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Molecularly imprinted microspheres as SPE sorbent for selective extraction of four Sudan dyes in catsup products

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ABSTRACT

A highly selective molecularly imprinted solid-phase extraction (MISPE) coupled with high performance liquid chromatography (HPLC) ultraviolet–visible detection was developed for the simultaneous isolation and determination of four Sudan dyes (I, II, III and IV) in catsup products. The novel molecularly imprinted microspheres (MIM) were synthesized by aqueous suspension polymerization using phenylamine and naphthol as template, which showed high affinity to Sudan dyes in aqueous solution. In order to develop a selective extraction protocol for simultaneous determination the four Sudan dyes from catsup products, the molecular recognition properties of MIM as a SPE sorbent were evaluated. Under the optimized condition, good linearity was obtained from 0.01 to 2.5 $\mu g\,g^{-1}$ ($r^2 \geq 0.9990$) with the relative standard deviations of less than 3.4%. This proposed MISPE-HPLC procedure eliminated the effect of template leakage on quantitative analysis and could be applied to direct determination of four Sudan dyes in complicated food samples.

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1. Introduction

Sudan dyes are synthetic industrial azo-dyes, traditionally used in waxes, plastics, oils, and polishes for their colorfastness and economical price. The presence of Sudan dyes in foodstuff is forbidden in any national and international food regulation act because they have been classified as a possible human carcinogen [1,2]. Notwithstanding, these dyes are often illegally used to enhance the appearance of products such as chili, tomato sauces, salami, olive oil, and many other frequently eaten foodstuffs [3–5]. In view of the above incidents, there is a pressing need to develop fast and sensitive methods for the simultaneous determination of Sudan dyes that contaminate food.

Several analytical methods based on liquid chromatography with ultraviolet/mass spectrometry [6–11] or on gas chromatography–mass spectrometry [12] have been developed for analysis of the Sudan dyes. Although these methods have been successfully applied to analysis of the Sudan dyes at trace levels in food products, they all suffered from the complexity of sample matrix and low concentration of Sudan dyes in real samples. Hence, a simple and effective sample pretreatment step is required for isolation and enrichment of the analytes. Conventional sample preparation method for Sudan dye analysis is liquid–solid extraction. However, this method suffers from the waste of organic solvents, is time-consuming and has potential toxicity hazards [13]. Other sample preparation methods, such as solid-phase extraction (SPE) [14,15], liquid phase microextraction [16], matrix solid-phase dispersion [17], dual solvent-stir bars microextraction [14], ultrasonic-assisted extraction [18] and centrifugal sedimentation [19,20] are also reported for Sudan dyes analysis. However, the selectivity of these pretreatment procedures needs to be further improved.

Molecular imprinting is considered as an elegant and convenient technology that can introduce special recognition and binding sites in imprinted materials, which are geometrically and chemically complementary to the template [21]. Compared to biological counterparts, molecularly imprinted polymers (MIPs) are more stable, less costly, and easier to produce [22]. Their use as sorbent material for SPE is one of the most exciting applications of MIPs because it would provide a simple and effective pretreatment method for complex samples. The traditional MIPs for Sudan dyes analysis are prepared by using one kind of Sudan dyes (most frequently Sudan I) as template[4,5,23,6], which may be influenced by template leaking when using MIPs as SPE sorbents to extract Sudan dyes from food samples.

In the present work, the new molecularly imprinted microspheres (MIM) was prepared through aqueous suspension polymerization using phenylamine–naphthol as template, methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the cross-linking agent. The obtained MIM showed high affinity to Sudan dyes and was successfully applied as special SPE

