



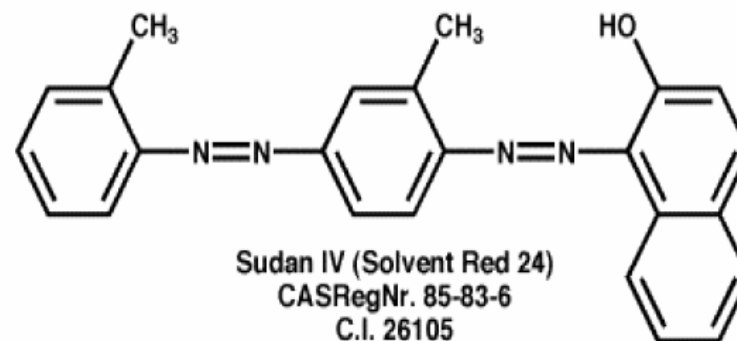
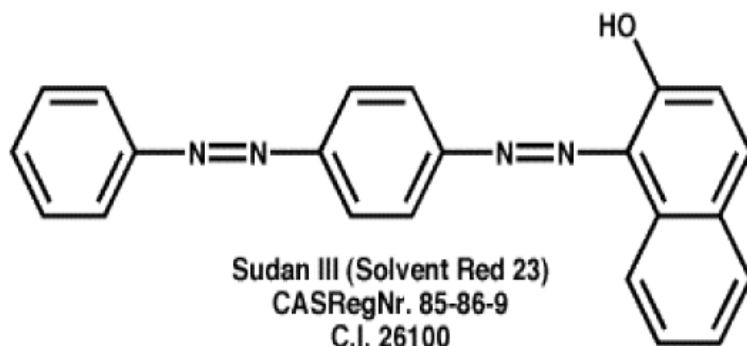
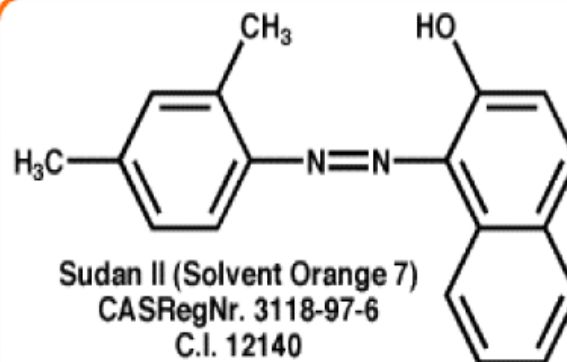
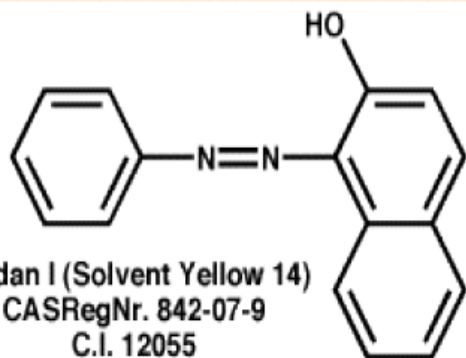
Totally Automated Method for the Determination of Sudan Dyes in Food via On-Line Filtration, SPE and HPLC Analysis

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Abstract:

Sudan dyes are categorized as Class 3 carcinogens by the International Agency for Research on Cancer (IARC), and, therefore, are illegal as food additives according to both FDA and the EU. The European commission requires products to have documentation confirming the absence of Sudan dyes. Since 2003, European nations have required random product testing and testing of suspected adulterated products. Items found to contain Sudan dyes must be disposed of as hazardous waste. The system presented offers a totally automated approach to the extraction of Sudan dyes from food products. The product is solubilized, filtered and SPE is performed prior to HPLC analysis. Detection limits set by the EU of 0.5-1 mg/kg are obtained from this method without manual intervention or human exposure. Data presented shows the efficacy of this approach relative to preexisting methodology.

