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ScienceDirect
Food Chemistry 102 (2007) 1027–1033
Food Chemistry
www.elsevier.com/locate/foodchem

Determination of banned sudan dyes in chili powder by capillary electrophoresis

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Received 5 May 2006; received in revised form 16 June 2006; accepted 21 June 2006

Abstract

A simple and fast method, based on the use of micellar electrokinetic capillary chromatography in combination with UV detection, was developed for the determination of Sudan dyes (I, II, III and IV). The separation of a mixture of the four standards was achieved using a background electrolyte consisting of 5 mM borate (pH 9.3), 20 mM sodium dodecyl sulfate and 20% acetonitrile. Under optimized conditions, the four azo-dyes were baseline separated in 20 min with limits of detection ranging from 96 to 610 μg/L ($S/N > 3$). The applicability of the method for rapid screening and determination of Sudan dyes (I, II and III) was studied by analyzing spiked chili powder samples from India, Pakistan, Mexico, United States, Canada, and China.

Keywords: Sudan dyes; Capillary electrophoresis; Chilli powder; MEKC

1. Introduction

Azo-dyes are a class of synthetic organic colorants (characterized by a chromophoric azo-group) that are typically used in many industrial applications including solvents, oils, fats, waxes, plastics, printing inks, and floor polishes (Habibi, Hassanzadeh, & Mahdavi, 2005; Nohynek, Fautz, Benech-Kieffer, & Toutain, 2004). The main reason for the widespread usage is their colorfastness and low price. However, azo-colorants are biologically active through their metabolites (Golka, Kopps, & Myslak, 2004; Pinheiro, Touraud, & Thomas, 2004) and have been associated with increased occurrence of bladder cancer in textile and leather dyers, painters and hairdressers (Ahlstrom, Sparr Eskilsson, & Bjorklund, 2005; Nohynek et al., 2004). Due to their potential carcinogenicity, many countries have banned the use of most azo-dyes at any level in products for human consumption (Ahlstrom et al., 2005).

Sudans I, II, III, and IV (Fig. 1) are non-ionic fat-soluble dyes used as additives in gasoline, grease, oils and plastics. These dyes are classified by the International Agency for Research on Cancer (IARC) as category 3 carcinogens because they can induce some forms of liver and bladder cancer in animals (International Agency for Research on Cancer, 1975). Despite the controversial level of risk, Sudan dyes are banned as food additives for humans. The recent contamination of hot chili and derived products from India and marketed in the European Union (Calbiani et al., 2004) demands the development of reliable and accurate analytical methods for the fast identification and quantification of such compounds in foodstuffs.

A wide variety of analytical methodologies have been developed for the determination of Sudan dyes in foodstuffs (Ahlstrom et al., 2005; Puoci et al., 2005), but the most popular are high performance liquid chromatography (HPLC) with optical (Cornet, Govaert, Moens, Van Loco, & Degroodt, 2006) or mass spectrometric (Ma, Luo, Chen, Su, & Yao, 2006) detection. Although a great amount of

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doi:10.1016/j.foodchem.2006.06.038

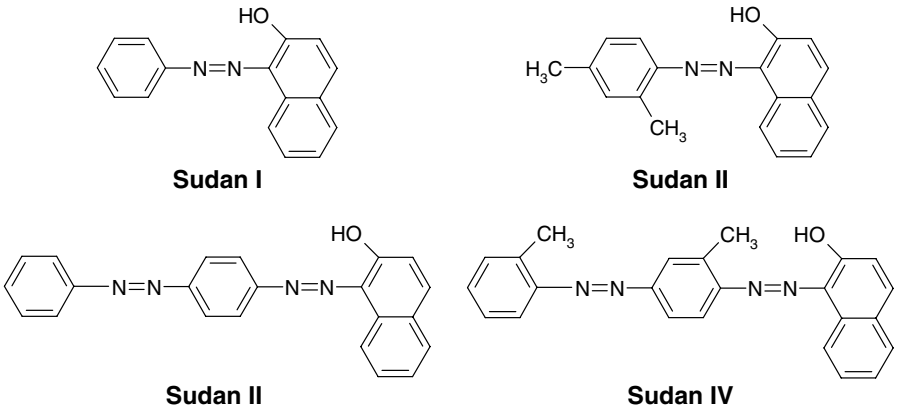

Fig. 1. Molecular structures of the selected Sudan dyes.