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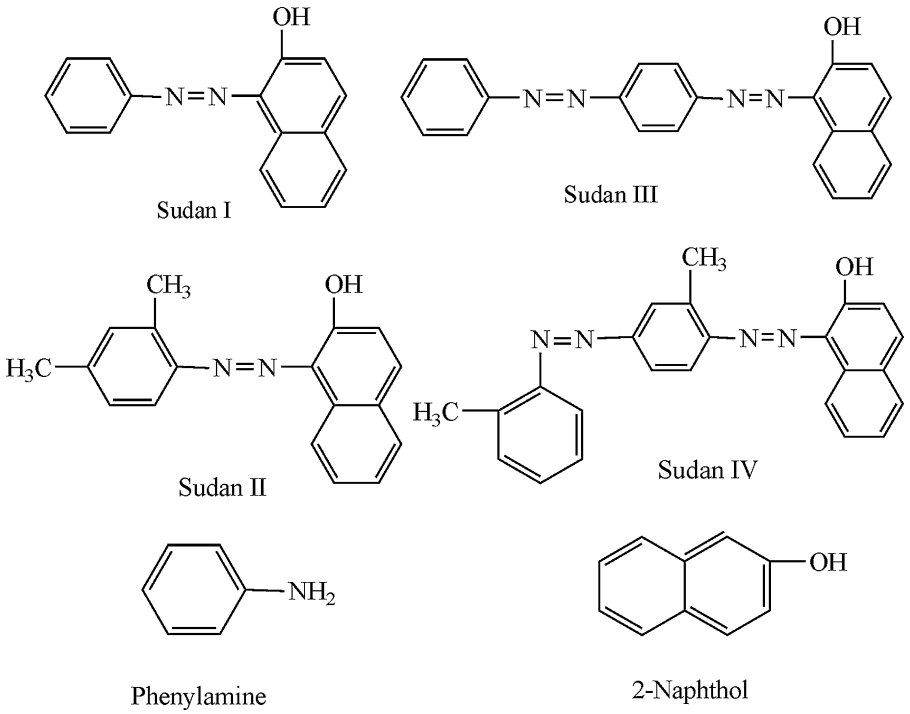

Fig. 1. Structures of template molecules and Sudan dyes.

sorbent for selective extraction of Sudan dyes from catsup products. The presented molecularly imprinted solid-phase extraction (MISPE) could be applied for the selective separation and quantitative determination of the four Sudan dyes in complicated food samples.

2. Experimental

2.1. Chemicals and reagents

Ethylene glycol dimethacrylate (EDMA) was from Shanghai Trading Co. Ltd. (Shanghai, China) and extracted with 2.0 mol L^{-1} NaOH solution then dried over anhydroxide magnesium sulfate. Methacryclic acid (MAA) from Tianjin No. 1 Chemical Reagent Factory (Tianjin, China) was purified by distillation to remove inhibitor. Sudan I, II, III and IV were purchased from Fuchen Chemical Co. Ltd. (Tianjin, China) (Fig. 1). Phenylamine, 2-naphthol, and 2, 2′-Azobisisobutyronitrile (AIBN) from Beijing Chemical Reagent Company (Beijing, China) were recrystallized prior to use. All the other reagents used in the experiment were of the highest grade commercially available. Double deionized water was filtered with 0.45 μm cellulose acetate filter membrane (Millipore, Billerica, MA, USA) before use.

2.2. Instrumentation and experimental conditions

High performance liquid chromatography (HPLC) analysis was performed using a LC-20A system equipped with two LC-20AT Solvent Delivery Units, a SUS-20A gradient controller and a SPD-20A Ultraviolet–Visible (UV) detector (Shimadzu, Kyoto, Japan). An N-2000 data workstation (Zheda Zhineng Co. Ltd., Hangzhou, China) was used as a data acquisition system. The HSE-12D SPE Apparatus was from Hengao Tech. Co. Ltd. (Tianjin, China) and an ultrasonic cleaner (KQ3200E, Kunshan Instrument Co., Jiangsu, China) was set at 40 kHz. The analytical column (Eclipse CSB-C18, 150 mm × 4.6 mm I.D., 5 μm) was purchased from Agilent Technologies Co. Ltd. (California, USA). The mobile phase was methanol–formic acid (99.9:0.1, v/v) and its flow rate was set at 1.0 mL min^{-1}. The injection volume was 10 μL for all the solutions and the wavelength of UV detector was set at 475 nm.