Sudan Dye Structures:



Outline:

- **Background Information on Sudan dyes**
- **Sample Preparation**
- **System and Procedure**
- **Data and Results**
- **Summary and Conclusion**

Background Information on Sudan Dyes:

- **Sudan dyes are red dyes that are used for coloring solvents, oils, waxes, petrol, shoe and floor polishes**
- **They have also been found in some chili powder imported from India, in a number of relishes, chutneys and seasonings containing this chili powder**
- **Sudan dyes are not allowed to be added to food in the USA, UK and the rest of the EU and Japan**
- **They are azo dyes, which when eaten, may be split into carcinogenic amines**

Sample Preparation:

- **4 gms of chili powder was mixed with 20 mL of acetone and vigorously shaken on an orbital shaker, 600 RPM**
- **The chili solution was filtered off-line with a 0.45 um PTFE filter**
- **1 ml of the chili solution was diluted to 5 mL with aqueous NaOH (pH 11)**
- **The diluted chili solution was spiked with 100 ppb standard solutions of Sudan I, II, III, IV in acetone**

- **The diluted chili solutions were filtered on the SPE-215 liquid handler via a syringe barrel type filter, 0.45 um**
- **The collected filtered solutions were subjected to automated SPE on the SPE-215**
- **SPE eluent was evaporated to dryness and reconstituted in 200 ul acetonitrile/water 90:10**
- **The SPE eluent was analyzed via analytical HPLC at 504 nm**

SPE Methodology:

- **215-SPE, 4 probe automated SPE and liquid handler**
- **Oasis MCX, 60 mgs, 3 ml, SPE cartridges**
 - **Condition Column: 2 mL ethyl acetate**
2 mL methanol
1 mL 0.1 NaOH
2 mL water
 - **Load 5 mL of diluted sample, in two 2.5 mL additions to the column**
 - **Wash column: 2 mL 70% methanol in water**
1 mL 1.0 M NaOH
2 ml methanol, 1 ml ethyl acetate
 - **Elute sample: 2 ml 89:9:2 ethyl acetate: methanol: formic acid**
 - **Dry down eluent, reconstitute with 200 uL 90:10 ACN, water**

