Fast determination of Sudan dyes in chilli tomato sauces using partial filling micellar electrokinetic chromatography

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ABSTRACT

A new method based on partial filling micellar electrokinetic chromatography (MEKC) for the quantitative determination of Sudan dyes (I, II, III and IV) in chilli sauces is presented. The separation is achieved filling 25 % of the capillary with a MEKC buffer composed of 40 mM NH_4HCO_3, 25 mM SDS and 32.5 % (v/v) acetonitrile (ACN). The rest of the capillary is filled using a capillary zone electrophoresis (CZE) buffer composed of 40 mM NH_4HCO_3 and 32.5 % (v/v) ACN. Under optimized conditions, the azo-dyes are baseline separated in less than 8 min with limits of detection (LODs) ranging from 0.57 to 0.71 μg mL^{-1} (S/N > 3). Using an internal standard the repeatability of the quantitative determination is improved almost four times. The applicability of the method for rapid screening and determination of Sudan dyes is corroborated by analyzing spiked chilli sauce samples with recoveries from 85 to 99%. The reported conditions are demonstrated to be compatible with mass spectrometry (MS) detection.

Keywords: dyes, capillary electrophoresis, MEKC, tomato chilli sauce, food analysis.

INTRODUCTION

Sudan dyes are a family of lipophilic synthetic organic colorants, characterized by a chromophoric azo-group, extensively used in industrial and scientific applications but banned as food colorants (*1, 2*). Sudan I, II, III, and IV (see Figure 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 (*3*). Moreover, these dyes can generate metabolites that are converted to active mutagens and carcinogens in humans (*4*). For instance, the azo group of these dyes can be reduced to aromatic amines that are confirmed or suspected carcinogenic compounds (*5*). Sudan dyes have been illegally added to foodstuffs to enhance the red-orange color of products and easily used because of their low cost and wide availability. Due to the continuing illicit use of Sudan dyes as food colorants including some recent episodes of contamination of hot chilli and derived products from India and marketed in the European Union (*6*), their determination in different food matrices, especially in different chilli and tomato sauces and related products, has received increasing attention during the last years (*7, 8*). As a result, development of new and fast analytical methods is still required for the 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 as recently reviewed by Rebane *et al.* (*7*). Among the different methodologies developed so far, the most popular are based on the use of high performance liquid chromatography (HPLC) with optical (*9-14*), or mass spectrometric detection (*6, 15-21*). Although a great amount of 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) has been shown as a powerful analytical technique to analyze additives and organic contaminants in foods (*22-24*) including the separation of Sudan dyes by MEKC with UV detection (*25*) and pressurized capillary electrochromatography (CEC) with amperometric detection (*26*). The work described by Liu et al. (26) showed the baseline separation of Sudan I, II, III, and IV in hot chilli powder within 7 min using capillaries of 20 cm packed with 1.5 μm octadecyl silica particles (ODS) with LODs from 0.8 to 1.2 μM. On the other hand, Mejia et al. (25) carried out the determination of Sudan I, II, III, and IV in chilli power using a MEKC method based on the use of borate buffer containing SDS and acetonitrile (ACN). The Sudan dyes were separated in 20 min with LODs from 0.1 to 0.6 $\mu g\ mL^{-1}$.

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). However, it typically suffers from low concentration sensitivity as a consequence of the limited sample volume and short path length for absorbance based detection. This paper describes the development and application of the first capillary electrophoresis method compatible with mass spectrometry (MS) for the simultaneous determination of Sudan dyes (I, II, III, and IV). The described method is based on the use of a straightforward sample preparation step followed by partial filling micellar electrokinteic chromatography allowing a fast and inexpensive screening of the four Sudan dyes in food samples. Moreover, the reported conditions are demonstrated to be compatible with mass spectrometry (MS) detection.

MATERIALS AND METHODS

Reagents and solutions

All reagents were of analytical grade, solvents were of chromatographic purity and water was purified using a Milli-Q system (Millipore, Bedford, MA, U.S.A.). ACN, acetone, dichloromethane and methanol were of chromatographic purity and obtained from Lab-Scan (Gliwice, Poland). Sodium hydroxide was obtained from Panreac (Barcelona, Spain).

Electrolyte solutions were prepared daily to the desired concentration from stock solutions of 100 mM of ammonium hydrogen carbonate (NH_4HCO_3, Fluka, Buchs, Switzerland) and sodium dodecyl sulfate (SDS) (Sigma Aldrich, St Louis, MO, U.S.A.).

Sudan I (1-(phenylazo)-2-naphthalenol) and Sudan II (1-[(2,4-dimethylphenyl)azo]-2-naphthalenol) were obtained from Sigma Aldrich (St Louis, MO, U.S.A.). Sudan III (1-(4-phenylazophenylazo)-2-naphthalenol) and Sudan IV (o-tolylazo-otolylazo-betanaphthalenol) were obtained from Fluka (Buchs, Switzerland). The molecular structures of these dyes are shown in Figure 1. Stock solutions of Sudan I and II were prepared at 1 mg mL^{-1} concentration and Sudan III and IV at 0.25 mg mL^{-1} all in acetone and stored at 4 °C until use. Working solutions containing 10 or 20 μg mL^{-1} of each dye were prepared diluting the stock solutions as described below. Likewise, for the calibration curves, standard solutions from 2.5 to 20 μg mL^{-1} of each dye were prepared by appropriate dilution of the stocks. Namely, the Sudan I-IV standard solution in acetone was first diluted in a MEKC buffer (50:50 v/v) composed of 30 mM NH_4HCO_3, 25 mM SDS and 30 % ACN. Since it was observed that an increase of the concentration of ammonium bicarbonate and SDS improved the peak shape, the concentration of the MEKC solution used to dilute the Sudan I-IV standard

acetone mixture (50:50 v/v) was raised to 60 mM NH_4HCO_3, 50 mM SDS and 30 % ACN.

Flumequine (Riedel-de Haën, Seelze, Germany) was used as internal standard. It was added to all samples at a concentration of 10 μg mL^{-1} prepared diluting a stock solution of 1 mg mL^{-1} in acetone.

