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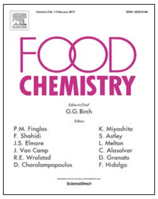

Analytical Methods

γ-Fe_2O_3 magnetic nanoparticle functionalized with carboxylated multi walled carbon nanotube for magnetic solid phase extractions and determinations of Sudan dyes and Para Red in food samples

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ABSTRACT

Hybrid nanostructures composed of γ-Fe_2O_3 (maghemite) and carboxylated-multi walled carbon nanotube (cMWCNT) were used for the magnetic solid phase extractions and determination of Sudan I, II, III, IV, Para Red, Sudan Black B and Sudan Red 7B in chili products. High performance liquid chromatography (HPLC) was employed for the measurements. Limit of quantification (LOQ) values were found in the range 0.44–2.82 ng mL^{-1} for analytes. The best extraction parameters were determined as pH 8.0, 40 mg of magnetic nanoparticle, 4.0 min of contact time, 0.3 mL desorption by acetonitrile. The samples were dissolved in acetone-dichloromethane-methanol (3:2:1, v/v/v) and diluted with acetonitrile-methanol (v/v; 80:20) before the method was applied. Concentrations of Sudan dyes and Para Red were determined in four samples of chili powder from less than LOQ to 31.21 ± 1.6 ng g^{-1}, two samples of chili tomato sauces (lower than LOQ) and two samples of ketchup (lower than LOQ).

1. Introduction

Sudan dyes have been categorized as class 3 carcinogens by the International Agency for Research on Cancer (IARC) (Mo, Zhang, Zhao, Xiao, Guo, & Zeng, 2010; Stiborova, Martinek, Rydlova, Hodek, & Frei, 2006). Despite this, Sudan dyes are used illegally for coloring foods, like ketchup and chili products, to improve their appearance. In 2014, they were detected in approximately 580 different food products bought in EU countries, and the products were withdrawn (Food Standard Agency, 2014).

The chemical structures, molecular formulas and CAS (Chemical Abstract Service) numbers of Sudan I, II, III, IV, Para Red, Sudan Black B and Sudan Red 7B are (Table 1) were reviewed recently (Yamjala, Nainar, & Ramisetti, 2016). Membrane filtration, solid phase extraction, liquid-liquid extraction, microwave, and ultrasound-assisted extraction have been carried out to clean up samples for analysis using spectrophotometry, thin layer chromatography, liquid chromatography with and without mass spectrometry (Yamjala et al., 2016). Molecularly imprinted solid-phase extraction coupled with liquid chromatography has also been used for the determinations of Sudan I, II, III and IV in 30 bean curd products (Yan et al., 2012). LODs were in the range of 5–9 μg kg^{-1}. A SPE-alumina-N cartridge was used to clean up the sample before HPLC analysis of Sudan I, II, III and IV in chili products. Here, however, LODs were found in the range 4.1–5.8 μg kg^{-1} (Qi, Zeng, Wen, Liang, & Zhang, 2011). Before liquid chromatography-photodiode array determinations of Sudan I, II, III and IV in chili-containing foodstuffs, supramolecular solvent-based microextraction was applied for sample clean up. The detection limits of the method were 4.2, 2.7, 6.5 and 7.4 μg kg^{-1} for Sudan I, II, III and IV, respectively (López-Jiménez, Rubio, & Pérez-Bendito, 2010). Using atmospheric pressure photoionization–tandem mass spectrometry, LODs ranged between 5 and 18 μg L^{-1} and LOQ between 10 and 24 μg L^{-1} for Sudan I, II, III and IV (Murty, Chary, Prabhakar, Raju, & Vairamani, 2009). Sudan I, II, III and IV were determined in candy using ionic liquid/anionic surfactant aqueous two-phase extraction coupledwith high-performance liquid chromatography (Yu, Liu, Li, Zhang, & Yu, 2015) in 80 red chili peppers (including Para Red) (Ertaş, Özer, & Alasalvar, 2007), in soft drink samples with an electrochemical detector (Chailapakul et al., 2008) and in chili products with gel permeation chromatography (GPC) clean-up (including Para Red and Sudan Red 7B) (Zhu et al., 2014). These methods are based on time-consuming procedures for pre-concentration of samples. Using magnetic nanomaterials would eliminate the need for such procedures whilst still achieving comparable LODs and LOQs.

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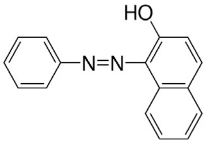
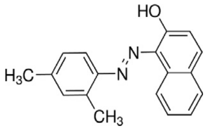
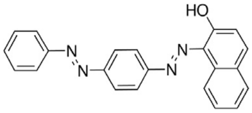
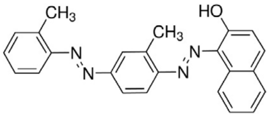
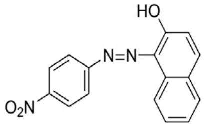
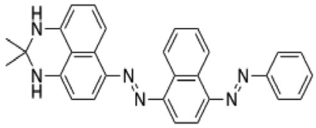
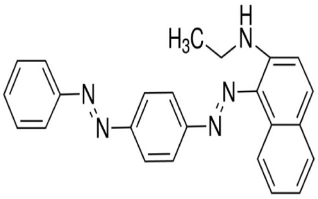
Table 1
Chemical informations of Sudan I, II, III, IV, Para Red, Sudan Black B and Sudan Red 7B.

