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INTRODUCTION

Recently there have been numerous reports regarding the potentially dangerous use of Sudan dyes for colorizing food products (structures shown in Figure 1). For example, Sudan I is an intense orange dye useful as a coloring agent for plastics and other industrial products but not intended for use in foods. However, Sudan I has been found in red chili products and in tomato-based products sold for human consumption. Sudan dyes are aromatic azo compounds and considered to be genotoxic carcinogens. This has necessitated the development of improved methods for their identification in foods.

Two complementary SPE procedures have been developed for rapid isolation and clean-up of Sudan dyes in chili and chili products. The first, more appropriate for non-oily matrices such as fresh chilies, is a mixed-mode anion-exchange procedure using Waters® Oasis® MAX sorbent. The second, more appropriate for oily matrices and dried chilies, is a normal-phase clean-up using Waters Sep-Pak® Alumina B. LC/MS analysis is accomplished with positive electrospray ionization. In most cases, the enrichment and cleanup obtained from SPE allows for mass spec screening (SIR) and confirmatory analysis with LOQ below 10 µg/kg using tandem LC/MS/MS (MRM). The types of chili products studied are shown in Figure 2. Some potential interferences removed using these SPE methods are shown in Figure 3.



Figure 2. Typical chili products studied.

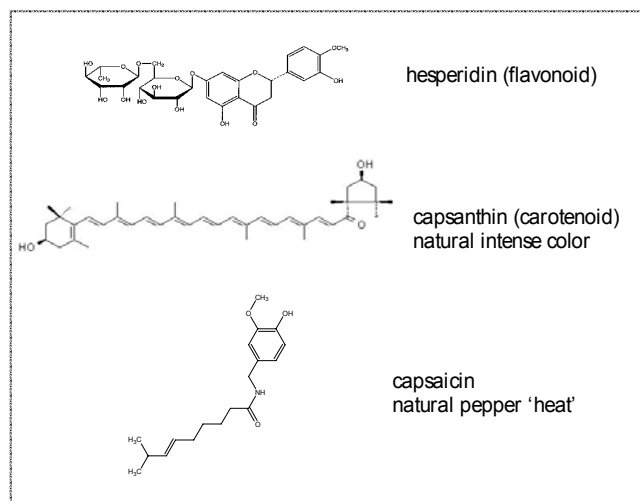


Figure 3. Natural pigments/potential interferences found in chilies.

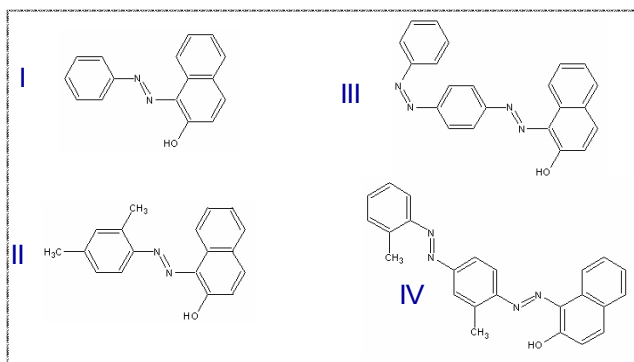


Figure 1. Sudan dye structures.

METHODS AND RESULTS

Oasis MAX (3cc, 60 mg)**Pre-extraction**

Chili products (1 g) are homogenized and extracted with 10 mL acetone. A 1 mL aliquot is diluted to 5 mL with aqueous NaOH (to pH 11).

Condition: 2 mL ethyl acetate 2 mL methanol, 1 mL 0.1 M NaOH, 2 mL water

Load: 5 mL of diluted acetone pre-extract

Wash: 2 mL 70% methanol in water, 1 mL 1 M NaOH in water, 2 mL methanol, 1 mL ethyl acetate

Elute: 2 mL 89:9:2 ethyl acetate/MeOH/formic acid - evaporate and reconstitute in 90:10 acetonitrile/water (200 µL)

Note: Polar phenolics such as capsaicin are not retained by ion-exchange at pH 11 and are removed with wash 1. Wash 2 (1 M NaOH) ionizes the retained Sudans prior to wash 3 and 4 (to remove non-polar neutrals and bases).