Sample preparation

Samples of chilli tomato sauce were acquired from local markets. The procedure of extraction used a mixture of acetone, dichloromethane and methanol (3:2:1, v/v/v) modifying the method proposed by Ertaş *et al.* (*11*). Namely, 1.0 g of sample was weighed into a sample tube and diluted with 10 mL of the above-mentioned three solvents mixture. Then, the sample was vortexed for 2 min, sonicated for 5 min and centrifuged for 5 min at 10000 rpm to precipitate the solids. The supernatant was collected and evaporated in a rotary evaporator. The dry residue was suspended in 1 mL of acetone containing the internal standard. This solution was diluted (50:50 v/v) in 60 mM NH_4HCO_3 with 50 mM SDS and 30 % ACN. Spiked samples were prepared by adding the Sudan dyes into the real samples before extraction. Recoveries were calculated using the average peak relative areas of the spiked samples to the internal standard and the obtained calibration curves.

Instrumentation

MEKC-UV: Partial filling MEKC experiments were conducted in a capillary electrophoresis system (model P/ACE MDQ, Beckman Instruments, Fullerton, CA, U.S.A.) equipped with a direct UV detector set at 214 nm. Acquisition and data treatment was done using System Gold® Software supplied by Beckman. Uncoated

fused-silica capillaries (Composite Metal Services, Worcester, UK) with 60.0 cm total length (50.0 cm effective length) x 50 μm i.d. were used. New capillaries were preconditioned by flushing the capillary (at 20 psi) with 0.1 M NaOH for 30 min followed by deionized water for 15 min. At the beginning of each day, the capillary was conditioned by flushing 0.1 M NaOH for 10 min, deionized water for 10 min and electrolyte solution for 5 min, whereas at the end of the day, the capillary was rinsed with 0.1 M NaOH and deionized water for 5 min each. Between runs, the capillary was conditioned with CZE buffer (containing the concentration of NH_4HCO_3 and ACN indicated in each case) for 5 min. Then the MEKC buffer (containing the concentration of NH_4HCO_3, SDS and ACN indicated in each experiment) was pushed in the capillary filling the percentage indicated in each case. The total length of the MEKC plug was varied from 20 % (12 cm) to 100 % (60 cm) of the total capillary length (calculated with CE Expert 1.0 Program, Beckman Instruments). The samples were injected into the capillary using nitrogen at 0.5 psi for 5 s. Finally, the separation was performed placing the CZE buffer solution at the inlet vial. The operating voltage was +30 kV and the temperature was 25 °C.

MEKC-MS: MEKC-MS studies were carried out using a CE system (P/ACE 5010 Beckman Instruments, Fullerton, CA, USA) controlled by a PC running System GOLD software from Beckman. The MS employed was an ion trap (IT) mass spectrometer (Esquire 2000, Bruker Daltonics, Bremen, Germany) equipped with an orthogonal electrospray interface (model G1607A from Agilent Technologies, Palo Alto, CA, USA). This instrument was controlled by a PC running the Esquire NT software from Bruker Daltonics. Electrical contact at the electrospray needle tip was established using isopropanol:water (50:50, v/v) with 0.1% formic acid as sheath liquid at a flow rate of 4 μL/min by a Cole Palmer syringe pump (Vernon Hills, IL, USA).

Nebulizer pressure, drying gas flow and drying temperature were 4.0 psi, 4.0 L/min, and 200 ºC respectively, and the electrospray operated in the positive ion mode (4.5 kV). The m/z range scanned by the mass spectrometer was from 100 – 400 m/z.

Separations were performed using uncoated fused-silica capillaries with a total length of 80 cm x 50 μm i.d. The total length of the MEKC plug was 25 % of the capillary length (20 cm). Sample injections were made at 0.5 psi for 6 s and the separation was achieved applying a voltage of 25 kV.

RESULTS AND DISCUSSION

Method development

Sudan dyes (see Figure 1) are lypophilic compounds and weak acids with pKa values around 11.65. Because of their neutral nature the separation of these dyes was studied by MEKC trying to develop a partial filling method compatible in the future with electrospray-MS detection. Different background electrolyte (BGE) compositions using volatile buffers (ammonium acetate and ammonium bicarbonate) plus SDS were investigated to separate the compounds by partial filling MEKC. Different organic solvents were also added to the BGE in order to increase the solubility of these hydrophobic analytes. First, Sudan I-IV solubilities were studied in mixtures of water and organic solvents, observing that the dyes were more soluble in ACN, acetone and isopropanol. In good agreement with Mejia *et al.* (*25*), ACN gave better separation when it was used as organic modifier in the MEKC buffer. The best separation conditions were obtained using NH_4HCO_3, SDS and ACN. Thus, the effect of different concentrations of NH_4HCO_3, SDS and percentage of ACN on the MEKC separation of the four investigated dyes was explored. As can be observed in Figure 2A, the four Sudan dyes were not separated when a concentration of 15 mM SDS was used, whereas

broader peaks were observed when 50 mM SDS was employed, obtaining a good compromise between separation and peak broadening using 25 mM of SDS. Next, using a concentration of 25 mM SDS the effect of the concentration of NH_4HCO_3 was investigated. As shown the Figure 2B, using a high concentration (40 mM NH_4HCO_3) broader peaks and longer migration times were obtained, while the use of a low concentration (20 mM NH_4HCO_3) decreased the resolution between peaks. Thus, a concentration of 30 mM NH_4HCO_3 containing 25 mM of SDS and 30 % ACN was chosen as the optimum conditions for the separation of the four Sudan dyes, observing that an increase of the percentage of ACN to either 35 or 40 % generated a decrease of resolution (data not shown). Although the selected BGE composed of 30 mM NH_4HCO_3, 25 mM of SDS and 30 % ACN enabled the separation of the four dyes, it could not be used in combination with MS detection because of the contamination of the ion source induced by the presence of SDS in the BGE, which causes low ionization efficiencies and loss of detection sensitivity (*27-29*). To avoid that SDS reaches the MS detector, the use of partial filling was investigated to make compatible the MEKC conditions with electrospray-MS detection (*29*). Then, the MEKC buffer composed of 30 mM NH_4HCO_3, 25 mM SDS with 30 % ACN was used to fill 20, 25, 50, 75 or 100 % of the capillary. The rest of the capillary was filled with a CZE buffer composed of 30 mM of NH_4HCO_3 and 30 % ACN. Filling the 20 % of the capillary with MEKC buffer led to the lost of the baseline resolution (data not shown). The best conditions in terms of analysis speed and peak shape were obtained filling 25 % of the capillary length with the MEKC buffer providing analysis time shorter than 7 min as can be observed in Figure 3.

