Method of Test for Sudan Dyes in Foods (2)

1. Scope

This method is applicable to the determination of 4 Sudan dyes (Sudan I, Sudan II, Sudan III and Sudan IV) in chili products and edible rose petals.

2. Method

After extraction and purification, analytes are determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph-tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: CORTEC C18, 1.6 μm, 2.1 mm × 15 cm or an equivalent product.

2.1.2. Centrifuge: centrifugal force > 5000 ×g.

2.1.3. Shaker.

2.1.4. Vortex mixer.

2.1.5. Nitrogen evaporator.

2.1.6. Solid phase extraction vacuum manifolds.

2.2. Chemicals

Acetonitrile, HPLC grade;
Formic acid, reagent grade;
Sodium methoxide solution, 25% in methanol, reagent grade;
Tetrahydrofuran, reagent grade;
n-Hexane, reagent grade;
Diethyl ether, reagent grade;
Sodium hydrogen citrate, AR grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
Sudan I, Sudan II, Sudan III and Sudan IV, reference standards;
Sudan I-d_5, Sudan II-d_6, Sudan III-d_6 and Sudan I-d_6, isotope-labeled internal standards.

2.3. Apparatus

2.3.1. Volumetric flask: 100 mL.

2.3.2. Centrifuge tube: 15 mL and 50 mL, PP.

2.3.3. Solid phase extraction cartridge: Sep-Pak® silica, 1 g, 6 mL, or an equivalent product.

2.3.4. Membrane filter: 0.22 μm, PVDF.

2.4. Reagents

2.4.1. Tetrahydrofuran: methanol (4:1, v/v)

Mix tetrahydrofuran and methanol at the ratio of 4:1.

2.4.2. 5% Sodium methoxide in methanol

Dilute 100 mL of 25% sodium methoxide in methanol with methanol to 500 mL.

2.4.3. 15% sodium hydrogen citrate

Dissolve and dilute 75 g of sodium hydrogen citrate with deionized water to 500 mL.

2.4.4. *n*-Hexane: diethyl ether (9:1, v/v)

Mix *n*-hexane and diethyl ether at the ratio of 4:1.

2.5. Mobile phase

2.5.1. Solvent A

Mix 1 mL of formic acid with 1000 mL of deionized water, and filter with a membrane filter.

2.5.2. Solvent B

Mix 1 mL of formic acid with 1000 mL of acetonitrile, and filter with a membrane filter.

2.6. Internal standard solution preparation

Transfer about 10 mg of Sudan I-d_5, Sudan II-d_6, Sudan III-d_6 and Sudan I-d_6 isotope-labeled internal standards accurately weighed to each 100-mL volumetric flask, dissolve and dilute to volume with acetonitrile as the internal standard stock solutions. Store at 4°C. When to use, mix appropriate volume of each internal standard stock solution, and dilute with acetonitrile to 0.1 μg/mL as the internal standard solution.

2.7. Standard solution preparation

Transfer about 10 mg of Sudan I, Sudan II, Sudan III and Sudan IV reference standards accurately weighed to each 100-mL volumetric flask, dissolve and dilute to volume with acetonitrile as the standard stock solutions. Store at 4°C. When to use, mix appropriate volume of each standard stock solution, and dilute with acetonitrile to 0.1 μg/mL for Sudan I, Sudan II and Sudan III, and 0.25 μg/mL for Sudan IV as the standard solution.

2.8. Sample solution preparation

2.8.1. Extraction

Transfer about 1 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube, add 0.1 mL of the internal standard solution and 5 mL of tetrahydrofuran: methanol (4:1, v/v), and mix well. Add 5 mL of 5% sodium methoxide in methanol, and shake for 5 min. Add 5 mL of *n*-hexane, shake for 5 min, then add 5 mL of 15% sodium hydrogen citrate, and mix well. Centrifuge at 5000 ×g for 5 min, take the supernatant into a 15-mL centrifuge tube, and evaporate to dryness by gently flushing with a stream of nitrogen at 40°C. Dissolve the residue with 2 mL of *n*-hexane for purification.

