Published, Nov 24, 2025
RA03D015.001

Method of Test for Sudan Dyes in Cosmetics

1. Scope

This method is applicable to the determination of Sudan I (CI 12055), Sudan II (CI 12140), Sudan III (CI 26100) and Sudan IV (CI 26105) in cosmetics.

2. Method

After extraction, 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: ACQUITY BEH Shield RP18, 1.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Methanol, HPLC grade;

Acetonitrile, HPLC grade;

Methylene chloride, HPLC grade;

Ammonium formate, GR grade;

Formic acid, GR 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 IV-d_6, isotope-labeled internal standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL and 20 mL.

2.3.2. Membrane filter: 0.22 μm, PTFE.

2.4. Reagents

2.4.1. Methylene chloride: methanol (2:8, v/v)

Mix methylene chloride and methanol at the ratio of 2:8 (v/v).

2.4.2. Extraction solution

Dilute 1 mL of formic acid with methylene chloride: methanol (2:8, v/v) to 1000 mL.

2.5. Mobile phase

2.5.1. Solvent A:

Dissolve and dilute 0.63 g of ammonium formate with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B:

Mix methanol and acetonitrile at the ratio of 1:1 (v/v), and filter with a membrane filter.

2.6. Internal standard solution preparation

Transfer adequate amounts of Sudan I-d_5, Sudan II-d_6, Sudan III-d_6 and Sudan Ⅳ-d_6 isotope-labeled internal standards accurately weighed to each 10 mL volumetric flask, dissolve and dilute to volume with extraction solution as the internal standard stock solutions. Store in a refrigerator. When to use, mix appropriate volume of each internal standard stock solution, and dilute with extraction solution to 0.5 μg/mL as the internal standard solution.

2.7. Standard solution preparation

Transfer adequate amounts of Sudan I, Sudan II, Sudan III and Sudan IV standards accurately weighed into each 10 mL volumetric flask, dissolve and dilute with appropriate extraction solution to the volume as the standard stock solutions. Store in a refrigerator. When to use, mix appropriate volume of each standard stock solution, and dilute with extraction solution to 0.01-0.2 μg/mL (contain an internal standard at a concentration of 0.05 μg/mL) as the standard solution.

2.8. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 20 mL volumetric flask. Add 2 mL of internal standard solution and 15 mL of extraction solution, and sonicate for 30 min. Add extraction solution to the volume. Filter with a membrane filter, and take the filtrate as the sample solution.

2.9. Standard curve establishment

Accurately inject 2 μL of the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Establish the standard curve of each Sudan dye by the ratios of the peak area of each Sudan dye to that of the internal standard vs. the concentrations of each Sudan dye.

LC-MS/MS operating conditions (note 1):

Column: ACQUITY BEH Shield RP18, 1.7 μm, 2.1 mm i.d. × 10 cm.

Column temperature: 30°C.

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

Time (min)	A (%)	B (%)
0 → 3	95 → 75	5 → 25
3 → 7	75 → 30	25 → 70
7 → 8	30 → 0	70 → 100
8 → 12	0 → 0	100 → 100
12 → 12.5	0 → 95	100 → 5
12.5 → 15	95 → 95	5 → 5

Flow rate: 0.3 mL/min.

Injection volume: 2 μL.

Capillary voltage: 2.65 kV.

Ion source temperature: 150°C.

Desolvation temperature：500°C.

Cone gas flow rate: 30 L/hr.

Desolvation flow rate: 650 L/hr.

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

No.	Analyte	Ionization mode	Ion pair: Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
1	Sudan I	ESI^+	249 > 128*	12	22
			249 > 156		10
2	Sudan II	ESI^+	277 > 156*	18	12
			277 > 260		8
3	Sudan III	ESI^+	353 > 197*	32	16
			353 > 156		22
4	Sudan IV	ESI^+	381 > 91*	30	40
			381 > 224		14
5	Sudan I-d_5 (I.S.)	ESI^+	254 > 156	12	10
6	Sudan II-d_6 (I.S.)	ESI^+	283 > 162	18	12

7	Sudan III-d_6 (I.S.)	ESI^+	359 > 162	32	22
8	Sudan IV-d_6 (I.S.)	ESI^+	387 > 225	30	14

*The quantitative ion pair.