information can be obtained by these methodologies, they are time consuming, need large sample volumes, generate large amounts of waste, or require bulky and expensive instrumentation. Capillary electrophoresis (CE) is an alternative method for the separation of dyes (Jager, Tonin, & Tavares, 2005). In CE, the separation takes place inside of a capillary filled with an electrolyte solution. When a potential difference is applied across the capillary, bulk flow of the solution is generated inside the capillary by a process referred to as electro-osmotic flow (EOF). The analytes, which are introduced as a sample plug at one end of the capillary, migrate towards the detector with a velocity that is dependent on their charge/mass ratio, the magnitude of the applied potential, and the solution conditions. CE provides high-speed, high-throughput, low waste generation, highly efficient and reliable separations, and it offers a simple way to handle very small samples (nL) without the use of pumps or valves. Another advantage of CE is the possibility of using a wide range of solution conditions to control the EOF and manipulate the separation of the analytes (Dolnik, 2004; Garcia, Dressen, Henderson, & Henry, 2005; Yin & Wang, 2005). Among many different alternatives to improve separations by CE, a commonly encountered mode is micellar electrokinetic chromatography (MEKC) (Watanabe & Terabe, 2000). MEKC can be advantageous over LC in terms of simplicity, resolution, and economy. Additionally, neutral analytes, which cannot be separated by capillary zone electrophoresis, are readily separated by MEKC (Ding, Mora, & Garcia, 2006; Hompesch, Garcia, Weiss, Vivanco, & Henry, 2005; Terabe, 2004). However, it typically suffers from low concentration sensitivity as a consequence of the limited sample volume and short path length for absorbance based detection (Molina & Silva, 2002).

MEKC is performed by adding surfactants to the background electrolyte (BGE) in amounts above the critical micelle concentration (CMC). Under these conditions, surfactant molecules aggregate and the resulting micelles form a pseudo-stationary phase (Pappas, Gayton-Ely, & Holland, 2005). The differential analyte/micelle interactions are the key factor determining the selectivity of MEKC. In general, more hydrophobic analytes will have an increased affinity for the micelles with respect to analytes of a more hydrophilic character. These interactions can be altered in many ways, such as changing the pH, the type of surfactant, or adding organic solvents to the BGE (James, 2003).

This paper describes the development and application of the first capillary electrophoresis method for the simultaneous determination of Sudan dyes (I, II, III, and IV). The described method requires a very simple sample preparation step and provides a fast and inexpensive process to screen for these dyes in food samples.

2. Experimental

2.1. Chemicals

Sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), sodium dodecyl sulfate (SDS), acetonitrile, acetone, Sudan I (1-(phenylazo)-2-naphthalenol), Sudan II (1-[(2,4-dimethylphenyl)azo]-2-naphthalenol), Sudan III (1-(4-phenylazophenylazo)-2-naphthalenol), and Sudan IV (*o*-tolylazo-*o*-tolylazo-betanaphthalenol) were obtained from Sigma (Saint Louis, MO, USA). The molecular structures of the selected dyes are shown in Fig. 1. Stock solutions of Sudan (I, II = 0.5 mg/mL, and III, IV = 1 mg/mL) were prepared in acetone and stored at room temperature. All chemicals were analytical reagent grade and used as received. Aqueous solutions were prepared using 18 MΩ cm water (NANOpure Diamond, Barnstead) and were filtered using a hollow fiber filter (0.2 μm, Barnstead). The pH of the BGE was adjusted using either 1 M NaOH or 1 M HCl (Fisher).

2.2. Apparatus and procedures

A Beckman-Coulter P/ACE MDQ (Fullerton, CA) capillary electrophoresis system and polyimide coated capillaries (50 μm ID × 375 μm OD × 57 cm long; Polymicro Technologies, Phoenix, AZ) were utilized in all the experiments, with the anode and cathode positioned at the inlet and outlet ends of the capillary, respectively. Data acquisition was performed using Karat 32 software (Beckman-

Coulter, Fullerton, CA) on an IBM personal computer. Unless otherwise noted, samples were introduced into the capillary by a 5 s, 0.5 psi pressure injection (~6.5 nL sample plug), (McLaren, Boulat, & Chen, 2002) and subsequently separated by MEKC with an applied potential of 20 kV, and a controlled temperature of 25 °C. Direct UV detection was performed with a wavelength of 214 nm, through the capillary at a window located 50 cm from the inlet. The selected wavelength allows direct detection of all four Sudan dyes (Zhang, Zhang, Gong, Gopalan, & Lee, 2005), as well as other possible contaminants.

