability were tested using the spiked samples. Calibration curves of the four Sudan dyes were constructed using the areas of chromatographic peaks measured at nine increasing concentrations, in a range of 0.05–4.0 $\mu\text{g/g}$. Good linearity was obtained throughout the concentration range with correlation coefficient (r^2) ≥ 0.9996 . The limit of detection (LOD) and the limit of quantification (LOQ) at the signal-to-noise ratios of 3 and 10 were 0.004–0.014 $\mu\text{g/g}$ and 0.015–0.048 $\mu\text{g/g}$, respectively (Table 1).

Table 1. Features of the ILPM-SPE-HPLC Method

compd	regression eq	r^2	linearity ($\mu\text{g/g}$)	LOD ($\mu\text{g/g}$)	LOQ ($\mu\text{g/g}$)
Sudan I	$Y = 1.28 \times 10^5 X + 1.03 \times 10^4$	0.9998	0.05–4.0	0.004	0.015
Sudan II	$Y = 1.55 \times 10^5 X + 3.22 \times 10^3$	0.9998	0.05–4.0	0.005	0.016
Sudan III	$Y = 1.28 \times 10^5 X - 4.32 \times 10^3$	0.9998	0.05–4.0	0.007	0.024
Sudan IV	$Y = 1.05 \times 10^5 X + 2.70 \times 10^4$	0.9996	0.05–4.0	0.014	0.048

Accuracy and precision of the ILPM-SPE-HPLC method were assessed by performing replicate analyses of the spiked samples in five replicates in the same day and consecutive three days. The intraday precision and accuracy of the method evaluated as RSD was $\leq 5.7\%$, and the interday reproducibility was below 6.7% in all cases. Moreover, there were no apparent influences after the ILPM was used several times.

Analysis of Real Foodstuff Samples. In order to validate the proposed ILPM-SPE-HPLC method, fifteen kinds of chilli powder, dry red pepper, ketchup, and sausage samples obtained from the local markets in Baoding were treated with the sample preparation process and then extracted by the ILPM-SPE procedure. Only for the case of tomato ketchup did a sample contain Sudan III at a concentration of 61 ng/g, while no Sudan dyes were found in the chilli powder, dry red pepper, and sausage samples. Moreover, the ILPM adsorbent could remove the potential matrix interference and preconcentrate the four Sudan dyes rapidly and effectively (Figure 9). The accuracy of the method was evaluated by a recovery test carried out with three different spiked levels of Sudan dyes in chilli powder samples. The results in Table 2 show that the recoveries ranged between 92.5% and 106.1% for the four Sudan dyes, which indicated that the method was reliable and could be used for

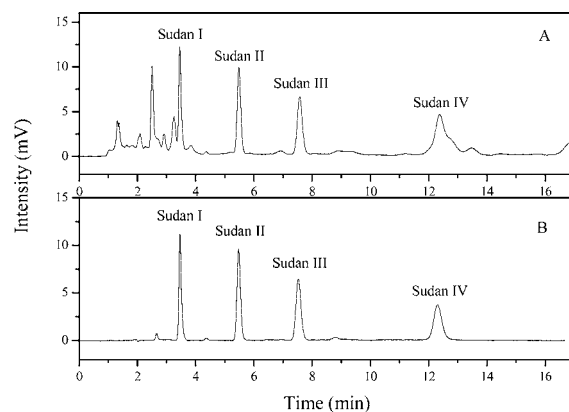


Figure 9. The chromatograms of chilli powder sample before and after ILPM-SPE: (A) before ILPM-SPE; (B) after ILPM-SPE.

Table 2. Recoveries of Sudan Dyes in Spiked Chilli Powders after ILPM-SPE

spiked levels	0.05 µg/g		0.5 µg/g		4.0 µg/g	
	recoveries (%)	RSD (%)	recoveries (%)	RSD (%)	recoveries (%)	RSD (%)
Sudan I	92.5	2.1	97.1	4.3	99.5	5.3
Sudan II	98.2	5.7	96.3	2.4	99.6	3.7
Sudan III	106.1	2.2	93.0	3.4	100.0	4.8
Sudan IV	101.7	5.6	101.9	4.1	100.6	3.0

the determination of trace levels of Sudan dyes in complicated foodstuffs.

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Notes

The authors declare no competing financial interest.

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