2.8.2. Purification

Transfer the solution for purification from section 2.8.1 into a solid phase extraction cartridge prerinsed with 10 mL of *n*-hexane, and discard the eluent. Wash the cartridge with 10 mL of *n*-hexane, and discard the eluent. Add 10 mL of *n*-hexane: diethyl ether (9:1, v/v) to the cartridge, and collect the eluent. Evaporate to near dryness by gently flushing with a stream of nitrogen at 40°C. Dissolve the residue with 0.2 mL of tetrahydrofuran, then add 0.8 mL of acetonitrile, and mix well. Filter with a membrane filter, and take the filtrate as the sample solution.

2.9. Matrix-matched calibration curve

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.8 to obtain the eluent from the purification procedure. Evaporate to near dryness by gently flushing with a stream of nitrogen at 40°C, dissolve the residue with 0.2 mL of tetrahydrofuran, separately add 0.04~0.5 mL of the standard solution and 0.1 mL of the internal standard solution, and dilute with acetonitrile to achieve a final volume of 1 mL. Mix well as the matrix-matched standard solutions. Operate LC-MS/MS according to the following conditions. Establish the matrix-matched calibration curve of each Sudan dye by the ratios of the peak area of each Sudan dye to that of the internal standard vs. the added concentrations (4~50 ng/mL for Sudan I, Sudan II and Sudan III; 10~125 ng/mL for Sudan IV).

LC-MS/MS operating conditions(note)

Column: CORTECS C18, 1.6 μm, 2.1 mm × 15 cm.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0 → 1.0	60 → 60	40 → 40
1.0 → 5.0	60 → 15	40 → 85
5.0 → 10.0	15 → 15	85 → 85
10.0 → 11.0	15 → 5	85 → 95
11.0 → 23.0	5 → 5	95 → 95
23.0 → 23.1	5 → 60	95 → 40
23.1 → 30.0	60 → 60	40 → 40

Flow rate: 0.25 mL/min.

Injection volume: 5 μL.

Ion spray voltage: 2.2 kV.

Ionization mode: ESI^+.

Ion source temperature: 120°C.

Desolvation temperature: 400°C.

Cone gas flow rate: 50 L/hr.

Desolvation flow rate: 850 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are shown as follows:

Analyte	Ion pair: Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Sudan I	249 > 156*	30	14
	249 > 128	30	28
Sudan II	277 > 260*	30	20
	277 > 156	30	24
Sudan III	353 > 197*	50	19
	353 > 156	50	25
Sudan IV	381 > 224*	50	22
	381 > 91	50	24

Sudan I-d_5 (I.S.)	254 > 156*	30	15
Sudan II-d_6 (I.S.)	283 > 121*	30	11
Sudan III-d_6 (I.S.)	359 > 162*	50	20
Sudan IV-d_6 (I.S.)	387 > 225*	50	20

*The quantitative ion.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.10. Identification and quantification

Accurately inject 5 μL of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.9. Identify each Sudan dye based on the retention time and the relative ion intensities(note). Calculate the amount of each Sudan dye in the sample by the following formula.

$$\text{The amount of each Sudan dye in the sample (ppb)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each Sudan dye in the sample solution calculated by the matrix-matched calibration curve (ng/mL)

V: the final make-up volume of sample (mL)

M: the weight of the sample (g)

Note: Relative ion intensities are calculated by peak areas of quantitative ions divided by peak areas of qualitative ions (≤100%). Maximum permitted tolerances of relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20 ~ 50	± 25
> 10 ~ 20	± 30
≦ 10	± 50

Remark

1. Limits of quantification (LOQs) are 4 ppb for Sudan I, Sudan II and Sudan III, and 10 ppb for Sudan IV.
2. Further validation should be performed when interference compounds

appear in samples.

Reference

Uematsu, Y., Ogimoto, M., Kabashima, J., Suzuki, K. and Ito, K. 2007. Fast cleanup method for the analysis of sudan I–IV and para red in various foods and paprika color (oleoresin) by high-performance liquid chromatography/diode array detection: focus on removal of fat and oil as fatty acid methyl esters prepared by transesterification of acylglycerols. J. AOAC Int. 90: 437-445.