Each BGE solution was prepared daily and degassed by sonication for 10 min before use. The capillary was conditioned daily by sequentially rinsing it with 0.1 M NaOH (5 min/20 psi), deionized water (5 min/20 psi), methanol (5 min/10 psi), deionized water (5 min/20 psi) and BGE (10 min/10 psi). Between consecutive analyses, the capillary was rinsed with BGE for 5 min at 20 psi. At the end of each day, the capillary was rinsed sequentially with 0.1 M sodium hydroxide (2 min/20 psi), deionized water (10 min/20 psi), methanol (2 min/20 psi), deionized water (5 min/20 psi), and then finally air-dried (10 min/20 psi). The capillary was always stored dry. Although this procedure increased the day-to-day and capillary-to-capillary reproducibility, slight shifts in t_M were observed and considered normal. For the same reason, each series of experiments was performed the same day and with the same capillary.

2.3. Sample preparation

Chili powders manufactured in different countries (see Table 2) were purchased in local stores. Initially, 50 mg of powder were weighed into a centrifuge tube. Then, 1 mL of acetone was added to perform an extraction. The sample was then vortexed for 2 min and centrifuged for 5 min at 13,500 rpm to precipitate the solids. The supernatant was diluted 10 times with the BGE and analyzed as described. Spiked samples were prepared by adding 50 μL of a stock Sudan solution (I, II ~0.5 mg/mL; III, IV ~1.0 mg/mL) to a dry powder sample before performing the extraction. Recovery assays were performed by comparing the average peak areas of the spiked samples with the corresponding peak areas of the original samples, as well as a standard Sudan sample.

3. Results and discussion

Although all of the Sudan dyes studied here are banned for use in human food, their biological activity differs significantly. Therefore, the separation step is particularly important in samples containing more than one dye. In order to optimize our method for the separation of a mixture of the four azo-dyes, the effects of the SDS concentration, the type and concentration of organic modifiers, the BGE concentration and pH, the temperature, the applied voltage and the sample volume (injection pressure and time) used for the analysis were studied.

3.1. Effect of SDS concentration

Sudan dyes (I, II, III, and IV) are non-ionic species, and can be separated using micellar electrokinetic capillary chromatography. The effect of the surfactant concentration was studied in the range of 5–50 mM, with 5 mM

Table 1
Analytical parameters corresponding to the calibration curves for the selected Sudan dyes

	t_M (s)	N[a]	Sensitivity (mL min/μg AU)	R^2	LOD[b] (μg/L)
Sudan I	14.0 ± 0.2	45,000	507.83	0.991	96.5
Sudan II	16.1 ± 0.3	93,400	327.17	0.996	149.8
Sudan III	15.5 ± 0.5	45,800	160.54	0.990	305.2
Sudan IV	17.7 ± 0.1	115,900	80.36	0.998	609.8

[a] N values calculates using the middle point of the calibration curve (~5 μg/mL).
[b] LOD values calculated as the concentration corresponding to 3 times the baseline noise.

Table 2
Brand, country of origin, and recovery results obtained for Sudan I, Sudan II and Sudan III in different chili powder samples

Brand	Manufactured country	Recovery (%)		
		Sudan I	Sudan II	Sudan III
Fiesta Brand	USA	99	97	98
Great Value	USA	98	99	97
Laxmi	India	90	91	87
Mari Cruz	Mexico	94	100	90
McCormick	USA	92	88	80
Nature's Selection	Canada	100	90	100
Oriental Mascot	China	99	100	96
Shan	Pakistan	100	95	91
Supreme Quality	Pakistan	97	93	93

borate at pH 9.3 and 20% acetonitrile (data not shown). No separation was achieved when only 5 mM SDS was dissolved in the BGE. This behavior was expected because the CMC of SDS is around 8 mM (in water) (Garcia et al., 2005). As the SDS concentration was progressively increased, the baseline separation of the four dyes was achieved. Using 20 mM SDS, well-defined peaks were obtained for Sudan I ($t_M = 14.5$ min), Sudan II ($t_M = 17.0$ min), Sudan III ($t_M = 16.4$ min), and Sudan VI ($t_M = 19.4$ min). At SDS concentrations greater than 20 mM, higher migration times and broader peaks were obtained (*e.g.* Sudan I $PW_{20\ mM} = 0.18$ min, $PW_{30\ mM} = 0.20$ min) with no concurrent increases in resolution. We believe that the observed increase in migration time is only a consequence of a decrease in EOF produced by the higher ionic strength of the BGE. Based on these results, 20 mM was selected as the optimum concentration of SDS.