Method repeatability and quantitative analysis

193 The selected conditions of Figure 3 were employed to study the method
194 repeatability. As can be observed in Table 1 the relative standard deviation (%RSD $_{n=10}$)
195 varied from 1.3 to 3.1 % for the analysis time and from 12.5 to 25.5 % for the relative
196 peak area. These latter values are unacceptable for quantitative analysis. For this reason,
197 flumequine was selected among different compounds as internal standard (IS). This
198 compound fulfills the requirements of no interfering with the analytes migration time
199 and it has similar solubility, electrophoretic mobility and extinction coefficient that the
200 studied compounds. Thus, the values of % RSD were improved more than 3 times for
201 Sudan I, II and III by using a relative peak area which was calculated dividing the
202 analyte corrected peak are by the I.S corrected peak area (see Table 1). However, the %
203 RSD value obtained for Sudan IV was still too high (25.4 %). This fact is probably due
204 to the low solubility of this analyte that causes poor peak shape (see Sudan IV peak in
205 Figure 4A). In order to improve this result an additional study of the analytes solubility
206 was carried out. The solubility of the dyes could be improved increasing the percentage
207 of ACN from 30 % to 35 % as could be deduced from their peak shapes. However,
208 using 35 % ACN, the separation was lost. Therefore, concentration of NH_4HCO_3 and
209 SDS were again varied to improve both solubility and resolution. Increasing the SDS
210 concentration and using 40 mM of NH_4HCO_3 and 35 % of ACN better peak shapes and
211 a slightly improvement of the resolution was observed. However, complete baseline
212 separation of the four analytes could not be obtained. A more subtle optimization of the
213 separation conditions was then carried out, concluding that a partial filling of 25 % with
214 a MEKC buffer composed of 40 mM NH_4HCO_3, 25 mM SDS and 32.5 % ACN
215 provided baseline separation of the four compounds with good peak shapes for all the
216 Sudan dyes as can be deduced from Figure 4B. Under these new conditions, both
217 repeatability and sensitivity of the method were improved as can be deduced comparing

218 the results from Table 1 and 2. The LODs for Sudan I-IV were improved from 1.28-
219 3.07 μg mL^{-1} to 0.57-0.71 μg mL^{-1}. In addition, the relative peak area repeatability for
220 Sudan IV improved from 25.4% to 8.8 %. Using these conditions, the inter-day
221 repeatability and the linearity of the method were studied. The results obtained for the
222 inter-day repeatability (see Table 2) provided % RSD values lower than 10 % in all
223 cases. With regards to the linearity, it was determined by plotting the peak areas as a
224 function of the Sudan I-IV concentration in μg mL^{-1}. The intercept, slope, correlation
225 coefficient (R^2), and the regression standard error for each of the Sudan dyes are shown
226 in Table 3. For the linear range studied from 2.5 to 20 μg mL^{-1} (namely, 2.5, 5, 7.5, 10,
227 12.5, 15, and 20 μg mL^{-1}), values of R^2 higher than 0.99 were obtained in all cases,
228 corroborating the good possibilities of this method for the quantitative analysis of Sudan
229 dyes in food additives.

230 **Application to real samples**

231 The proposed method was applied to the identification and determination of
232 Sudan dyes in three different samples of chilli tomato sauces. No presence of Sudan dye
233 was detected in any of the studied samples. Thus, in order to demonstrate the
234 applicability and accuracy of this new method, the samples were spiked with known
235 amount of Sudan dyes.

236 Accuracy of the method was evaluated as the recovery obtained by each Sudan
237 dyes when spiking the samples of chilli tomato sauces with 2.5 μg mL^{-1} of Sudan I and
238 II and 5.0 μg mL^{-1} of Sudan III and IV. Table 4 shows that the recovery values obtained
239 for Sudan I and II were from 92.1 to 99.0 % while the values obtained for Sudan III and
240 IV were lower (from 85.2 to 92.1 %). These data are in agreement with the literature
241 where the recoveries values depend on the solvent utilized for the extraction and the
242 matrix of the samples, being generally lower for Sudan IV (*7*).

Figure 5 shows the electropherograms obtained under the optimized conditions for the separation of Sudan I, II, III and IV from a spiked chilli tomato sauce, namely, the chilli tomato sauce was spiked with 2.5 μg mL^{-1} of Sudan I and II and 5 μg mL^{-1} of Sudan III and IV. LOD slightly higher than those obtained for the standard samples were obtained for chilli tomato sauce. Namely, the LOD was 0.68, 0.63, 0.94 and 1.25 μg mL^{-1} for Sudan I, II, III, and IV, respectively. This fact can be explained by both the recoveries mentioned above (from 85 to 99%) and the presence of interferences from the matrix that can negatively affect the separation and detection of the dyes. This figure demonstrates the selectivity of the method developed in this work since it provides an adequate separation between the Sudan dyes studied and the rest of constituents from the complex matrix of the chilli tomato sauce.

Preliminary MEKC-MS results.