Results from spiked chili sauce (n = 6, 80 µg/kg)

Sudan I	83% recovery (9% RSD)
Sudan II	83% recovery (1% RSD)
Sudan III	77% recovery (3% RSD)
Sudan IV	75% recovery (4% RSD)

A representative LC/MS chromatogram is presented in Figure 4.

Sep-Pak Alumina B (3 cc, 500 mg)**Pre-extraction**

Chili oils (0.1 g) are diluted to 1 mL with hexane. Chili products (1 g) are homogenized and extracted with 10 mL acetone. A 1 mL aliquot is evaporated to complete dryness and the residue is taken up in 1 mL hexane.

Condition: 2 mL methanol, 2 mL ethyl acetate, 3 mL hexane

Load: 1 mL of pre-extract in hexane

Wash: 3 mL hexane, 1 mL ethyl acetate

Elute: 4 mL 90:10 ethyl acetate/methanol - evaporate and reconstitute in methanol (200 µL)

Note: Fats are completely removed with the hexane and ethyl acetate washes. Carotenoids are removed with the ethyl acetate wash. The Sudan dyes are eluted with 10% methanol in ethyl acetate while more polar interferences, such as capsaicin, are retained.

Results from spiked chili oil (n = 6, 80 µg/kg)

Sudan I	99% recovery (11% RSD)
Sudan II	91% recovery (11% RSD)
Sudan III	93% recovery (6% RSD)
Sudan IV	122% recovery (11% RSD)

A representative LC/MS chromatogram is presented in Figure 5.

LC/MS Determination of Sudan Dyes in Chili Products**LC Conditions**

Column:	Atlantis dC18 2.1 x100 mm, 3 µm
Gradient A:	0.1% formic acid in water 80% B to 95% B in 10 min
Gradient B:	acetonitrile
Flow Rate:	0.4 mL/min
Injection Volume:	15 µL
Temperature:	30 °C
Instrument:	Waters® Alliance® 2695
Software:	All data collection and processing carried out using Waters MassLynx™ Software.

The calibration curve prepared in spiked chili sauce was linear in the range from 8 to 100 µg/kg (R² = 0.995).

MS Conditions

Instrument:	Waters® Micromass® Quattro micro™ API
Ion Mode:	Electrospray positive ionization

Analyte	SRM (m/z)	Cone Voltage (V)	Collision Energy (eV)
Sudan I	249 → 156	20	15
	249 → 93	20	24
	249 → 128	20	25
Sudan II	277 → 156	28	15
	277 → 121	28	18
	277 → 106	28	40
Sudan III	353 → 77	45	28
	353 → 120	45	23
	353 → 196	45	23
Sudan IV	381 → 91	45	26
	381 → 106	45	40
	381 → 224	45	20