2.3. Synthesis of the MIM

The template phenylamine (0.18 mL), 2-naphthol (0.29 g) and MAA (0.69 mL) were dissolved in 20 mL of chloroform, and put into a refrigerator for 30 min to facilitate template-monomer self-assembly, and then EDMA (3.76 mL) and AIBN (200 mg) were added and sonicated for 5.0 min to fully dissolve. Then it was dropped slowly in 120 mL of water solution (contained 3.0 g of polyvinylpyrrolidone). The polymerization was performed at 60 °C for 24 h. After polymerization, the solution was filtered and the MIM were packed into a chromatography column, followed by washing with methanol–acetic acid (9:1, v/v) at the rate of 0.3 mL min^{-1} for 24 h, then it was rinsed by water and methanol respectively at the same rate for another 12 h, thus ensure to remove the template molecules and residual monomers completely. The non-imprinted microspheres (NIM) was prepared and processed in the same manner, but the template omitted.

2.4. Binding experiments

To evaluate the binding capacity of the MIM, static adsorption test was carried out in acetonitrile solution. 30 mg of MIM or NIM polymer was equilibrated with 5.0 mL of acetonitrile solution containing Sudan dyes at various concentrations ranging from 0.0625 to 10.0 μg mL^{-1}. The mixture was slightly shaken on a horizontal shaker for 12 h at room temperature. Finally, the concentrations of Sudan dyes were determined by HPLC-UV detection. The adsorption quantity (B) was calculated by subtracting the amount of free Sudan dyes in the supernatant from the initial amount of Sudan dyes.

2.5. The pretreatment procedure of catsup products

The catsup samples which were obtained from the local supermarkets in Baoding were spiked with different levels of Sudan dyes in a range of 0.01–2.0 μg g^{-1} (based on the detection limit (10 μg kg^{-1}) of National Standard of China GB/T 19681-2005). 1.0 g of spiked sample was homogenized in 5.0 mL of acetonitrile and ultrasonicated for 3.0 min at room temperature. The homogenate was centrifuged at 4000 rpm for 5.0 min and the obtained supernatant solution was collected. The extraction process was repeated in triplicate, all the obtained supernatant solution were mixed together and concentrated to 1.0 mL for further MISPE procedure.

2.6. MISPE procedures

500 mg of MIM and NIM particles were packed into empty polypropylene cartridges (60 mm × 10 mm) with two glass–wool frits at each end. The cartridges were rinsed with 5.0 mL of chloroform and methanol respectively, and then conditioned with 3.0 mL acetonitrile before use. 1.0 mL of sample solution was loaded onto MISPE and NISPE cartridges, respectively. The cartridges were washed with 3.0 mL of methanol–water (3:7, v/v) to eliminate interferences that retained by non-specific adsorption and eluted with 5.0 mL of acetonitrile–dichloromethane–acetic acid (47.5:47.5:5, v/v/v). The eluents were evaporated to dryness under vacuum and reconstituted with 0.5 mL mobile phase for further HPLC analysis.

3. Results and discussion

3.1. Preparation and characteristic of the MIM

A highly selective MIM for Sudan dyes was prepared by aqueous suspension polymerization using MAA as functional monomer and EDMA as the cross-linking agent in which the thermopolymerization procedure was employed at 60 °C for 24 h. Though several MIPs had been synthesized using one kind of Sudan dyes as template [4,5,23,6], the template leaking was almost inevitable in its real application, which affects the results of quantitative analysis. Considered the basic structure of Sudan dyes including benzene, naphthol and azo group, phenylamine and naphthol were selected as template to synthesize the MIM, which eliminated the effect of template leaking on quantitative analysis of Sudan dyes. Generally, the affinity and imprinting effect of MIM toward its template molecule was affected by the molar ratios between template and monomer in the synthesis of MIM. The porogen volume was also an important factor that influenced both the solubility of template-monomer mixture and the morphology of polymers. Therefore, different ratios of template-monomer-crosslinker with various porogen solvents were tested in our experiment and the results revealed that MIM prepared at ratio of 1:4:10 with 20 mL of chloroform showed satisfactory mechanical strength and better affinity to the four Sudan dyes. The scanning electron microscope image indicated the MIM was monodispersed and uniformly spherical with regular morphology, which could confer better mass transfer and improved the extraction efficiency of MISPE.