The compatibility of the developed MEKC method with MS detection was studied for the simultaneous determination of Sudan dyes. Due to instrumental constrains the coupling MEKC-MS needs longer capillary, thus, the injection time of samples and the plug were adapted to the dimension of the capillary in order to carry out the analysis in comparable condition with MEKC-UV. Besides, several analytical parameters, such as ESI voltage, nature of sheath liquid, temperature and flow of dry gas were optimized in order to obtain the higher intensity in the MS signal by flushing the standards by low pressure (0.5 psi) towards the MS instrument. The highest intensity of the MS signals was obtained using the ESI source in positive mode (4.5 kV), isopropanol:water (50:50 v/v) with 0.1 % formic acid as sheath liquid and a temperature of dry gas of 200 ºC flowing at 4 L/min. Figure 6 depicts the partial filling MEKC-MS extracted-ion electropherograms (EIE) for the standard mixture of Sudan I, II, III and IV

dyes. As can be observed in this figure, the $[M-H]^+$ obtained for each dye was: 249.2, 277.1, 353.2 and 381.2 m/z for Sudan I, II, III, and IV, respectively (values that are in good agreement with the expected molecular weight for these compounds: 248.3, 276.3, 352.4 and 380.4 g mol^{-1}, respectively). Under these conditions, the LODs obtained were ranging from 0.52 to 1.67 µg/mL for the four dyes. These LODs are similar to those obtained by MEKC-UV, although they could be improved by using MS/MS in multiple reaction monitoring (MRM) mode. This possibility is currently being under study at our laboratory.

In summary, a new method for the determination of Sudan dyes by partial filling micellar electrokinetic chromatography was developed. After a fine optimization of the analytical conditions (BGE composition and ionic strenght, surfactant concentration, type and percentage of organic modifier, and percentage of capillary filled with the MEKC solution) a fast method was achieved that allows the simultaneous determination of Sudan dyes (I, II, III, and IV) in less than 8 min with minimum instrumentation needs and cost. The LODs provided by this method were ranging from 570 to 710 ng mL^{-1}. Although samples containing Sudan dyes were not found, the method was successfully applied for the analysis of Sudan dyes (I, II, III and IV) in spiked chilli tomato sauces samples with good recoveries, showing the potential of the method for quality control of food samples. Moreover, the partial filling MEKC method is also demonstrated to be compatible with MS detection.

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FIGURE CAPTIONS

Figure 1. Structures of the four Sudan dyes analyzed in this work.

Figure 2. Effect of SDS and ammonium bicarbonate concentration on MEKC separation of Sudan I to IV (10 μg mL^{-1} each) by partial filling with 25 % buffer MEKC and 75 % CZE buffer. (A) MEKC buffer composed of 30 mM NH_4HCO_3 and 30% v/v ACN with 15 mM, 25 mM and 50 mM SDS and CZE buffer composed of 30 mM NH_4HCO_3 and 30 % v/v ACN (B) MEKC buffer composed of 20, 30 and 40 mM NH_4HCO_3, 25 mM SDS and 30 % v/v ACN and the respective CZE buffer contained the same concentration of NH_4HCO_3 and 30 % v/v ACN. Other conditions: run voltage 30 kV, detection wavelength 214 nm; sample injected at 0.5 psi for 5 s; capillary with 50 μm i.d., 60 cm of total length and 50 cm of detection length.

Figure 3. Electropherograms of a standard mixture of Sudan I-IV (20 μg mL^{-1} each) partially filling a percentage of the capillary with the MEKC buffer of (A) 100%, (B) 75 %; (C) 50 %, and (D) 25 %. MEKC buffer: 30 mM NH_4HCO_3, 25 mM SDS and 30 % (v/v) ACN; CZE buffer: 30 mM NH_4HCO_3 and 30 % (v/v) ACN. Other conditions as in Figure 2.

Figure 4. Effect of acetonitrile percentage on the separation of a standard mixture of Sudan I to IV (10 μg mL^{-1} each) plus the internal standard (I.S., 10 μg mL^{-1} of flumequine) by partial filling 25 % of the capillary with MEKC buffer and 75 % with CZE buffer. (A) MEKC solution contained 30 mM NH_4HCO_3, 25 mM SDS and 30 % (v/v) ACN; CZE buffer composed of 30 mM NH_4HCO_3 and 32.5 % (v/v) ACN; (B)

MEKC solution contained 40 mM NH_4HCO_3, 25 mM SDS and 32.5 % (v/v) ACN; CZE buffer composed of 40 mM NH_4HCO_3 and 32.5 % (v/v) ACN. Other conditions as in Figure 2.

Figure 5. Electropherograms of chilli tomato sauce (A) and the same chilli tomato sauce spiked with 2.5 μg mL^{-1} of Sudan I and II and 5 μg mL^{-1} of Sudan III and IV (B). Other conditions as in Figure 4B.

Figure 6. Partial filling MEKC-MS extracted ion electropherograms (EIE) of a standard mixture containing 5 μg/mL of each Sudan dye. Conditions: uncoated fused-silica capillary, 50 μm ID × 80 cm total length; CZE buffer composed of 40 mM ammonium bicarbonate and 32.5 % ACN (v/v); the capillary was partially filled (25 %) with a MEKC solution composed of 40 mM ammonium bicarbonate, 25 mM SDS and 32.5 % ACN (v/v); sample injected at 0.5 psi for 6 s; applied voltage, 25 kV; temperature, 25 ºC. ESI conditions: positive ion mode; spray voltage, 4.5 kV; sheath liquid, isopropanol/water (50/50 v/v) with 0.1% formic acid at 4 μL/min; drying gas flow, 4 L/min; drying temperature, 200 ºC; nebulizer pressure, 4 psi.

Table 1. Repeatability and limit of detection (LOD) obtained for the four Sudan dyes using the partial filling-MEKC method.

	Repeatability (%RSD, n=10)			LOD ($\mu g\ mL^{-1}$)
Analyte	**Migration Time**	**Corrected Peak Area[1)]**	**Relative Peak Area[2)]**	
Sudan I	1.3	24.5	8.8	1.28
Sudan II	1.5	25.5	7.5	1.53
Sudan III	2.2	23.6	9.6	2.19
Sudan IV	3.1	12.5	25.4	3.07

[1)] Corrected peak area calculated as (peak area)/(migration time)
[2)] Relative peak area calculated as (analyte corrected peak area)/(internal standard corrected peak area). Flumequine was used as internal standard.