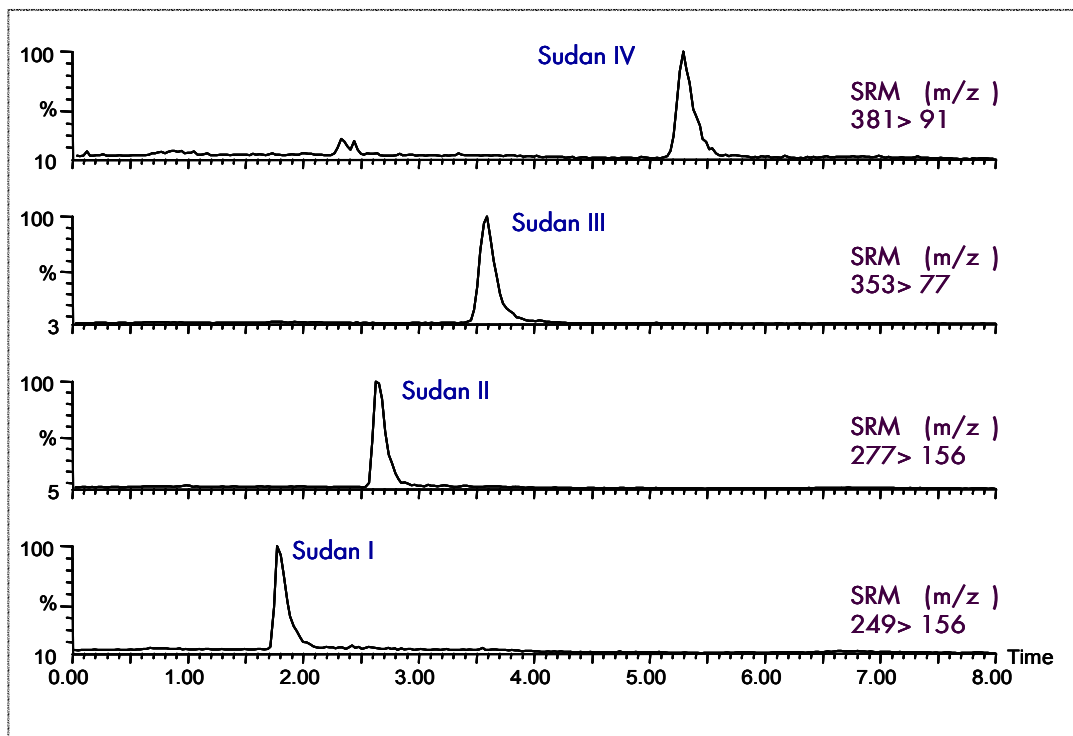


Figure 4. LC/MS Spiked Chili Sauce (80 µg/kg) Oasis MAX Method.

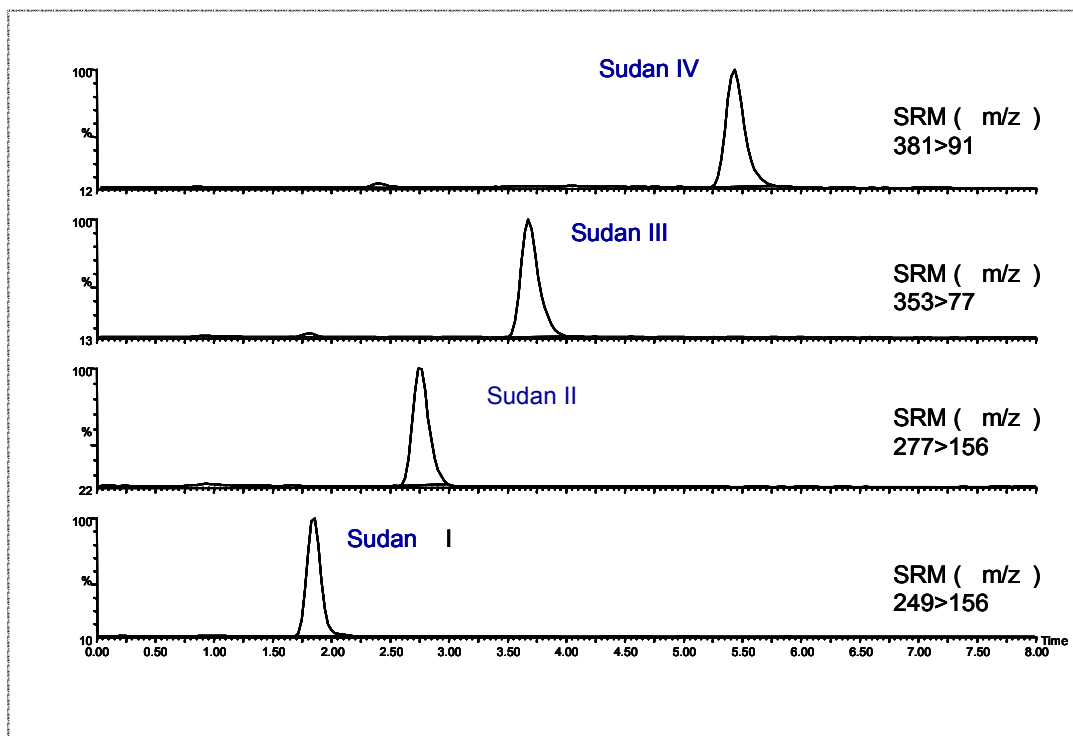


Figure 5. LC/MS/MS Spiked Chili Oil (80 µg/kg) Sep-Pak® Alumina B Method.

