Solid Phase Extraction for Detection of Sudan Dye Contaminants in Spices with HPLC/UV Detection

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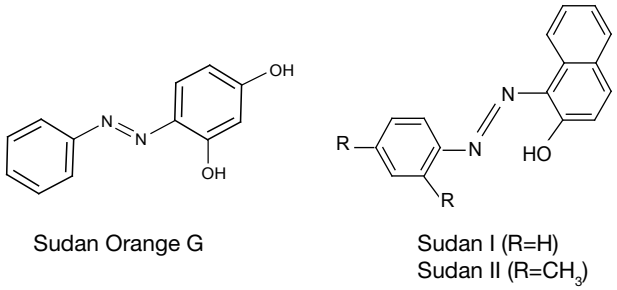
Introduction

Synthetic dyes are used in industries for coloring mineral oils, waxes, solvents, textile and leather garments, but are not permitted to be used in food products owing to health concerns relating to their ingestion. In 2003, an alert was issued in France about Sudan dye contamination of chili products imported from Asia, and by the end of the year hundreds of notifications were provided to the RASFF (Rapid Alert System for Food and Feed)[1] and prompted investigations in USA.[2] HPLC with UV or mass spectrometric detection is the staple method for detection/quantitation of Sudan dyes.[3,4] However, in several instances a sample clean up step is necessary to eliminate contaminants.[5] In this communication we present a solid phase extraction (SPE) protocol for isolating Sudan dyes from chili powder (see Figure 1 for structures of dyes used).



Sudan Orange G

Sudan I (R=H)
Sudan II (R=CH_3)

Figure 1: Structures of the Sudan Dyes studied

Experimental Conditions

Sudan I, Sudan II and Sudan Orange were from Aldrich. Chili powder was from a local grocery store. All solvents used were ACS reagent grade. HPLC was done on a HP 1100 system (Agilent Technologies, Palo Alto, CA) using either a Gemini® C18 or Synergi™ Polar-RP® column (150 x 4.6 mm, 5 µm, silica-based) from Phenomenex, Inc., Torrance, CA.

Solid Phase Extraction: The chili powder (500 mg), spiked with Sudan dye (1 mg), was extracted with 2 mL of isopropanol and filtered with a syringe. The extract (10 mL) was added to 990 mL of 30 % isopropanol in water and the resulting solution loaded on to SPE cartridge (strata™-X or -X-CW, 30 mg/1 mL, from Phenomenex). After washing with water (1 mL) and methanol/water (50:50, 1 mL), elution was done with dichloromethane/isopropanol/formic acid (78:20:2 by volume, 1 mL). Part of the extract (300 mL) was diluted with methanol (700 mL) and 10 mL of this solution was injected into the HPLC (conditions shown in Figure 2).

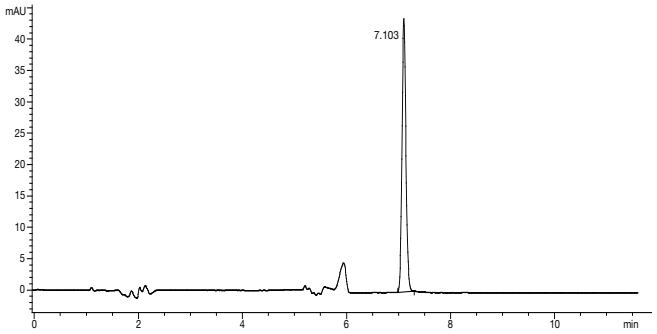


Figure 2: HPLC-UV chromatogram of Sudan Orange G (extracted with strata-X-CW from chili powder); Conditions: water (A)/acetonitrile (B) at 40:60 for 3 min, then to 15:85 in 12 min; flow rate = 1 mL/min, detector wavelength = 480 nm; Synergi™ Polar-RP® column (150 x 4.6 mm)

Table 1:
Recovery data of Sudan Dyes from Chili Powder on strata-X and strata-X-CW