Table 2. Intra-day and inter-day precision and limit of detection obtained.

Analyte	Intra-day precision (%RSD, n=10)		Inter-day precision (%RSD, n=15, 3 days)		LOD ($\mu g\ mL^{-1}$)
	Migration Time	Relative Peak Area [1)]	Migration Time	Relative Peak Area [1)]	
Sudan I	2.3	3.2	2.3	4.1	0.57
Sudan II	2.5	5.9	2.5	5.8	0.63
Sudan III	2.8	5.2	2.9	6.9	0.66
Sudan IV	3.2	8.8	3.3	9.9	0.71

[1)] Relative peak area calculated as (analyte corrected peak area)/(internal standard corrected peak area). Flumequine was used as internal standard.

Table 3. Results of calibration of peak area ratio versus concentration of Sudan I-IV (concentration interval from 2.5 to 20 µg mL^{-1}). Flumequine was used as internal standard at 10.0 µg mL^{-1}.

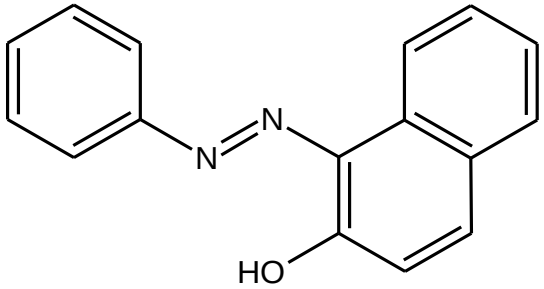
Analyte	Intercept	Slope	R^2	SE
Sudan I	0.040 ± 0.008	0.052 ± 0.001	0.997	0.017
Sudan II	0.110 ± 0.013	0.061 ± 0.001	0.993	0.028
Sudan III	0.085 ± 0.011	0.051 ± 0.001	0.994	0.023
Sudan IV	0.038 ± 0.021	0.057 ± 0.001	0.994	0.026

SE, regression standard error.

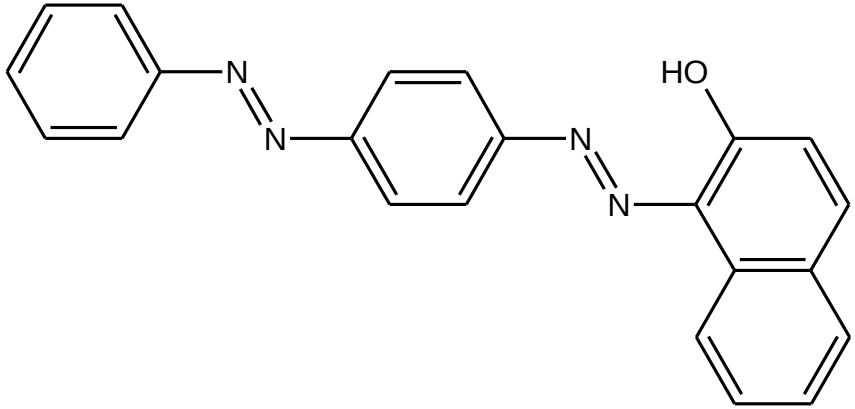
Table 4. Recovery values of Sudan I-IV from different chilli tomato sauces. Chilli samples were spiked with 2.5 μg mL^{-1} of Sudan I and II and 5 μg mL^{-1} of Sudan III and IV.

	Recoveries (%)		
Analyte	**Chilli tomato sauce 1**	**Chilli tomato sauce 2**	**Chilli tomato sauce 3**
Sudan I	98.2 ± 1.0	95.2 ± 2.5	95.3 ± 6.1
Sudan II	99.0 ± 2.7	97.2 ± 1.1	92.1 ± 1.2
Sudan III	90.4 ± 1.2	92.1 ± 2.2	88.4 ± 4.2
Sudan IV	85.5 ± 5.2	90.4 ± 3.4	85.2 ± 2.5

