

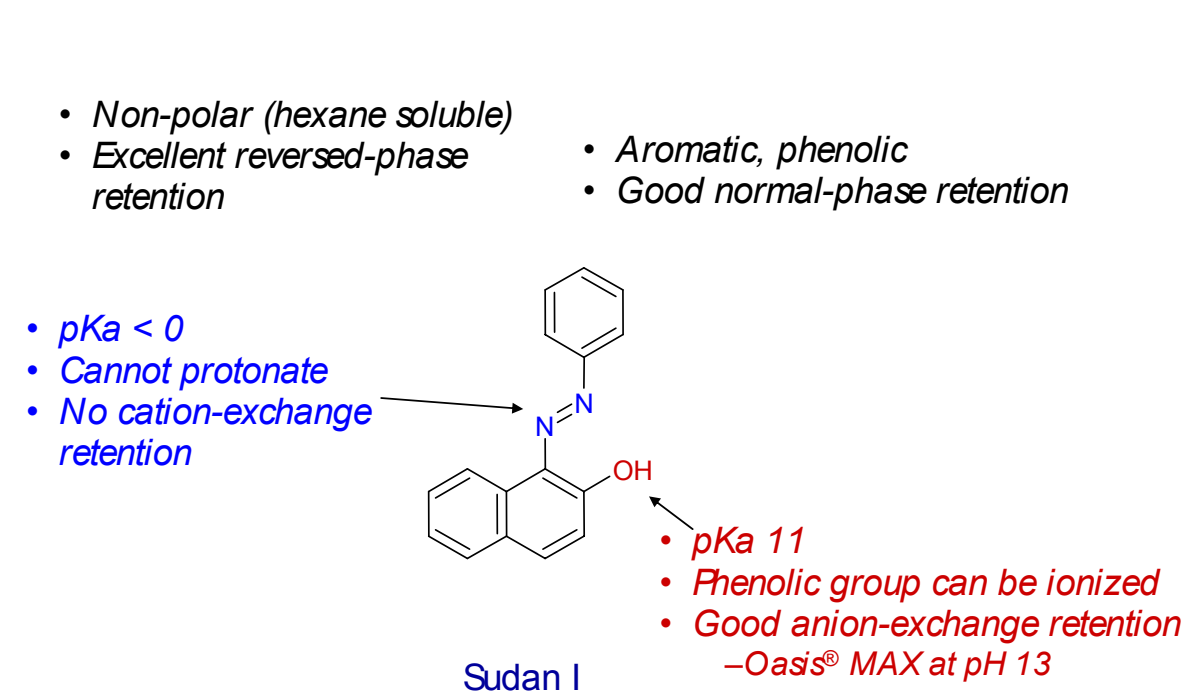
INTRODUCTION

Recently there have been numerous reports regarding the potentially dangerous use of Sudan dyes for colorizing food products (see structures below). 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 are presumed to be carcinogenic. Therefore, there is an urgent need for improved methods for determination of these compounds in foods.

Two complementary SPE procedures have been developed for rapid isolation and cleanup 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 Oasis® MAX sorbent. The second, more appropriate for oily matrices and dried chilies, is a normal-phase cleanup using 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 single quad screening (SIR) and confirmatory analysis with LOQ below 10 µg/kg using tandem LC-MS (MRM).