The imprinting effect was evaluated by the static binding experiments in which the MIM and NIM were incubated with different concentrations of Sudan dyes (0.0625–10.0 μg mL^{-1}). Fig. 2 showed that the binding amount of MIM increased with the increasing of the initial concentration of Sudan dyes, and displayed higher binding amount for target analytes than the NIM. Furthermore, when MIM and NIM be used as SPE sorbents for Sudan dyes, though the maximum sample loading volume was similarly, methanol could elute more than 82% Sudan dyes out from NIM cartridge, while only few

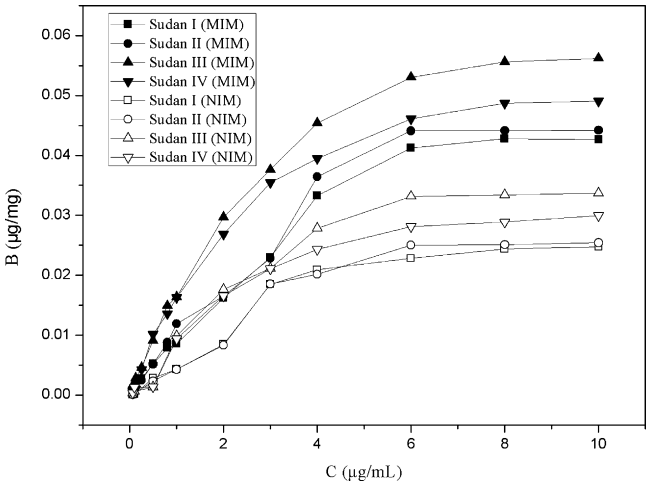

Fig. 2. Binding capacity of the MIM and NIM.

Sudan dyes were eluted out the MIM cartridge. The weak adsorption of analytes on NIM was due to non-specific interaction with the polymer matrix. Additionally, the acid had obviously effect on the affinity between analytes and MIM, and methanol–acetic acid (9:1, v/v) could completely destroy the imprinted recognitions and obviously decreased the affinity of MIM to similar value with NIM.

3.2. Optimization of the MISPE procedures

In order to obtain the optimum selectivity and recovery, the selection and volume of loading, washing and elution solvents were optimized. Different loading solvents such as acetonitrile, chloroform, dichloromethane and hexane were investigated and the results in Fig. 3 showed that the most loaded Sudan dyes were retained on MIM when acetonitrile as loading solvent.

To interrupt the non-selective interactions with the interferences present in the sample matrix, different washing solvents including methanol, acetonitrile, water, acetonitrile–water (1:1, and 3:7, v/v), and methanol–water (1:1, and 3:7, v/v) were evaluated. The results in Fig. 4 showed that methanol and acetonitrile had higher elution strength, which resulted in a lower recovery. Although water as the washing solvent provided the best recoveries nearly 100%, the impurities elimination efficiency was not sufficient. Moreover, the recoveries of the four Sudan dyes were not