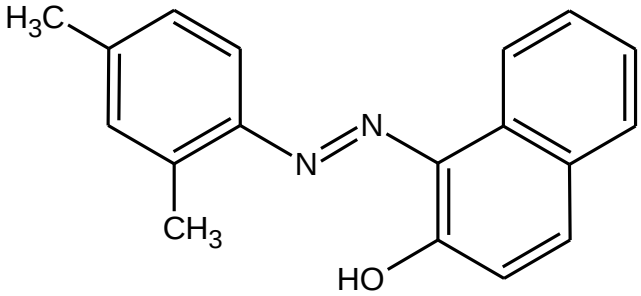
Figure 1



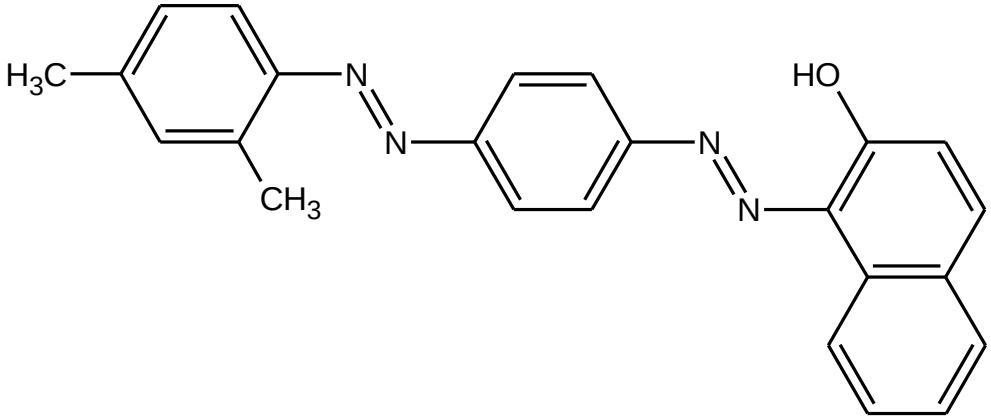
Sudan I



Sudan III



Sudan II



Sudan IV

Figure 2

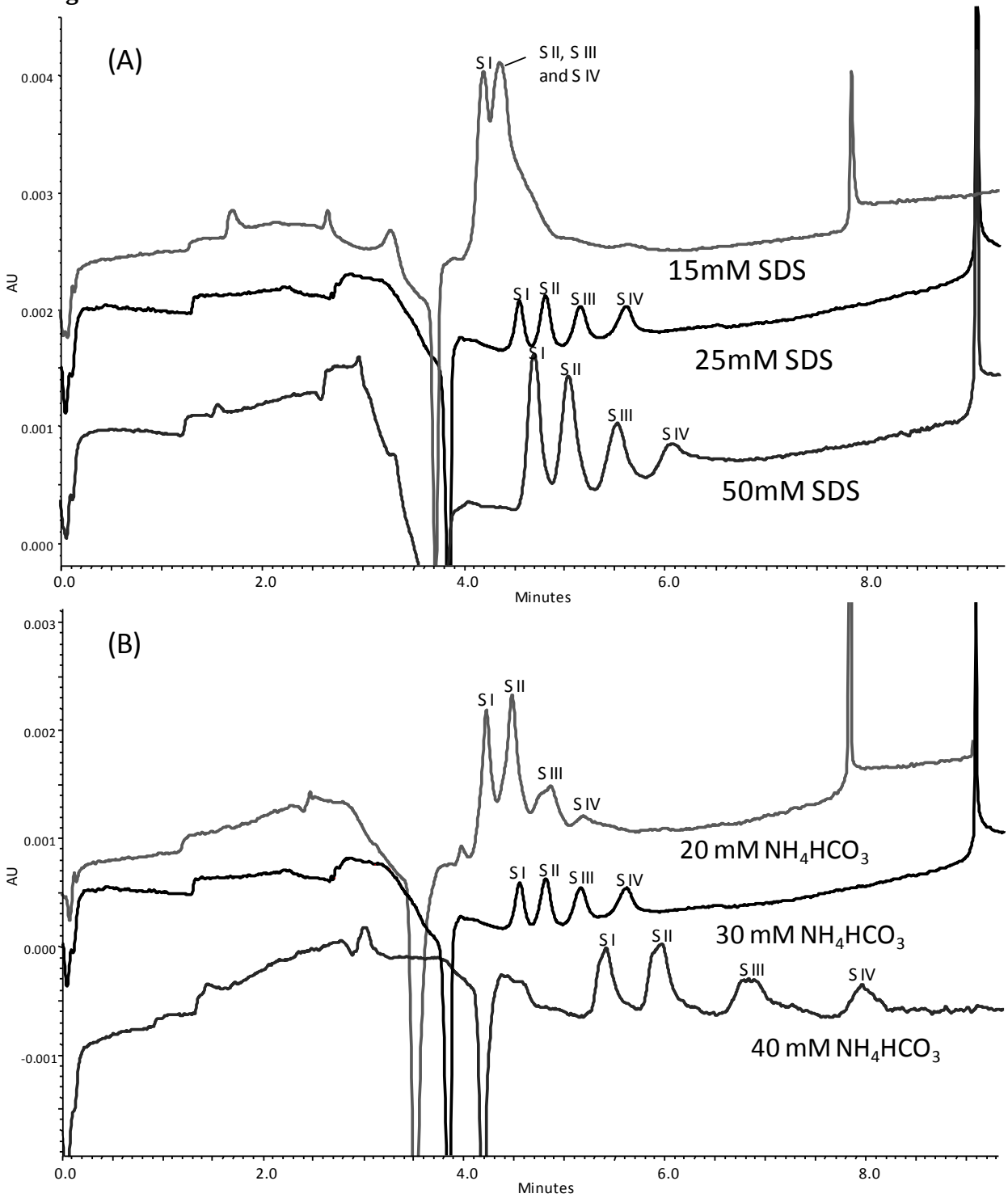

(A)
S I
S II, S III
and S IV
15mM SDS
S I
S II
S III
S IV
25mM SDS
S I
S II
S III
S IV
50mM SDS
AU
0.004
0.003
0.002
0.001
0.000
0.0
2.0
4.0
6.0
8.0
Minutes
(B)
S I
S II
S III
S IV
20 mM NH4HCO3
S I
S II
S III
S IV
30 mM NH4HCO3
S I
S II
S III
S IV
40 mM NH4HCO3
AU
0.003
0.002
0.001
0.000
-0.001
0.0
2.0
4.0
6.0
8.0
Minutes