Dye	Molecular formula	CAS No	Structure
Sudan I	$C_{16}H_{12}N_2O$	842-07-9	
Sudan II	$C_{18}H_{16}N_2O$	3118-97-6	
Sudan III	$C_{22}H_{16}NO$	85-86-9	
Sudan IV	$C_{24}H_{20}N_4O$	85-83-6	
Para Red	$C_{16}H_{11}N_3O_3$	6410-10-2	
Sudan Black B	$C_{29}H_{24}N_6$	4197-25-5	
Sudan Red 7B	$C_{24}H_{21}N_5$	6368-72-5	

In this study, carboxyl functionalized MWCNT-γ-Fe_2O_3 MNPs synthesized previously (Kılınç, 2016) was used for magnetic solid phase extractions and determination of Sudan I, II, III, IV, Para Red, Sudan Black B and Sudan Red 7B in foods using HPLC. Using the magnetic nanomaterials overcame problems with traditional solid phase extraction and time-consuming procedures, such as filtration and centrifugation on SPE, were eliminated, allowing large numbers of samples to be tested for Sudan dyes.

2. Materials and methods

2.1. Reagents and standards

Multiwalled carbon nanotube (o.d. 10–15 nm, i.d. 2–6 nm, length 0.1–10 μm), toluene, NH_4OH and Sudan I (≥95), Sudan II (≥96), Sudan III (≥96), Sudan IV (≥96), Para Red (> 95), Sudan Black B and Sudan Red 7B (≥96) were supplied from Sigma-Aldrich (St Louis, MO, USA). $FeCl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$ were bought from Merck (Darmstadt, Germany) and Fluka (St Louis, MO, USA). All chemicals were of analytical reagent grade. Doubly distilled water was used for the entire experiments. Solvents were of HPLC grade and obtained from Merck. PTFE membrane filters (0.2 μm) were supplied by Waters (CITY, Massachusetts, USA).

After the required amounts of each dye were mixed with acetone-dichloromethane-methanol (3:2:1, v/v/v), the solution was heated to 40 °C for 10 min to dissolve the dyes completely. Acetonitrile-methanol (v/v; 80/20) was used to make up the final volume (100 mL).

2.2. Instrumentation

The chromatography equipment with a Dionex Ultimate 3000 LC system consisted of a quaternary pump, degasser, auto sampler, DAD detector and thermostated column compartment (Agilent, Waldbronn, Germany). The ACE 5 C18 column (250 × 4.6 mm stainless steel, particles size as 5 μm) was used for separation of Sudan dyes and Para Red. Acetonitrile-methanol (v/v; 80/20) was used as a mobile phase with a flow rate of 1.0 mL min^{-1}. The targeted analytes were monitored at 504 nm.

2.3. Sample preparation for magnetic solid phase extraction

10 g of chili powder, chili tomato sauces and ketchup samples were weighed into a 50 mL glass bottle. After the addition of 30 mL of acetone-dichloromethane-methanol (3:2:1, v/v/v), the mixtures were ultrasonicated for 15 min at 40 °C. Then, acetonitrile-methanol (v/v; 80:20) was added to a final volume of 50 mL. The final solutions were subjected to SPE and passed through a 0.2 μm syringe filter before injection on to the HPLC column. Spiked samples were prepared in the same way. A mixed stock standard solution was added to samples before extraction.

2.4. Magnetic solid phase extraction procedure

For the magnetic solid phase extraction, 100 μL of methanol and 100 μL of water were added sequentially to activate 20 mg of cMWCNT-γ-Fe_2O_3.This was dispersed in 50 mL of sample and the mixture shaken on a slow-moving platform shaker for 15 min. The magnetic adsorbent was isolated from the solution by means of a strong magnet (Nd–Fe–B); 100 mm100 mm20 mm) at the bottom of the beaker and most of the supernatant was poured away. Then, the magnetic nanoparticles and residual solution were transferred to a 15-mL centrifuge tube. The desorption of analytes was realized with the addition of 0.5 mL of acetone twice with vortexing for 1 min. These solutions were combined and transferred to a 2 mL microcentrifuge tube before being dried under nitrogen. The residue after drying was reconstituted in 250 μL of acetonitrile-methanol (v/v; 80/20) and passed through a PTFE filter (0.2 μm). 15 μL was injected on to the HPLC system for analysis.

3. Results and discussions

3.1. Investigation of extraction parameters for magnetic solid phase extraction of Sudan dyes and Para Red

50 mL portions of double-distilled water spiked with 10 ng mL^{-1} of each dyes (model solution) were used to determine the best experimental conditions. The effects of pH, amounts of magnetic nanoparticles, extraction time, and type and the volume of desorber were investigated. All experiments were performed in triplicate.pH is one of the most important parameters that influences adsorption and desorption behaviors (Özdemir, Okumuş, Dündar, & Kılınç, 2013). The effect of pH on magnetic solid phase extraction of Sudan dyes was investigated in the range 3–9. pH values was adjusted with NaOH and HNO_3. As shown in Fig. 1, the percentage adsorption increased with increasing pH to a maximum at pH 8. Adsorption decreased at higher pH values, suggesting the negatively charged sites on the adsorbent surface did not favor Sudan dyes.

The amount of magnetic nanoparticle, as a solid phase adsorbent, was examined for quantitative extraction and removal of Sudan dyes in the range 10–100 mg of cMWCNT-γ-Fe_2O_3. Maximum adsorption capacities were achieved when 40 mg of cMWCNT-γ-Fe_2O_3 was in contact with a model solution for 15 min. The results are shown in Fig. 2.