Method of Identification for Sudan Dyes in Foods

1. Scope

This method is applicable to the identification of 4 Sudan dyes (Sudan I, Sudan II, Sudan III and Sudan IV) in foods such as dried plant products and condiments.

2. Method

After sample pretreatment using the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, Safe), Sudan dyes are identified 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 i.d. × 15 cm, or an equivalent product.

2.1.2. High speed dispersing device: SPEX SamplePrep 2010 Geno/Grinder®, ≥ 1000 rpm, or an equivalent product.

2.1.3. Centrifuge: centrifugal force ≥ 5000 ×g, temperature control ≤ 10°C.

2.1.4. Nitrogen evaporator.

2.1.5. Vortex mixer.

2.2. Chemicals

Acetonitrile, HPLC grade;
Acetone, HPLC grade;
Formic acid, reagent grade;
Tetrahydrofuran, reagent grade;
Primary secondary amine (PSA), AR grade;
Octadecylsilane, end-capped (C18 EC), AR grade;
EMR-Lipid extraction powder, AR grade;
Sodium citrate anhydrous, AR grade;
Disodium hydrogen citrate sesquihydrate, AR grade;
Magnesium sulfate anhydrous, AR grade;
Sodium chloride, AR grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
Sudan I, Sudan II, Sudan III and Sudan IV, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL.

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

2.3.3. Ceramic homogenizer(note 1): Bond Elut QuEChERS P/N 5982-9312, or an equivalent product.

2.3.4. Extraction powder(note 2): Containing 1 g of sodium citrate anhydrous, 0.5 g of disodium hydrogen citrate sesquihydrate, 4 g of magnesium sulfate anhydrous and 1 g of sodium chloride.

2.3.5. Clean-up centrifuge tube I(note 2): Containing 150 mg of PSA, 150 mg of C18 EC and 900 mg of magnesium sulfate anhydrous.

2.3.6. Clean-up centrifuge tube II(note 2): Containing 1 g of EMR-Lipid extraction powder, or an equivalent product.

2.3.7. Clean-up centrifuge tube III(note 2): Containing 1.6 g of magnesium sulfate anhydrous and 0.4 g of sodium chloride.

2.3.8. Membrane filter: 0.22 μm, PVDF.

Note 1: The ceramic homogenizer can be used depending on the viscosity of the sample.

Note 2: The commercial extraction/clean-up kit can be used as needed.

2.4. Acetonitrile: tetrahydrofuran (4:1, v/v)

Mix acetonitrile and tetrahydrofuran at the ratio of 4:1 (v/v).

2.5. Mobile phase

2.5.1. Solvent A

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

2.5.2. Solvent B

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

2.6. Standard solution preparation

Transfer about 1 mg of Sudan I, Sudan II, Sudan III and Sudan IV reference standards accurately weighed to each 10-mL volumetric flask, dissolve and dilute to volume with acetonitrile as the standard stock solutions. Store under refrigeration. When to use, mix appropriate volume of each standard stock solution, and dilute with acetonitrile to 100 ng/mL for Sudan I, Sudan II and Sudan III, and 250 μg/mL for Sudan IV as the standard solution.

2.7. Sample solution preparation

2.7.1. Dried plant products

2.7.1.1. Extraction

Transfer about 2 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube. Add 10 mL of pre-chilled deionized water, vortex-mix, and add 10 mL of acetonitrile, a ceramic homogenizer and the extraction powder in order. Cap the centrifuge tube, and shake vigorously by hands several times to prevent salt coagulation. Shake at 1000 rpm for 5 min by the high speed dispersing device, centrifuge at 5000 ×g for 5 min at 10°C, and collect the supernatant for purification.

2.7.1.2. Purification

Transfer the supernatant for purification from section 2.7.1.1(note 3) into a clean-up centrifuge tube I, cap the centrifuge tube, and shake vigorously by hand several times to prevent coagulation. Shake at 1000 rpm by the highs speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 5000 ×g for 5 min at 10°C. Transfer 5 mL of the supernatant into a 15-mL centrifuge tube, and evaporate to dryness under a gentle stream of nitrogen at 40°C. Dissolve the residue with 1 mL of acetonitrile: tetrahydrofuran (4:1, v/v), vortex-mix, and filter with a membrane filter. Take the filtrate as the sample solution.

Note 3: If a significant matrix interference was observed, an additional purification step may be performed as follows: Transfer the supernatant for purification from section 2.7.1.1 into a clean-up centrifuge tube II pretreated with 2 mL of deionized water, cap the centrifuge tube, and shake vigorously by hands several times to prevent salt coagulation. Shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 5000 ×g for 5 min at 10°C. Transfer the supernatant into a clean-up centrifuge tube I, and perform the same procedure described in section 2.7.1.2.

2.7.2. Condiments

2.7.2.1. Extraction

Transfer about 2 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube, and add 5 mL of acetone, 5 mL of acetonitrile and a ceramic homogenizer. Cap the centrifuge tube, and shake at 1000 rpm for 10 min by the high speed dispersing device. Centrifuge at 5000 ×g for 5 min at 10°C, and collect the supernatant for purification.

2.7.2.2. Purification

Transfer the supernatant for purification from section 2.7.2.1 into a clean-up centrifuge tube II pretreated with 2 mL of deionized water, cap the centrifuge tube, and shake vigorously by hands several times to prevent salt coagulation. Shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 5000 ×g for 5 min at 10°C. Transfer the supernatant into a clean-up centrifuge tube III, cap the centrifuge tube, and shake vigorously by hands several times to prevent salt coagulation. Shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 5000 ×g for 5 min at 10°C. Transfer 5 mL of the supernatant into a 15-mL centrifuge tube, and evaporate to dryness under a gentle stream of nitrogen at 40°C. Dissolve the residue with 1 mL of acetonitrile: tetrahydrofuran (4:1, v/v), vortex-mix, and filter with a membrane filter. Take the filtrate as the sample solution.