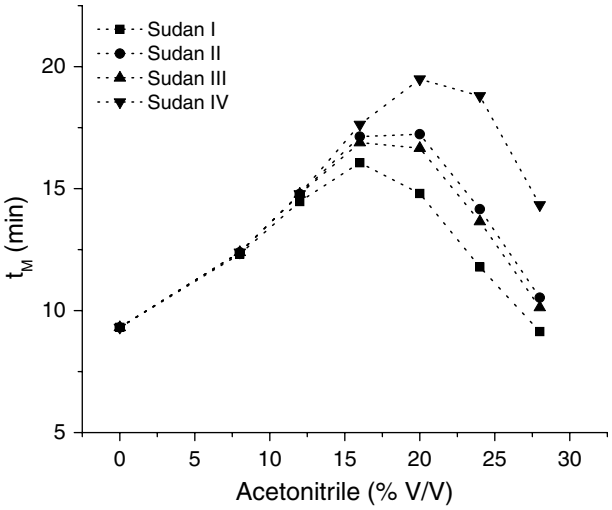

Fig. 2. Effect of the concentration of acetonitrile on the separation of Sudan dyes. Other conditions: 5 mM borate at pH 9.3 and 20 mM SDS, $E_{SEPARATION} = +20{,}000$ V, injection = 5 s, 0.5 psi, temperature = 25 °C.

3.2. Effect of organic solvents

In MEKC, organic solvents are commonly added to the BGE in order to improve separations. In addition to increasing the solubility of hydrophobic compounds, organic solvents affect the EOF by altering the polarity of the aqueous phase, the electrolyte viscosity and the zeta potential (Berzas Nevado, Castaneda Penalvo, & Pinilla Calderon, 2002; James, 2003; Wang, Wu, Yao, & Shen, 2004). Using a BGE consisting of 5 mM borate at pH 9.3 and 20 mM SDS, the effect of organic solvents (methanol, ethanol, acetone, and acetonitrile, all at 18% v/v) on the separation efficiency was studied. Poor resolution was obtained with methanol ($Rs_{Sudan\ I/III} = 0.99$) and no peaks were observed when either ethanol or propanol were used. However, both acetone ($Rs_{Sudan\ I/III} = 5.84$) and acetonitrile ($Rs_{Sudan\ I/III} = 7.02$) produced a significant improvement in the separation of the Sudan dyes. Although this behavior does not correlate with the dielectric constant or the polarity of the selected organic solvents (Lee, Park, & Whitesides, 2003), indicates that a series of specific interactions are affecting both the solubility and the partitioning of these analytes into the micelles.

Because better separations were obtained, acetonitrile was chosen as the most effective organic modifier, and the effect of its concentration on the separation was analyzed from 0% to 28% v/v. It was observed (Fig. 2) that consecutive increases in migration time and separation efficiency (N) occurred in the range of 0% to 20% v/v acetonitrile. This effect was reverted at concentrations greater than 20%. Considering the separation efficiency and analysis time, 20% v/v acetonitrile was selected as the optimum organic modification to the BGE. Similar compositions of modified electrolytes have been also reported for the separation by SDS-MEKC of flavonoids (Tonin, Jager, Micke, Farah, & Tavares, 2005), herbicides (Nuñez, Kim, Moyano, Galceran, & Terabe, 2002), amino acids (Roman, McDaniel, & Culbertson, 2006), and carbohydrates (Zhang, Xu, Zhang, Zhang, & Zhang, 2003).

3.3. Effect of borate concentration and pH

It is well known that the BGE concentration has a significant effect on separations by CE. The BGE concentration not only influences the zeta potential of the capillary tube, but also the CMC of the surfactant used. In order to study the effect of borate concentration on the baseline separation of the four Sudan dyes, solutions in the range of 5–30 mM were studied (pH 9.3, 20 mM SDS, 20% v/v acetonitrile). The results showed that higher borate concentrations produced longer migration times and had little effect on the separation efficiency and migration order of the analytes (data not shown). In order to minimize the analysis time, 5 mM was selected as the optimum borate concentration and used throughout the rest of the experiments.

