Analysis of Sudan I, Sudan II, Sudan III and Sudan IV using LC UV and LC MS

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Introduction

Sudan dyes are synthetic industrial dyes traditionally used in waxes, plastics, oils, and polishes. Although recognized as carcinogens, Sudan dyes have recently been found in food products in some European countries. Sudan dyes are added to foods such as chili powders to mimic, intensify, and prolong the appearance of natural red hues for the shelf life of the product. In addition to chili products, more than six hundred other products containing Sudan dyes have been recalled in the UK, making for the largest food recall in British history.

Sudan dyes are azo-dyes, which are recognized as carcinogens. The International Agency for Research on Cancer (IARC) classifies Sudan dyes as Class 3 carcinogens and, therefore, it is illegal to use them as food additives according to both the FDA and the EU. The European Commission passed a directive stating that products must 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 are considered to be hazardous waste and must be disposed of accordingly. Laboratories performing analyses for Sudan dyes are not required to follow defined methods.

Purpose

This work describes a reversed phase HPLC separation of Sudan I, Sudan II, Sudan III, and Sudan IV (Scarlet Red). UV-visible spectroscopy is used to detect all four dyes with two fixed wavelengths. Calibration curves are generated to determine the linearity of the absorbance-concentration relationship. LCMS is used for detection and characterize spectrum. LCMS is utilized to determine typical spectra of Sudan dyes as well as optimize electrospray parameters to minimize in source fragmentation.

Materials and methods

Sudan I, Sudan II, Sudan III, and Sudan IV and ethyl acetate were purchased from Sigma-Aldrich. Acetonitrile and methanol were of HPLC grade. A Barnstead ultra pure system supplied deionized water. Stock solutions of 1mg/mL Sudan I and Sudan II were prepared in methanol; equivalent solutions of Sudan III or Sudan IV were prepared in ethyl acetate. To avoid oxidative cleavage, the solutions were stored at 4°C in the dark (containers covered with foil). 20µg/mL solutions were prepared by diluting stock solutions with methanol, to match the mobile phase. Mixtures containing all four dyes were prepared by combining stock solutions and diluting to 20µg/mL of each dye.

LC UV-Visible Parameters

An Ultra Aqueous C18 column, 150 x 4.6mm, 5µm was used at room temperature, in conjunction with a Waters 717plus autosampler, 1525 binary pump, and 2487 dual wavelength absorbance detector. An isocratic mobile phase of 70:30 (v/v) acetonitrile: methanol with a 1mL/min flow rate was used. Injection volume was 10 µL. Detection was performed by two schemes: each dye was detected using the corresponding wavelength of maximum absorption and also with only two fixed wavelengths. Sudan I was detected at 476nm, Sudan II at 493nm, Sudan III at 512nm, and Sudan IV at 357nm. Sudan I and II were monitored at 488nm and Sudan III and IV were monitored at 520nm. Chromatographic peaks were identified based on matching retention times to times for individual reference standards. Calibration concentrations included: 1 ppm, 10 ppm, 50 ppm, 100 ppm, n=4.

LC MS Parameters

An Ultra Aqueous C18 column, 150 x 2.1mm, 5µm was used at room temperature, in conjunction with a Waters 717plus autosampler, 1525 binary pump, and Waters ZMD mass spectrometer. An isocratic mobile phase 70:30 (v/v) acetonitrile: methanol, each containing 0.1% formic acid with a 0.15 mL/min flow rate was used. Injection volume was 10 µL.

The Waters ZMD mass spectrometer used the following settings: Positive mode electrospray ionization, 80-400 amu range, 3.5 kVolts capillary voltage, 10 V cone voltage (to deter fragmentation in the ESI source), rf lens 0.4 V, 150 ˚C source temperature and 200 ˚C desolvation temperature. MassLynx 3.0 was used for data analysis.

Results

Figure 1. Chromatogram produced by changing the detection wavelength at appropriate times to detect each dye at it's corresponding wavelength of maximum absorbance. **1**. Sudan I, 476 nm, **2**. Sudan II, 493 nm, **3**. Sudan III, 512 nm, **4**. Sudan IV, 357 nm.

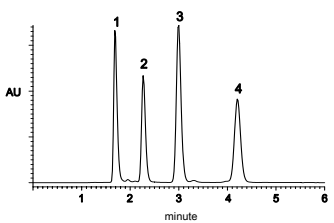


Figure 2. Chromatogram produced by detecting all dyes at two fixed wavelengths. **1**. Sudan I, 488 nm, **2**. Sudan II, 488 nm, **3**. Sudan III, 520 nm, **4**. Sudan IV, 520 nm.

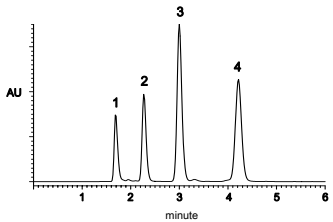


Figure 3. Calibration curves include 1 ppm, 10 ppm, 50 ppm, 100 ppm, (n=4, linear regression) concentrations. Sudan dyes detected at wavelengths as seen in Figure 1.

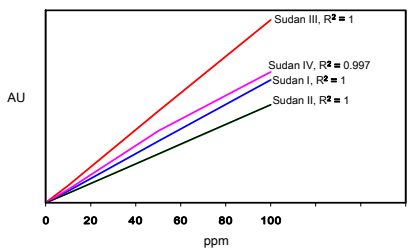


Figure 4. Extracted ion chromatograms for Sudan I (m/z 249), Sudan II (m/z 277), Sudan III (m/z 353) and Sudan IV (m/z 381). 1 ppm

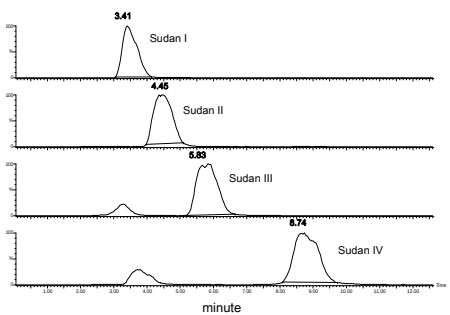
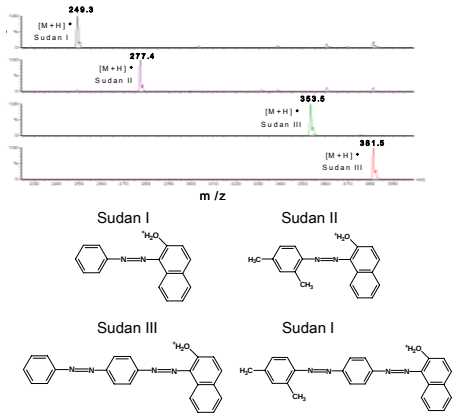


Figure 5. Mass spectra of dominate $[M+H]^+$ for Sudan I, Sudan II, Sudan III and Sudan IV.



Conclusions

Separation of the four Sudan dyes was accomplished in less than five minutes for the UV detection method and in less than 10 minutes for the mass spectrometric method. The chromatographic method is simple yet efficient, requiring only a simple mobile phase and isocratic elution. The Ultra Aqueous C18 phase provides the selectivity needed to assure the separation. This method can be used with UV visible detectors that only have the ability for detect at two fixed wavelengths. Excellent linearity for 1 ppm to 100 ppm allows quantification of Sudan dyes.

The mass spectrometry analysis was optimized for short analysis time and minimization of in ESI source fragmentation.

Future work

Work will continue by evaluating extraction of the dyes from food matrices including methods generated by international food safety organizations.

References

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