2.8. Standard comparison solution preparation

Take about 2 g of a blank sample, add 80 μL of the standard solution, and follow the procedure in section 2.7 to prepare the standard comparison solution.

2.9. Identification

Accurately inject 5 μL of the sample solution and the standard comparison solution into the LC-MS/MS separately, and operate according to the following conditions. Identify each Sudan dye based on the retention time, peak area and relative ion intensities(note 4).

LC-MS/MS operating conditions(note 5)

Column: CORTECS C18, 1.6 µm, 2.1 mm i.d. × 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 as follows$^{(note\ 6)}$:

Analyte	Ion pair Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Sudan I	249 > 93	15	30
	249 > 156	15	14
	249 > 128	15	28
	249 > 232	15	8
Sudan II	277 > 156	20	20
	277 > 128	20	24
	277 > 106	20	26
	277 > 260	20	20

Sudan III	353 > 197	30	17
	353 > 156	30	21
	353 > 196	30	17
	353 > 128	30	30
Sudan IV	381 > 224	30	22
	381 > 276	30	20
	381 > 106	30	22
	381 > 244	30	22

Note 4: Relative ion intensities are calculated by the ratio of peak areas of two ion pairs (≤ 100%). Maximum permitted tolerances are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

Note 5: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Note 6: Detection ion pairs may be selected at least two pairs depending on the matrix.

Remark

1. Limits of detection (LODs) are 4 ppb for Sudan I, Sudan II and Sudan III, and 10 ppb for Sudan IV.
2. Further validation should be performed when interfering compounds appear in samples.

Reference

1. Piątkowska, M., Jedziniak, P. and Żmudzki, J. 2014. Determination of illegal dyes in eggs by liquid chromatography-tandem mass spectrometry. Bull. Vet. Inst. Pulawy 58: 247-253.
2. Adjei, J. K., Ahormegah, V., Boateng, A. K., Megbenu, H. K. and Owusu, S. 2020. Fast, easy, cheap, robust and safe method of analysis of Sudan dyes in chilli pepper powder. Heliyon 6: e05243.

Reference chromatogram

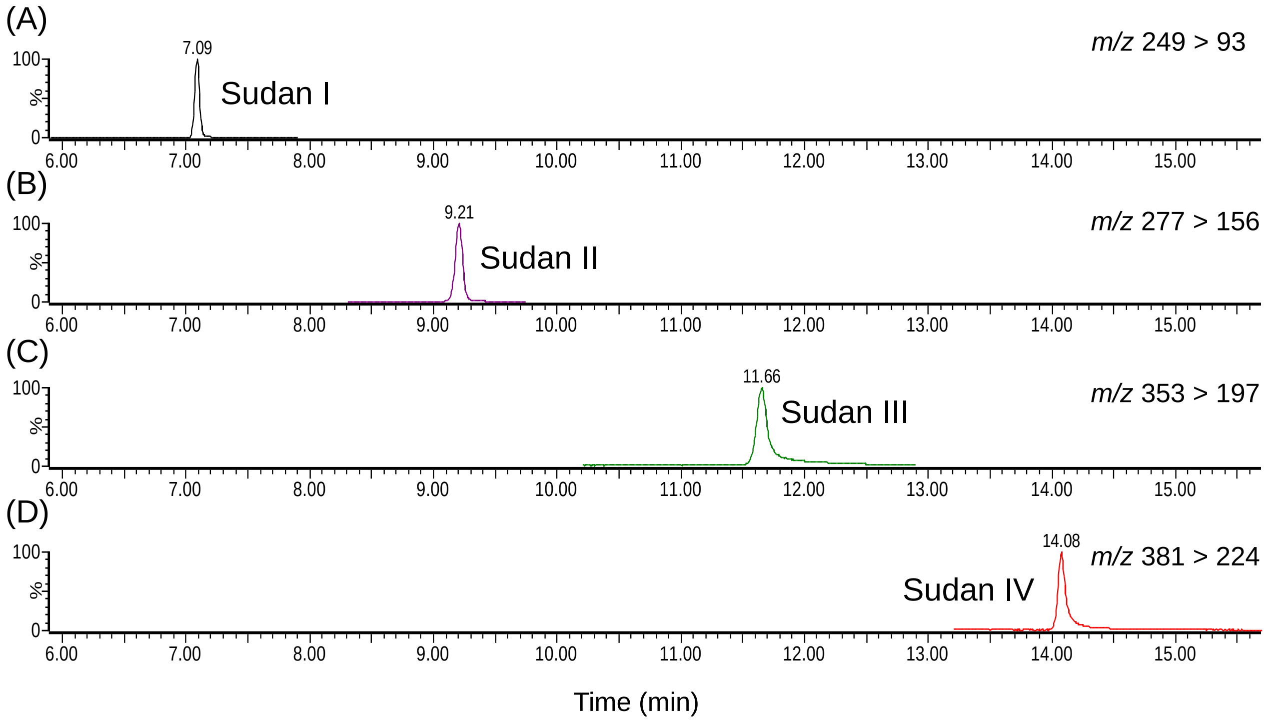

Figure. MRM chromatograms of Sudan I (A), Sudan II (B), Sudan III (C) and Sudan IV (D) analyzed by LC-MS/MS.