Considerations for SPE

- Sudan dyes are well retained on all Oasis sorbents by reversed-phase.
- Oasis MAX allows for ion-exchange retention at pH 13.
- Sudan dyes are also well suited for normal-phase retention (i.e. on Alumina B).

Analysis of chili products obtained from local stores

Seven powdered products and two oils were analyzed using Sep-Pak Alumina SPE for sample preparation. Two water-based chili sauces were analyzed using Oasis MAX SPE for sample preparation. Results are summarized in Table 1 and representative chromatograms are shown in Figures 6 and 7.

Chili Product	Sudan I	Sudan II	Sudan III	Sudan IV	SPE Method
Chili Powder 1	88000 µg/kg	nd	nd	nd	Alumina B
Chili Powder 2	450 µg/kg	nd	nd	nd	Alumina B
Chili Powder 3	300 µg/kg	nd	nd	nd	Alumina B
Chili Powder 4	nd	nd	nd	nd	Alumina B
Chili Powder 5	nd	nd	nd	nd	Alumina B
Chili Powder 6	nd	nd	nd	nd	Alumina B
Chili Powder 7	nd	nd	nd	nd	Alumina B
Chili Sauce 1	nd	88 µg/kg	nd	nd	Oasis MAX
Chili Sauce 2	nd	98 µg/kg	nd	nd	Oasis MAX
Chili Oil (soybean)	nd	nd	nd	nd	Alumina B
Hot Sesame Oil	nd	nd	nd	nd	Alumina B

Table 1. Analysis of Commercial Chili Products.