Sudan Dye	Log P	pK_a	% Recovery strata-X (RSD)	% Recovery strata-X-CW (RSD)
Sudan I	5.07	11.65	91 (6.7)	78 (5.5)
Sudan II	6.00	11.65	92 (7.2)	75 (5.1)
Sudan Orange G	3.78	8,19, 11.67	71 (4.9)	93 (4.8)

Results and Discussion

Sudan dyes are hydrophobic molecules (see Table 1 for log P values) with strong absorbance in the UV-visible region, which facilitates their quantitation by UV even at low concentrations. However, Sudan Orange G is considerably more polar and is not well retained on silica-based C18 SPE sorbents during strong organic wash. We investigated the retention capacity of a neutral polar functionalized polymer (strata™-X) and a weak cation exchange polymer (strata™-X-CW) for these dyes. strata-X interacts predominantly by π-π interactions, while strata-X-CW shows strong hydrogen bonding interactions. The phenolic moieties on Sudan I and II are intra-molecularly hydrogen bonded to the azo nitrogen and hence cannot undergo H-bonding with strata-X-CW. On the other hand, Sudan Orange G has an isolated phenolic moiety, which is more acidic and can show strong hydrogen bonding. Thus, Sudan I and II are more retained on strata-X than on strata-X-CW owing to stronger hydrophobicity of the former, but Sudan Orange G is more retained on the latter due to stronger hydrogen bonding. These features are reflected in the capability of each sorbent to withstand 50 % methanol wash and ultimately in the elution recoveries (see Table 1).

Conclusion

Recovery of Sudan dyes from chili powder extracts is facile with both polymeric sorbents strata-X and strata-X-CW. However, a subtle difference in retention due to H-bonding is noticed depending on the number of phenolic groups on the dyes.

References

1. RASFF Annual Report 2005, Office for Publication of the European Communities, Luxemberg, 2006, pp. 15-17.
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3. P. Botek, J. Poustka, J. Hajslova, Czech *J. Food Sci.* 2005, **25**, 17-24.
4. Y.P. Zhang, Y.J. Zhang, W.J. Gong, A.I. Gopalan, K. Lee, *J. Chromatogr. A*, 2005, **1098**, 183-187.
5. C. Baggiani, L. Anfossi, C. Giovannoli, *Anal. Chim. Acta,* 2007, **591**, 29-39.

High-Throughput Screening Ordering Information

High-Throughput 96-Well Plates (2/box)

Phase	10 mg	30 mg	60 mg
strata-X	8E-S100-AGB	8E-S100-TGB	8E-S100-UGB
strata-X-C	8E-S029-AGB	8E-S029-TGB	8E-S029-UGB
strata-X-CW	8E-S035-AGB	8E-S035-TGB	8E-S035-UGB
strata-X-AW	8E-S038-AGB	8E-S038-TGB	8E-S038-UGB

Traditional Screening Ordering Information*

1 mL Tubes (100/box)

Phase	30 mg
strata-X	8B-S100-TAK
strata-X-C	8B-S029-TAK
strata-X-CW	8B-S035-TAK
strata-X-AW	8B-S038-TAK

3 mL Tubes (50/box)

Phase	60 mg	200 mg	500 mg
strata-X	8B-S100-UBJ	8B-S100-FBJ	8B-S100-HBJ
strata-X-C	8B-S029-UBJ	8B-S029-FBJ	8B-S029-HBJ
strata-X-CW	8B-S035-UBJ	8B-S035-FBJ	8B-S035-HBJ
strata-X-AW	8B-S038-UBJ	8B-S038-FBJ	8B-S038-HBJ

6 mL Tubes (30/box)

Phase	100 mg	200 mg	500 mg
strata-X	8B-S100-ECH	8B-S100-FCH	8B-S100-HCH
strata-X-C	8B-S029-ECH	8B-S029-FCH	8B-S029-HCH
strata-X-CW	8B-S035-ECH	8B-S035-FCH	8B-S035-HCH
strata-X-AW	8B-S038-ECH	8B-S038-FCH	8B-S038-HCH

*Contact Phenomenex about tabless or Teflon® coated tubes. Contact Phenomenex for additional formats available.

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