Reference chromatograms

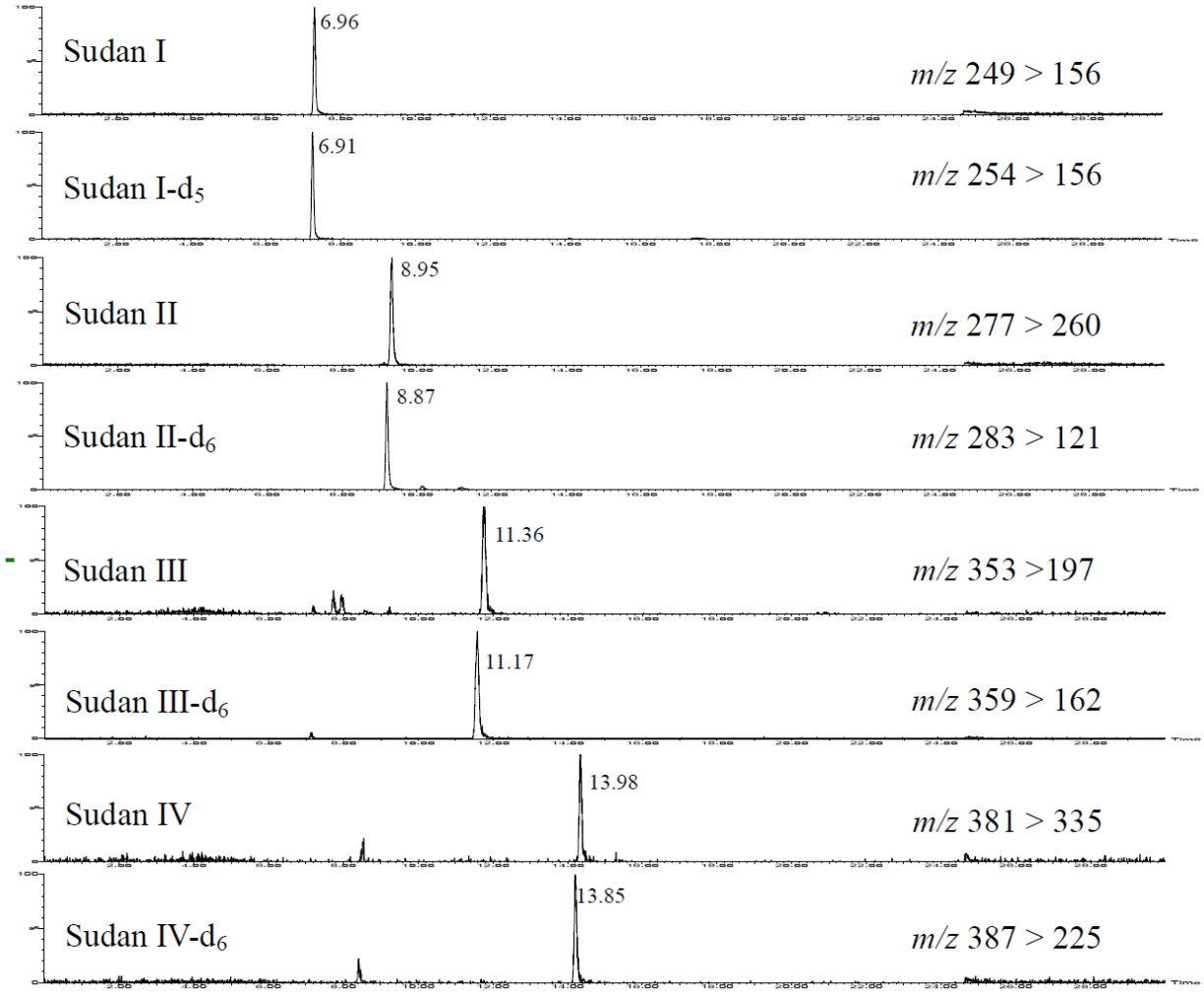

Figure. MRM chromatograms of Sudan dyes and internal standards analyzed by LC-MS/MS.