Changes in the buffer pH also affect the zeta potential, the EOF, the separation selectivity and the resolution. In order to study the effect of pH on the separation of the Sudan dyes, a series of buffer solutions were prepared at pH values ranging from 8 to 10, using a BGE consisting of 5 mM borate, 20 mM SDS, and 20% v/v acetonitrile. No significant enhancements in the separation efficiency (Fig. 3) were observed at pH values either higher or lower than native pH for borate of 9.3. However, a slight increase in migration times was obtained at pH values above 9.3. The Sudan dyes are neutral in this pH range (Bailey & Dorsey, 1999; Marting & Breen, 1998), so the changes in migration time as a function of pH can be attributed to reductions in EOF, as the result of increases in the ionic strength of the BGE. Accordingly, a pH value of 9.3 was selected as the optimum pH value for the BGE, at which

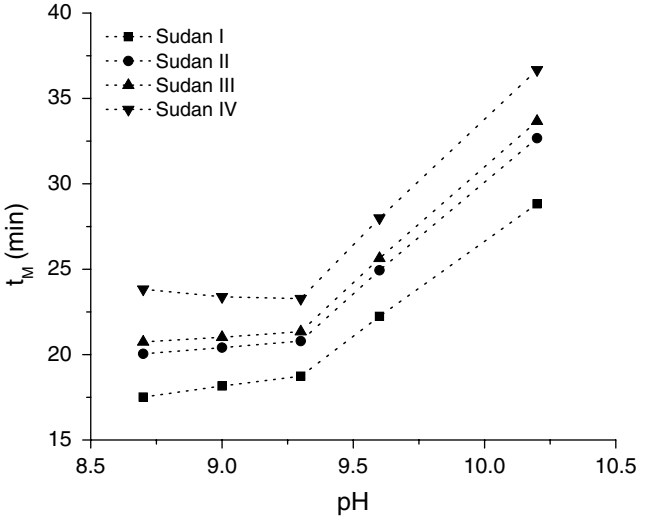

Fig. 3. Effect of the pH on the separation of Sudan dyes. Other conditions: 5 mM borate, 20 mM SDS, and 20% acetonitrile, $E_{SEPARATION} = +20{,}000$ V, injection = 5 s, 0.5 psi, temperature = 25 °C.

the four Sudan dyes can be baseline separated with the lowest analysis time.

3.4. Effect of temperature

Temperature is an important parameter in optimizing separations by CE. It has been reported that temperature considerably affects resolution, efficiency and analysis time. Therefore, the effect of the capillary temperature was investigated between 20 and 45 °C in the presence of 5 mM borate buffer (pH 9.3), 20 mM SDS and 20 % v/v acetonitrile. The migration time (Fig. 4) and resolution (data not shown) of all investigated Sudan dyes decreased with increasing temperature. This behavior can be mainly attributed to a decrease in BGE viscosity, resulting in higher electrophoretic and electro-osmotic mobilities. Although a significant decrease (about 50%) in analysis time was achieved at higher temperatures, a lower temperature (25 °C) was chosen as optimum in order to prioritize separation efficiency over analysis time and to avoid the detrimental formation of bubbles.

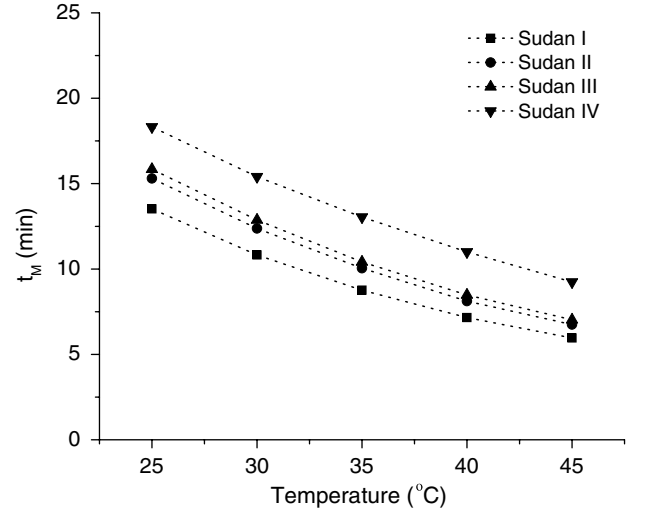

Fig. 4. Effect of the capillary temperature on the separation of Sudan dyes. Other conditions: 5 mM borate (pH 9.3), 20 mM SDS, and 20% acetonitrile, $E_{SEPARATION} = +20{,}000$ V, injection = 5 s, 0.5 psi.