215-SPE: Automated SPE Instrument



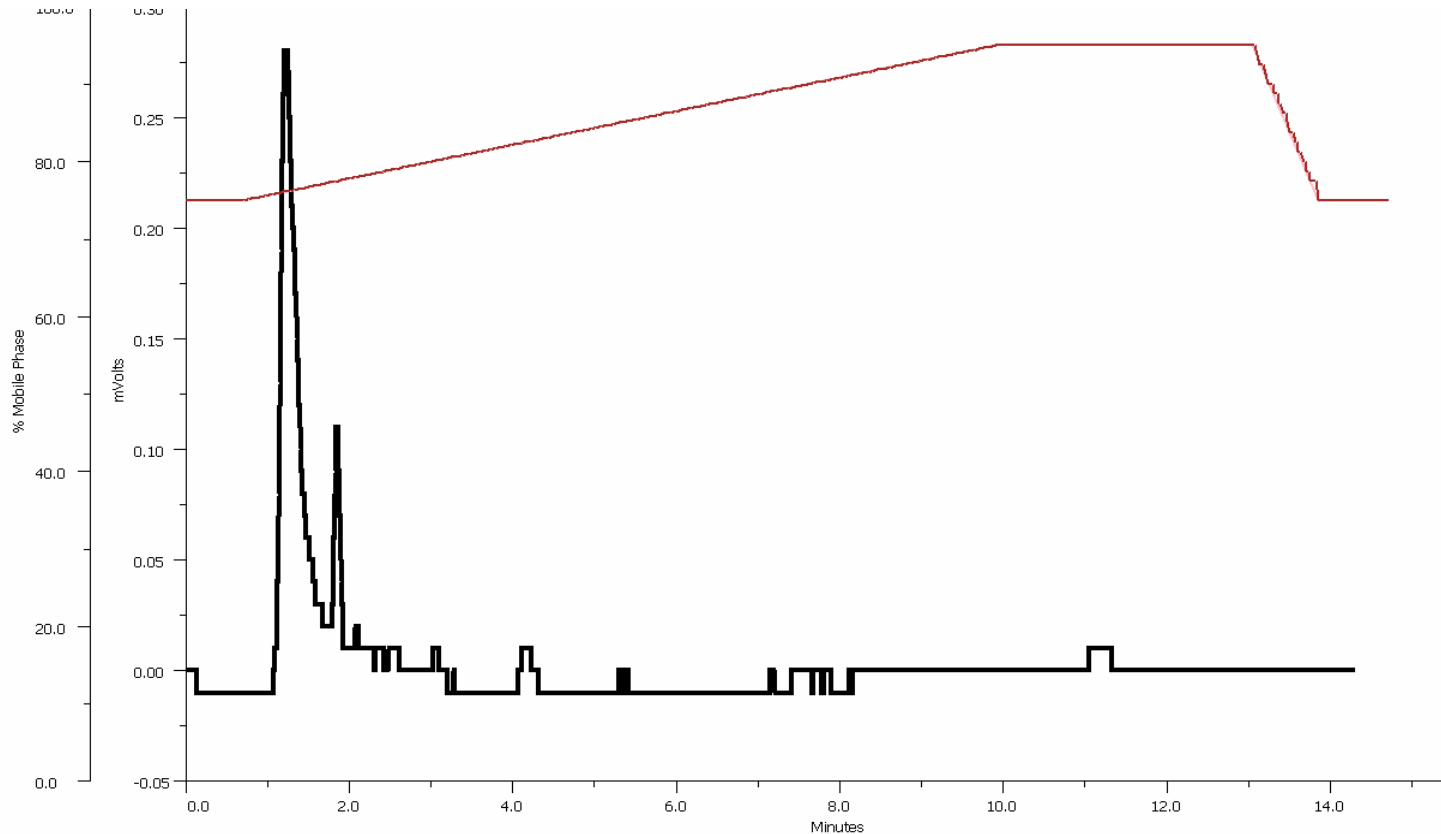
The SPE-215 uses positive pressure to push the solvents and sample through the SPE cartridges, can accommodate

