INTRODUCTION

Recently there have been numerous reports regarding the potentially dangerous use of Sudan dyes for coloring 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 azoic color 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 quadrupole screening (SRM) and confirmatory analysis with LOQ below 10 µg/kg using tandem LC-MS (MRM).

CHILIES AND CHILI PRODUCTS

Natural pigments/potential interferences

**CHILIS**

- Capsaicin (capsaicinoids) - natural internal color
- carotenoids (fluorescent) - natural red/purple "flame"

**Typical Chili Products Studied**

Sudan Dye Structures

- Sudan I
- Sudan II
- Sudan III
- Sudan IV

**SPE METHODS**

**Oasis® MAX (2 cc, 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 (pH 11).

**Condition:** 2 or 4 ml acetone 2 ml methanol, 1 ml 0.1 M NaOH, 2 ml water

**Load:** 5 ml of diluted acetone pre-extraction

**Wash:** 2 to 70 % methanol in water

**1 ml 0.1 M NaOH in water**

**2 ml methanol**

**1 ml acetate**

**Elute:**

2 or 89:12 2 ml acetate/Meth/Formic acid

-vaporize and reconstitute in 90:10 acetone/h2o

**Comments:** Polar pigments such as capsaicin is not retained by ion-exchange at pH 11 and are removed with wash 2. Wash 2 (0.1 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 (95% RSD)
- Sudan II: 83% recovery (95% RSD)
- Sudan III: 77% recovery (35% RSD)
- Sudan IV: 75% recovery (10% recovery or enhancement < 10%)

**Cap-Sep® Alumina B (3 cc, 500 mg)**

**Pre-extraction**

Chili solids (1 g) are diluted to 1 ml with hexane.

**Condition:** 2 ml methanol, 2 ml 0.1 M acetate, 3 ml hexane

**Load:** 1 ml of pre-extraction in hexane

**Wash:** 3 ml hexane

**1 ml acetate**

**Elute:**

4 ml 90:10 acetate/methanol

-vaporize and reconstitute in methanol

**Comments:** Fats are completely removed with the heaxane and acetate wash. These materials are removed with the acetate wash while the acetate wash is eluted with 10% methanol in 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 (1% RSD)
- Sudan II: 91% recovery (1% RSD)
- Sudan III: 93% recovery (8% RSD)
- Sudan IV: 122% recovery (1% RSD)

-ion suppression or enhancement < 20%

CONCLUSIONS

- The Sep-Pak® Alumina B SPE Procedure provides enrichment and cleanup for oils and oily matrices such as chili sauce 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 ng/kg (ppb) of Sudan I
  - two chili sauces contained over 80 µg/kg (ppb) of Sudan II

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