Figure 3

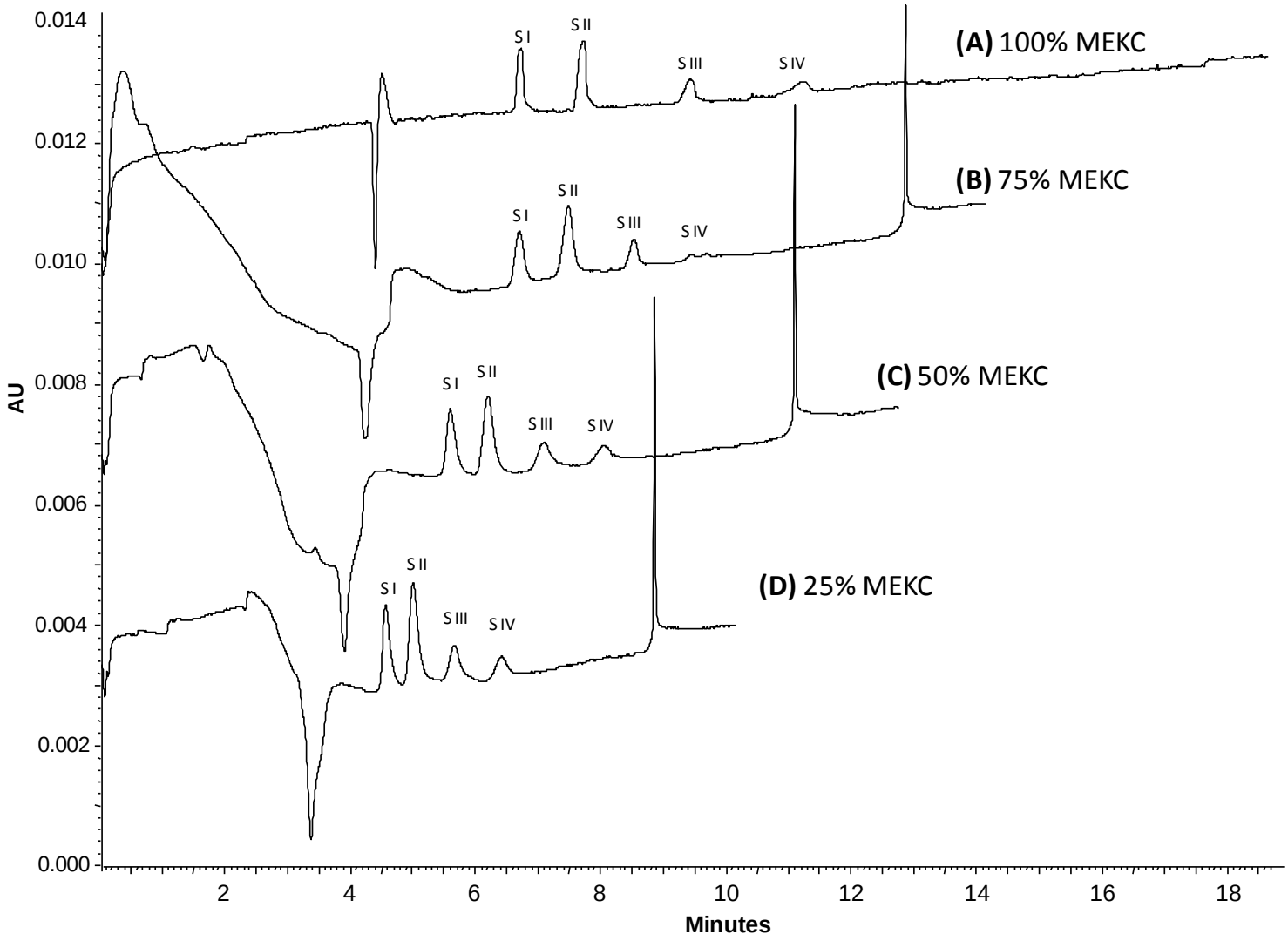

0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
AU
2
4
6
8
10
12
14
16
18
Minutes
S I
S II
S III
S IV
(A) 100% MEKC
(B) 75% MEKC
(C) 50% MEKC
(D) 25% MEKC

Figure 4

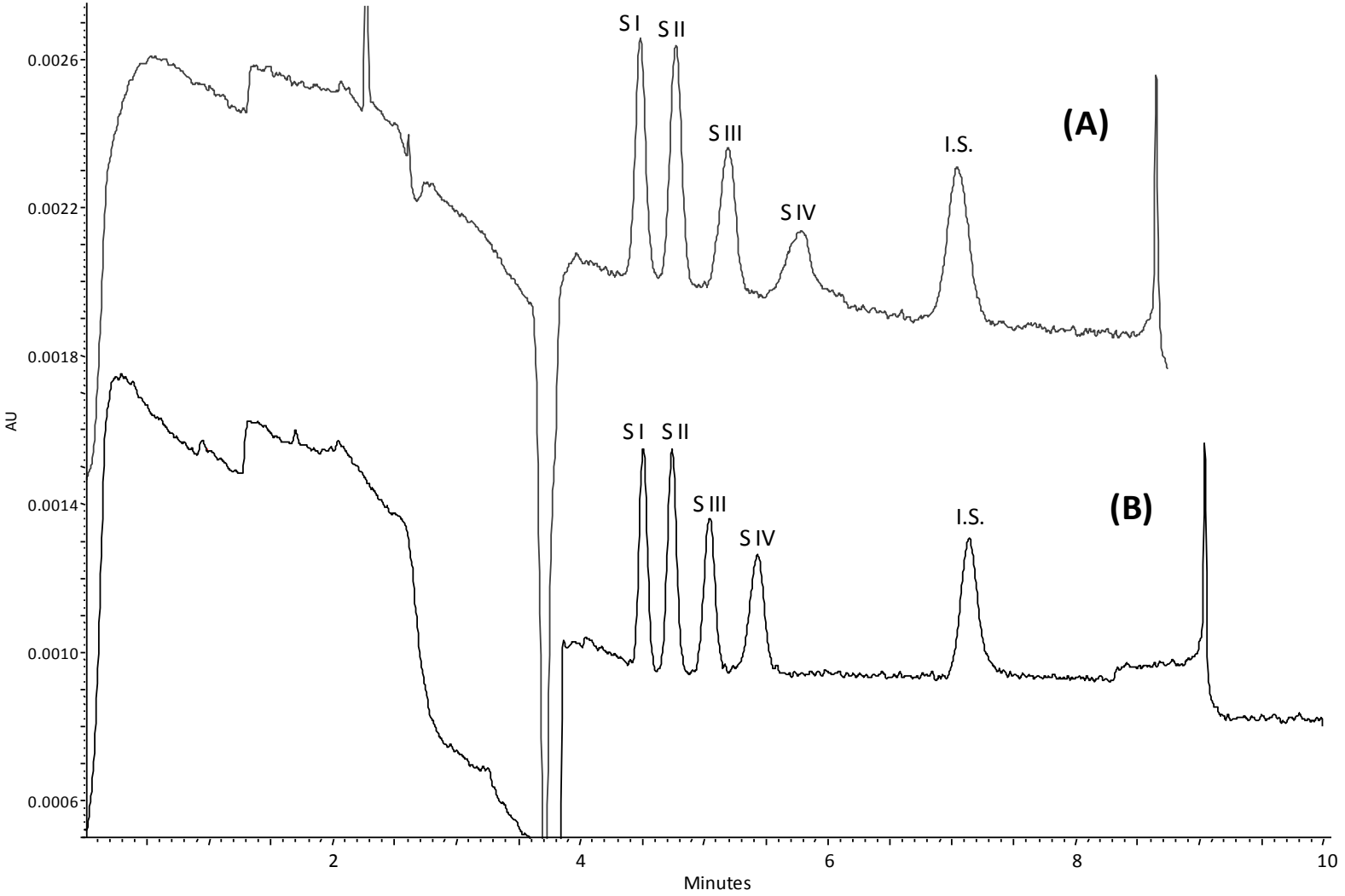

S I
S II
S III
S IV
I.S.
(A)
S I
S II
S III
S IV
I.S.
(B)
AU
0.0026
0.0022
0.0018
0.0014
0.0010
0.0006
2
4
6
8
10
Minutes

Figure 5.