1, 3, 96 well formats

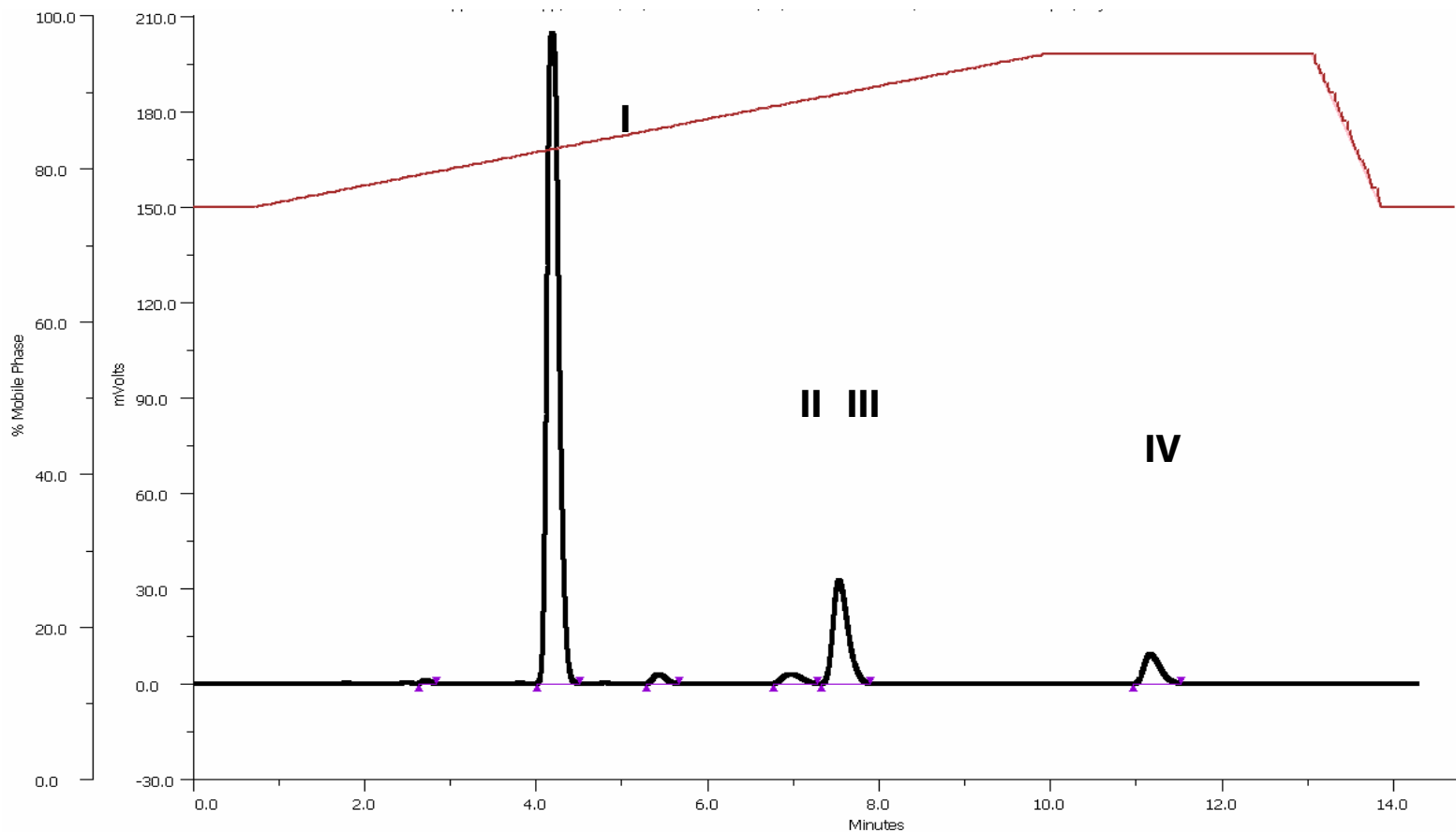
HPLC Methodology:

- **Solvent A: Water, 0.1% TFA**
Solvent B: ACN, 0.1% TFA
- **Flow: 1.5 mL/min**
- **Gradient conditions: 75% (B) 0 -1 min**
75 – 95% (B) 1 – 10 min
95% (B) 10 -12 min
95 - 75% (B) 12 – 12.5 min
75% (B) 12.5 – 13.5 min
- **Injection volume: 25 uL, Column: Thermo BetaBasic C-18, 4.6 x 150 mm, 3 um**
- **UV/Vis Detector 504 nm, sensitivity 0.1 AUFS**