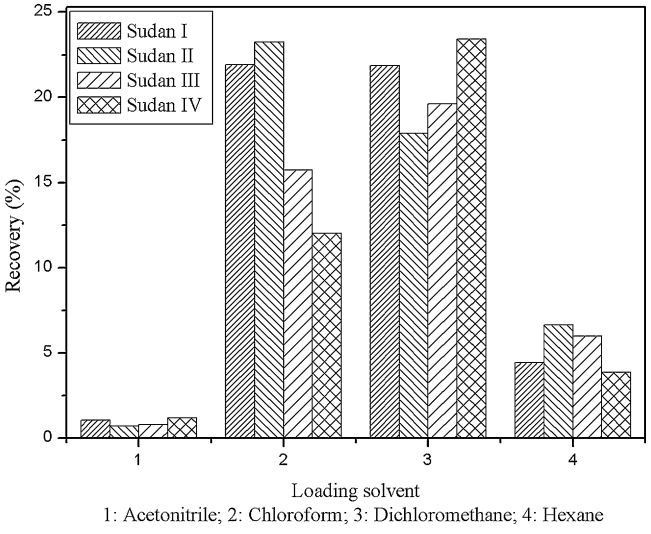

Fig. 3. Loss ratio of Sudan dyes in loading fraction.

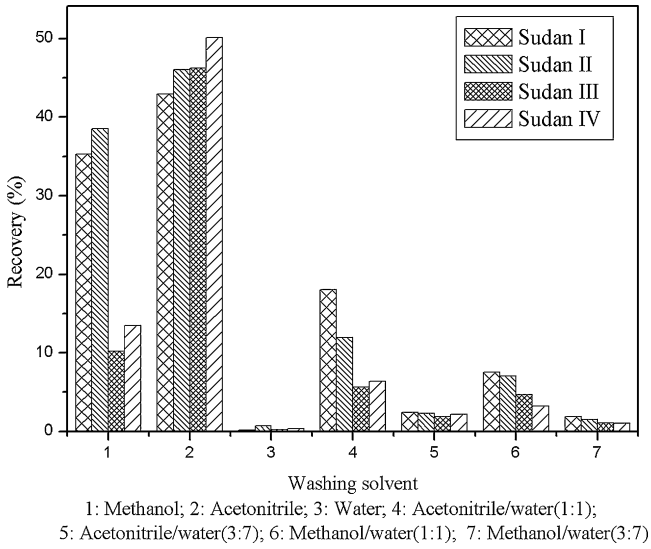

Fig. 4. Effect of washing solvents on the loss of Sudan dyes.

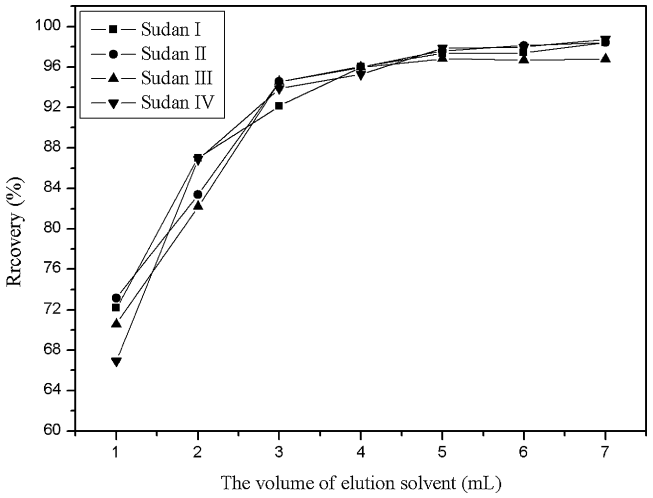

Fig. 6. Effect of volume of elution solvent on extraction efficiency of MISPE.

obviously decreased (ranged from 96.6% to 98.7%) with the increasing volume of methanol–water (3:7, v/v) from 1.0 mL to 5.0 mL. Considering the recoveries, impurities elimination efficiency and economic factors, 3.0 mL of methanol–water (3:7, v/v) was used as the washing solution for further work.

The elution solvent was also a key factor that affected the recovery. Different elution solvents were investigated to identify its influence on desorption of Sudan dyes from the MIM cartridges and the results of Fig. 5 indicated that acetonitrile–dichloromethane–acetic acid (47.5:47.5:5, v/v/v) as elution solvent had the best elution ability. Different volumes of acetonitrile–dichloromethane–acetic acid (47.5:47.5:5, v/v/v) (1.0–7.0 mL) were tested and the results of Fig. 6 demonstrated that its volume lower than 5.0 mL was not sufficient to elute the analytes completely from the cartridges. Therefore, 5.0 mL acetonitrile–dichloromethane–acetic acid (47.5:47.5:5, v/v/v) was chosen as the elution solvent for further work.