SUDAN DYES

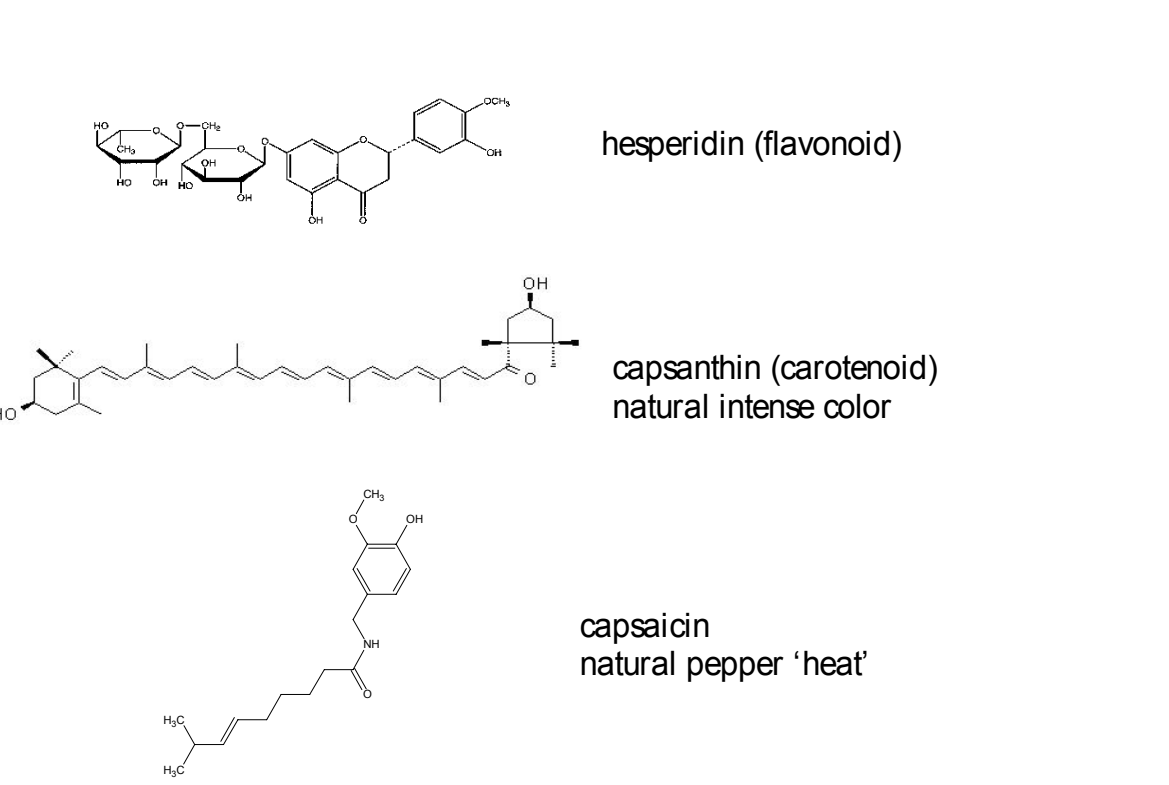
Considerations for SPE



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

CHILIES AND CHILI PRODUCTS

Natural pigments/potential interferences



SPE METHODS

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

Comments: *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).*

Typical 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)

ion suppression or enhancement < 10%

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

Comments: *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.*

Typical 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)

ion suppression or enhancement < 20%

LC-MS DETERMINATION OF SUDAN DYES IN CHILI PRODUCTS

LC-MS-MS Conditions

Waters Quattro micro™

Column: Atlantis™ dC₁₈ 2.1 x 100 mm , 3µm
Gradient: A: acetonitrile B: 0.1% formic acid in water
80% A to 95% A in 10 min

Flow Rate: 0.4 mL/min

Injection Volume: 10 µL

Temperature: 30 °C

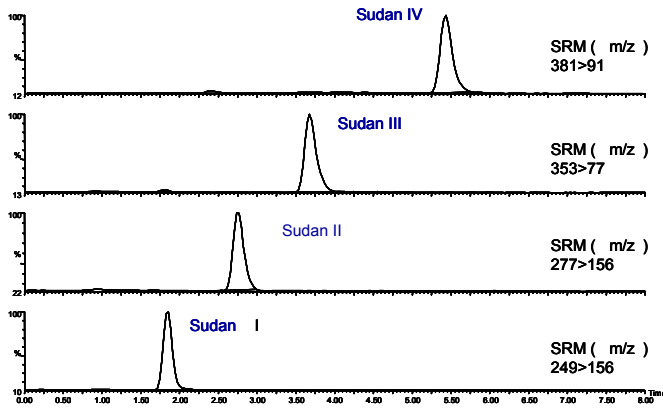
Instrument: Alliance® 2695

LC-ESI–MS/MS parameters

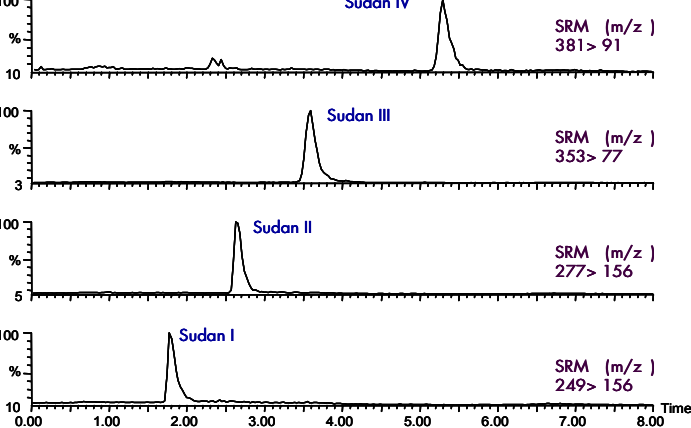
Analyte	SRM (m/z)	Cone voltage (V)	Collision energy (eV)
Sudan I	249 → 156	25	15
Sudan II	277 → 156	28	15
Sudan III	35 → 77	45	28
Sudan IV	381 → 91	45	26

LC-MS-MS Spiked Chili Products (80 µg/kg)

Spiked Chili Oil (80 µg/kg) Sep-Pak® Alumina B Method



Spiked Chili Sauce (80 µg/kg) Oasis® MAX



CONCLUSIONS

- The Sep-Pak® Alumina B SPE Procedure provides enrichment and cleanup for oils and oily matrices such as chili sesame oil and for dried chili products
- The Oasis® MAX SPE procedure provides enrichment and cleanup for water based samples such as chili sauces
- For this study, eleven commercial samples were analyzed and five were found to be contaminated with Sudan dyes
 - one dried chili product contained 88 mg/kg (ppm) of Sudan I
 - two chili sauces contained over 80 µg/kg (ppb) of Sudan II

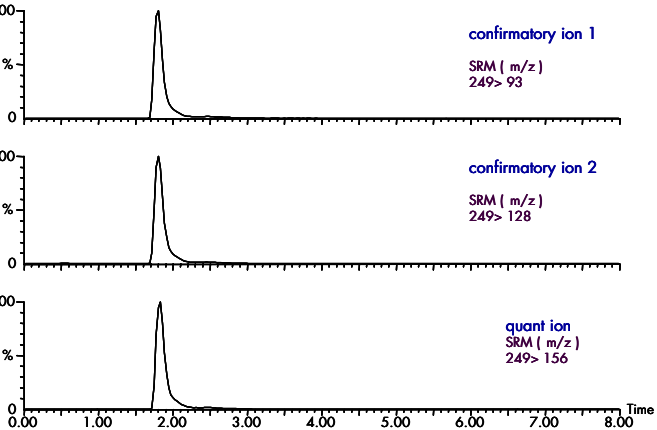
Analysis of Contaminated Chili Products

Results From Products Obtained From Local Stores

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

LC-MS-MS Contaminated Chili Products

Commercial Chili Powder 1 Sep-Pak® Alumina B Method



Commercial Chili Sauce 2 Oasis® MAX Method