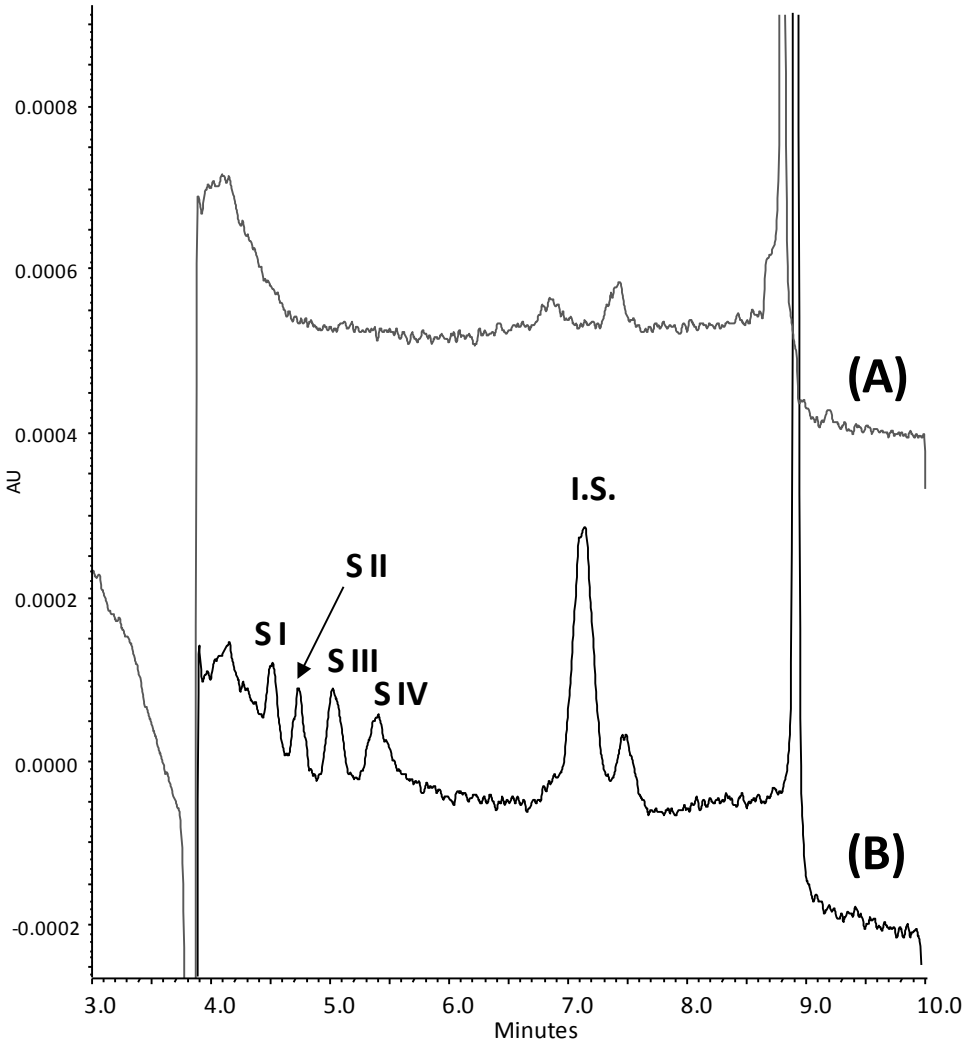
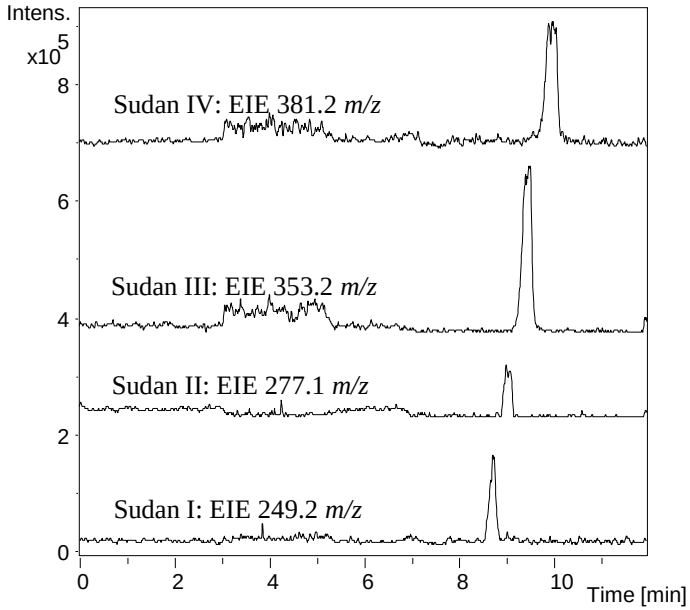

0.0008
0.0006
0.0004
0.0002
0.0000
-0.0002
AU
(A)
(B)
I.S.
S II
S I
S III
S IV
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
Minutes

Figure 6.


Intens.
x10^5
8
6
4
2
0
Sudan IV: EIE 381.2 m/z
Sudan III: EIE 353.2 m/z
Sudan II: EIE 277.1 m/z
Sudan I: EIE 249.2 m/z
0
2
4
6
8
10
Time [min]