3.3. Methodology of the MISPE-HPLC method

The developed MISPE-HPLC method was evaluated by the linearity, precision, repeatability, recovery, detection limits,

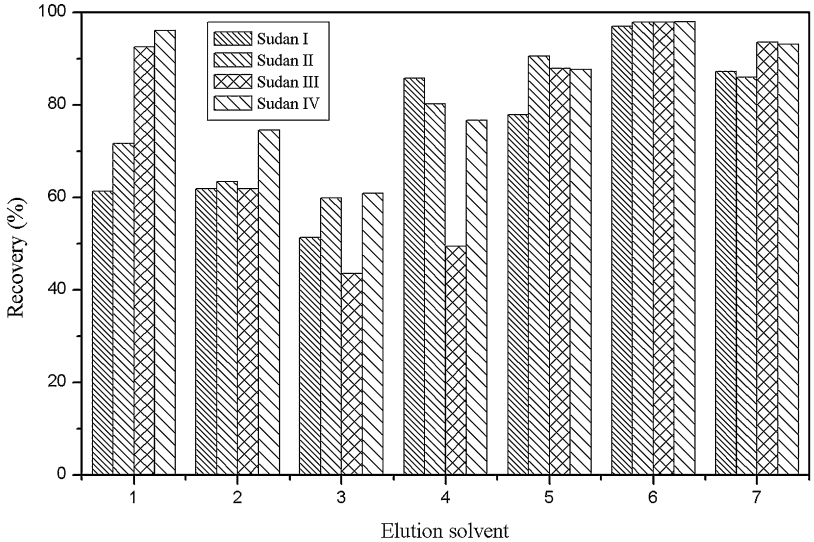

Fig. 5. Effect of elution solvents on the recovery of Sudan dyes.

Table 1
Features of the MISPE-HPLC method.

Analytes	Regression equation	r^2	RSD (%)	LOD ($\mu g\,g^{-1}$)	LOQ ($\mu g\,g^{-1}$)
Sudan I	$Y = 1.10 \times 10^4 X - 1.25 \times 10^3$	0.9990	3.4	0.002	0.007
Sudan II	$Y = 1.63 \times 10^4 X - 2.81 \times 10^3$	0.9990	2.7	0.004	0.012
Sudan III	$Y = 1.64 \times 10^4 X - 2.57 \times 10^3$	0.9994	2.4	0.005	0.016
Sudan IV	$Y = 1.18 \times 10^4 X - 1.01 \times 10^3$	0.9993	2.0	0.007	0.021

Table 2
Recoveries of Sudan I–IV in catsup samples after MISPE-HPLC.

Spiked level of analytes	0.01 μg g^{-1}		0.50 μg g^{-1}		2.0 μg g^{-1}	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Sudan I	101.5	4.2	102.1	2.3	98.9	2.4
Sudan II	86.3	3.5	96.7	4.6	95.2	3.9
Sudan III	94.8	3.1	98.5	3.4	96.1	5.1
Sudan IV	102.9	3.9	91.4	2.7	102.2	4.6

inter-assay and intra-assay deviation under the optimum condition. Calibration curves were established by plotting the chromatographic peak areas versus the concentrations (0.01–2.5 μg g^{-1}) of each standard solution to perform a linear regression analysis (Table 1). Good linearity was obtained for the four Sudan dyes with the correlation coefficient (r^2) ≥0.9990 and the detection limits based on S/N = 3 was between 0.002 and 0.007 μg g^{-1}, which bellowed the detection limits of the national standards of China GB/T 19681-2005. Accuracy and precision of the MISPE-HPLC method were assessed by performing replicate analyses of the spiked samples in five replicates in the same day and consecutive three days. The intra-day precision and accuracy of the method evaluated as RSD were ranged from 2.1% to 4.6% and the inter-day reproducibility was less than of 6.2%. Additionally, chromatogram (Fig. 7) of spiked sample indicated that MISPE exhibited cleaner eluents without interferences, which demonstrated the high selectivity and affinity of the MIM toward the target analytes.