3.5. Calibration curves and application

Using the optimized conditions (5 mM borate buffer, pH 9.3, 20 mM SDS and 20%, v/v acetonitrile) the relationship between the response and the analyte concentration was studied by analyzing mixtures of the Sudan dyes (I, II, III, and IV) at different concentrations. The results are summarized in Table 1. Briefly, linear relationships were obtained in the 0.5 to 36 μg/mL range with calculated limits of detection (signal/noise ratio > 3) ranging from 96.5 μg/L (for Sudan I) to 609.8 μg/L (for Sudan IV). A typical electropherogram obtained with a mixture of standards is shown in Fig. 5a. As can be observed, a clear separation of the four components was obtained. Additionally, a yet unidentified peak was also observed at 17.2 ± 0.2 min.

Since interferences deriving from different components of the matrix can negatively affect the separation and detection of analytes, a variety of real samples were analyzed. The MEKC method developed was applied to the identification and determination of Sudan dyes in several hot chili products from different countries. Because none of the chili samples contained Sudan dyes above the limit of detection, dry samples were spiked with a known amount of Sudan and the recovery assays were performed following the described procedure (see Section 2). As can be observed in Table 2, recovery values above

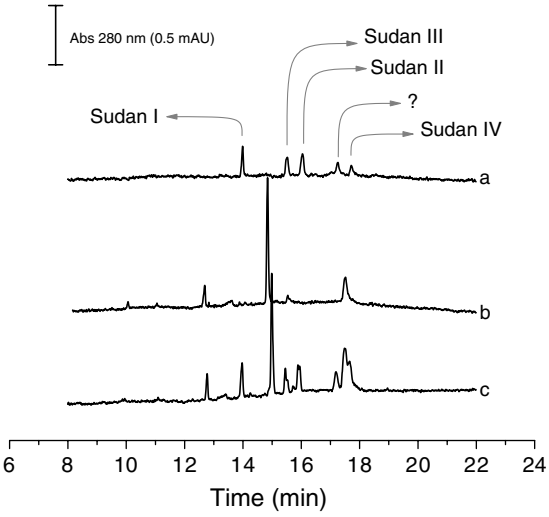

Fig. 5. Electropherograms corresponding to a mixture of standards (a), a chili powder sample (b), and the chili powder sample spiked with three Sudan dyes. Conditions: 5 mM borate (pH 9.3), 20 mM SDS, and 20% acetonitrile, $E_{SEPARATION} = +20{,}000$ V, injection = 5 s, 0.5 psi, temperature = 25 °C.

90% were obtained for Sudan I, II, and III in all the studied samples. As an example of the results typically obtained, the electropherograms corresponding to a standard mixture, a sample and the sample spiked with Sudan dyes (I, II, III, and IV) are shown in Fig. 5. It was also observed that the quantification of Sudan IV was particularly challenging because some of the chili powder samples showed an additional peak, which matched the t_M of Sudan IV. Although this important contribution of the matrix precluded the quantification of Sudan IV in some of the samples, the method showed great potential for the most commonly used Sudan dye (Sudan I) as well as the lesser used dyes (Sudan II and Sudan III).

4. Conclusions

After optimization of the analytical conditions (buffer concentration and pH, surfactant concentration, organic modifier, and temperature), a method for the determination of Sudan dyes by micellar electrokinetic capillary chromatography was developed. The method allows the simultaneous determination of Sudan dyes (I, II, III, and IV) with minimum instrumentation needs, analysis time and cost. Although no samples containing Sudan dyes were found, the method was successfully applied for the analysis of Sudan dyes (I, II and III) in spiked samples from six different countries, showing the potential for quality control of food samples.

Acknowledgements

Authors thank The University of Texas at San Antonio and the San Antonio Area Foundation for financial support. E. Mejia also thanks MBRS-RISE program for the research fellowship (NIH/NIGMS MBRS-RISE GM60655).

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