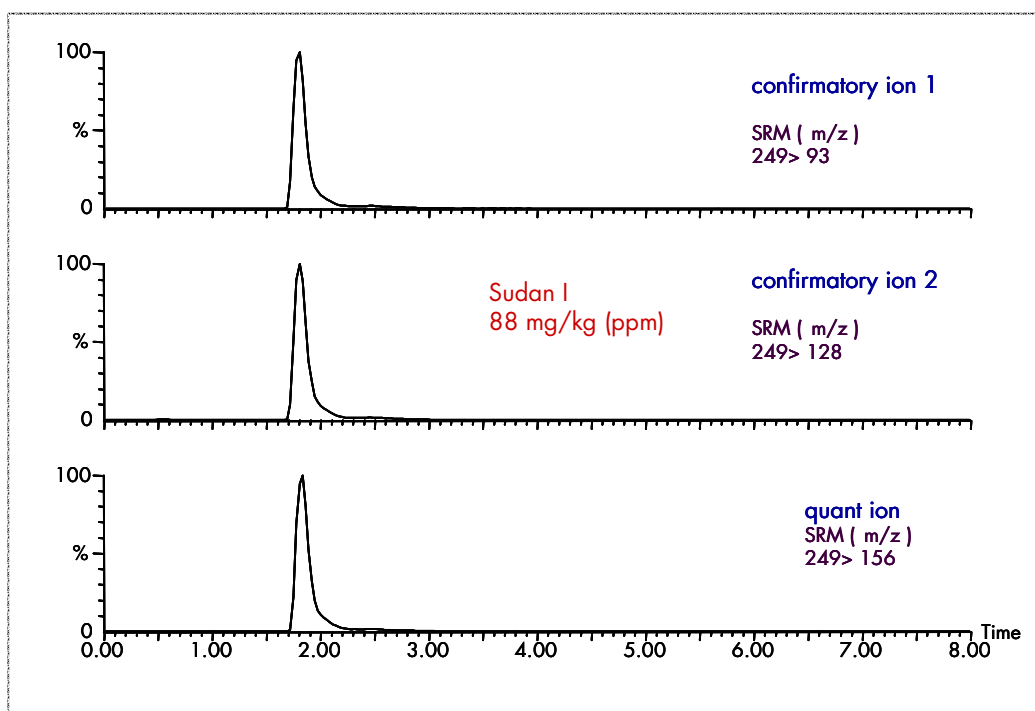


Figure 6. Commercial Chili Powder 1 Sep-Pak Alumina B Method.

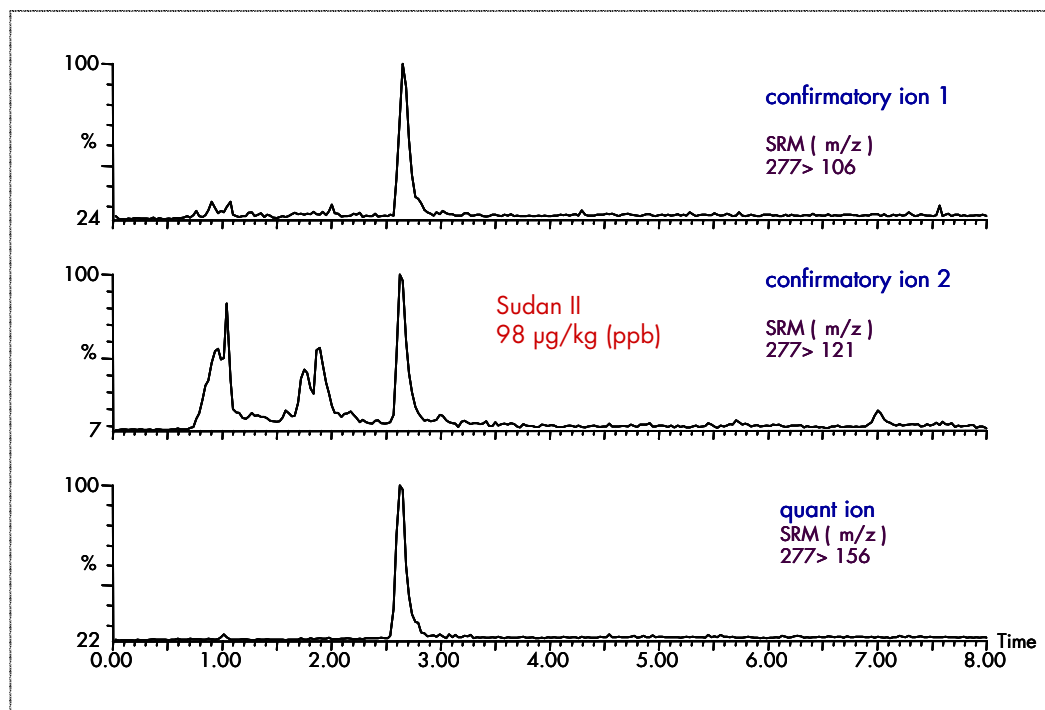


Figure 7. Commercial Chili Sauce 2 Oasis MAX Method.

CONCLUSION

The Sep-Pak Alumina B SPE Procedure provides enrichment and clean-up for oils and oily matrices such as chili sesame oil and dried chili products. The Oasis MAX SPE procedure provides enrichment and clean-up for water-based samples such as chili sauces.

In this study, five out of the 11 commercial samples analyzed were found to be contaminated with Sudan dyes. Three dried chili products contained greater than 250 µg/Kg (ppb) of Sudan I; one sample measured 88 mg/kg (ppm) of Sudan I. Two chili sauces contained over 80 µg/kg of Sudan II.

The high incidence and concentration of Sudan dyes in the test samples provides evidence of the need for further investigation of Sudan dyes in U.S. food products.

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