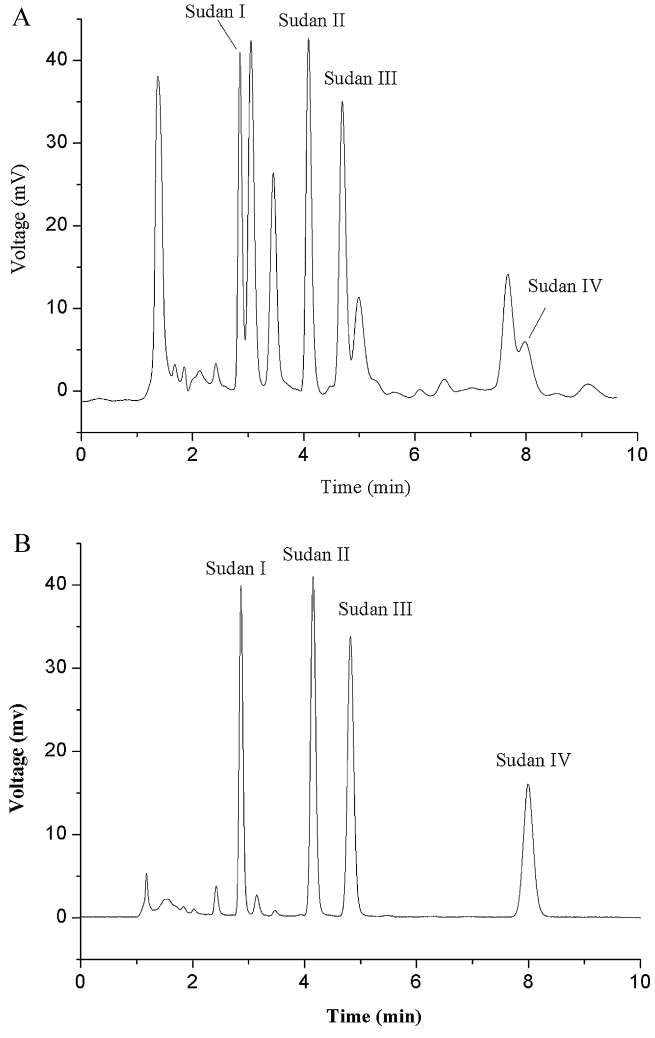

Fig. 7. Chromatograms of the spiked catsup sample (A: before MISPE; B: after MISPE).

3.4. Application to catsup products

To evaluate the accuracy and application of the MISPE-HPLC method for the selective extraction and determination of Sudan dyes in real samples, five different brands of catsup products were purchased from local markets and dealt with the proposed method. No Sudan dyes were observed in any of the catsup products, which demonstrated that the misapplication of the four kinds of Sudan dyes in catsup products at local area was not extensive. Therefore, the catsup products spiked with Sudan dyes at 0.01, 0.5 and 2.0 μg g^{-1} were analyzed. The results in Table 2 indicated that the recoveries of the four Sudan dyes were ranged from 86.3% to 102.9% with RSD less than 5.1%. Three blank catsup samples were also processed and no interferences from the endogenous components of catsup matrix were observed at the retention time of the analytes, which demonstrated the good application of the MISPE-HPLC method.

4. Conclusion

A highly selective MISPE-HPLC method was developed for the simultaneous isolation and determination of four Sudan dyes (I, II, III and IV) in catsup products. The novel MIMs were synthesized by aqueous suspension polymerization using phenylamine and naphthol as template, which showed high affinity to Sudan dyes in aqueous solution. The presented MISPE-HPLC method combined the advantages of MIM and SPE, which could be potentially applied for the simultaneously determination of Sudan dyes in complicated food samples.

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