Note 1: 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 2 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 (note2) by multiple reaction monitoring. Calculate the amount of each Sudan dye in the sample by the following formula:

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

where,

C: the concentration of each Sudan dye in the sample solution calculated by the standard curve (μg/mL)

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

M: the weight of sample (g)

Note: 2. Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤ 100%). Maximum permitted tolerances for relative ion intensities by LC-MS/MS are as follows:

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

Remark

1. Limits of quantitation (LOQs) are 0.2 μg/g for Sudan I, Sudan II, Sudan III and Sudan IV.

2. Further validation should be performed when interference compounds appear in samples.

Reference

Tsai, C.F., Kuo, C.H. and Shih, Y.C. 2015. Determination of 20 synthetic dyes in chili powders and syrup-preserved fruits by liquid chromatography/tandem mass spectrometry. J Food Drug Anal. 23: 453-462.

Reference chromatogram

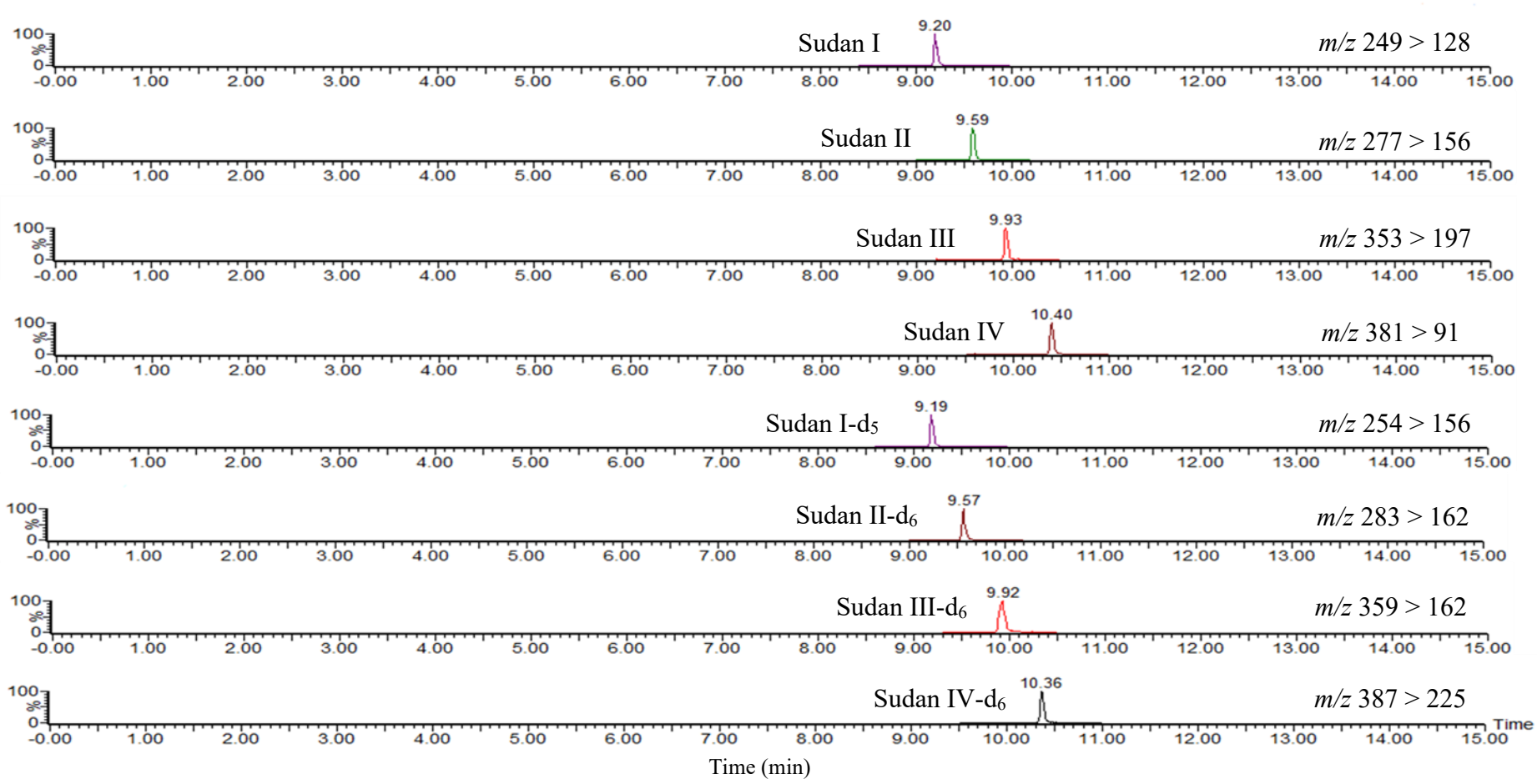

Figure. MRM chromatograms of 4 Sudan dye standards and 4 isotope-labeled internal standards analyzed by LC-MS/MS.