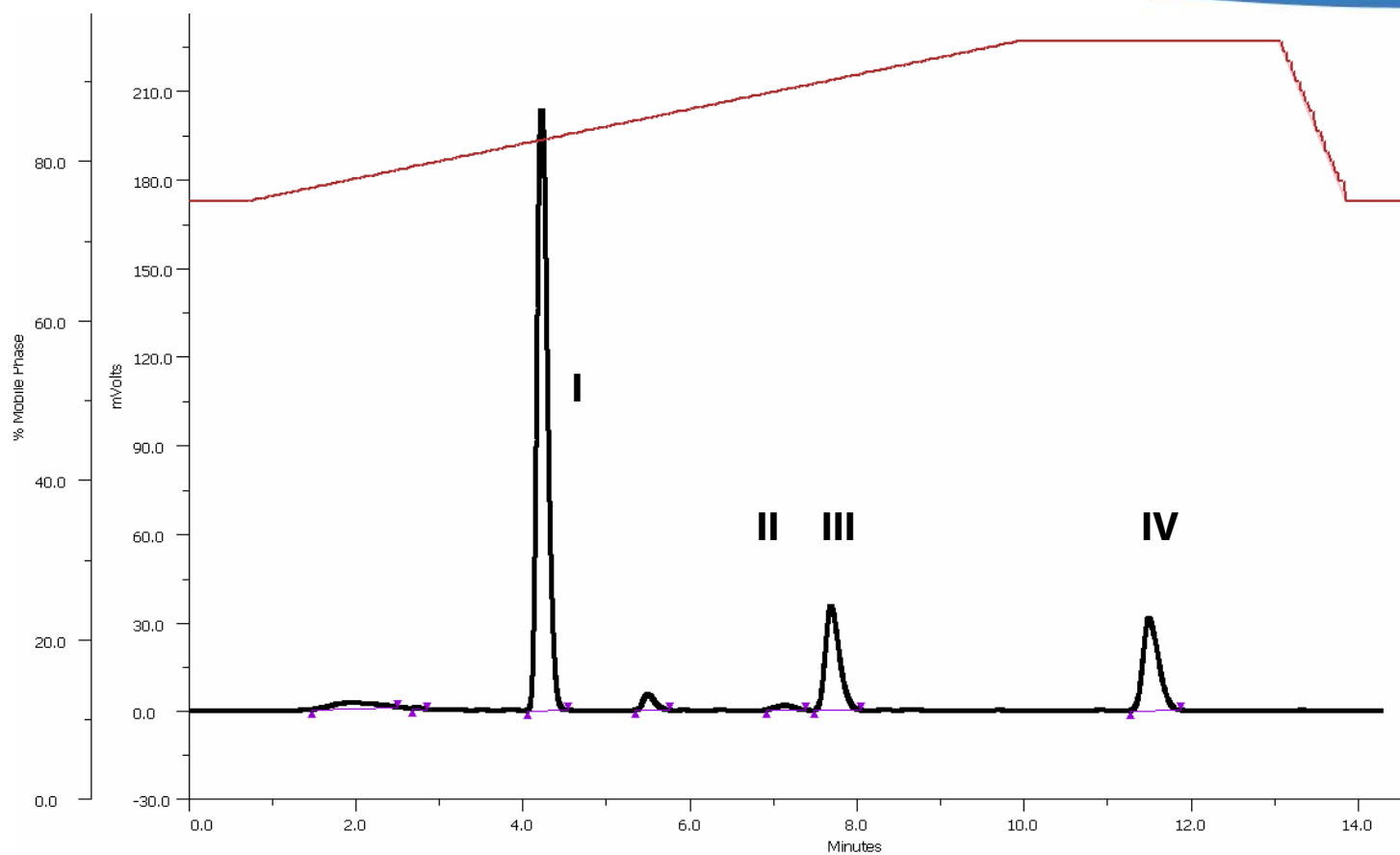
Data and Results:



Graph 1: Chromatogram of McCormick's chili powders, all three types (chili powder, hot Mexican, and chipotle) gave the same results, no detection of Sudan I or its analogues.



Graph 2: Chromatogram representing the results from a spiked chili powder solution with Sudan I, II, III, IV, 7 -11 ug on column



Graph 3: Chromatogram of a mixture of Sudan I and its analogues in acetone/NaOH dilution mix, not chili powder extract

Recovery:

Recovery (n = 10, 7 – 11 ug on column)

- Sudan I 95% (4% RSD)
- Sudan II 85% (2% RSD)
- Sudan III 93% (3% RSD)
- Sudan IV 75% (3% RSD)
- Limit of detection have been determined to be in the 0.1 – 0.5 ppm range without LC/MS

Conclusion:

- A versatile method was developed, which would determine Sudan I, Sudan II, Sudan III and Sudan IV in food stuffs, within the same chromatogram
- A chromatographic analysis time of 10 minutes, and total SPE time of less than 5 minutes per sample was achieved, thereby increasing sample throughput
- The recovery and LOD proved that LC/MS was not required to test food stuffs for Sudan I and its analogues
- We could continue to use our chili powders from the US, safe in the knowledge that there had been no trace of Sudan I or its analogues, found in off the shelf chili powders.....PHEW!