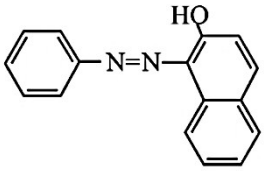
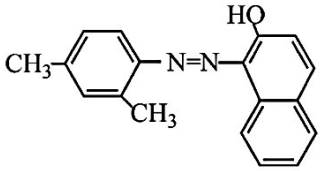
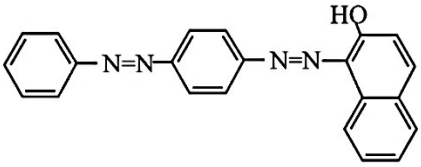
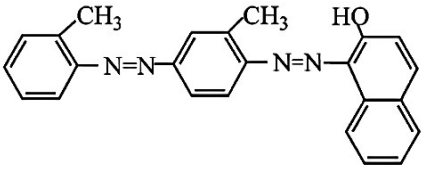
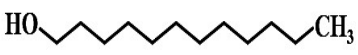
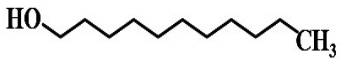
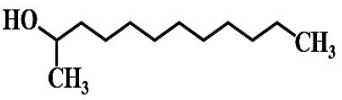
1 **Supplementary data**

2 **Electrolyte-assisted microemulsion breaking in vortex-agitated solidified floating**
3 **organic drop microextraction for preconcentration and analysis of Sudan dyes in**
4 **chili products**

5

6 **Table S1** Chemical formula and structures of Sudan dyes and alcohols used in this study.

Compound	Formula	MW (g/mol)	log K_{ow}	Structure
Sudan I	$C_{16}H_{12}N_2O$	248.3	5.51	
Sudan II	$C_{18}H_{16}N_2O$	276.3	6.60	
Sudan III	$C_{22}H_{16}N_4O$	352.4	7.63	
Sudan IV	$C_{24}H_{20}N_4O$	380.4	8.72	
1-dodecanol	$C_{12}H_{26}O$	186.3	5.13	
1-undecanol	$C_{11}H_{24}O$	172.3	4.72	
2-dodecanol	$C_{12}H_{26}O$	186.3	4.70	

7 MW: molecular weight; K_{ow}: octanol-water partition coefficient

Supplementary data 1

Sample preparation by ultrasonic optimization

Sample amount

The sample amount is a key criterion for the contact area of sample (solid) and solvent (liquid), which consequently influences its extraction efficiency. The maximum contact area can be obtained when the sample is saturated with the solvent. The methanol volume was fixed at 10 mL, whereas the sample amounts of the chili products varied between 0.1 and 0.5 g. The average contents of all the dyes in the samples (0.1–0.5 g) were significantly similar (Fig. S1(a)). Therefore, 0.1 g samples were used for further optimization experiments.

Volume of the extraction solvent

In general, an appropriate volume of the extraction solvent is needed for a suitable sample and solvent ratio. The samples (0.1 g) were studied with different volumes of methanol (5, 7.5, 10, and 12.5 mL). As shown in Fig. S1(b), the average contents of Sudan I–IV were almost similar at different volumes of methanol. Therefore, 5 mL of the extraction solvent was used for the further extraction procedure.

Extraction time

During methanol extraction (1–20 min), the contents of the Sudan dyes increased with an increase in sonication time between 1 and 5 min (Fig. S1(c)). The complete extract was obtained within 5 min, and the yield was nearly constant up to 20 min. However, further prolongation of the extraction process may have certain disadvantages. Therefore, a sonication time of 5 min was chosen as the optimal time.

Extraction temperature

The effect of extraction temperature was studied at different temperatures, ranging from ambient temperature (30°C) to 50°C. In general, tests are not performed at higher temperatures, possibly due to the loss of organic solvents. Similar results were observed for

the tested temperature range (Fig. S1(d)), indicating that the effect of extraction temperature is negligible. Therefore, any temperature could have been chosen; however, the experiment was set at 30°C.

Extraction solvent

To test the extracting efficiency of different solvents, six typical organic solvents (methanol, ethanol, acetone, acetonitrile, hexane, and DMSO) were used for the extraction of Sudan dyes in chili products (Fig. S1(e)). Methanol, ethanol, acetone, acetonitrile, and DMSO are the most effective solvents that could extract these dyes from the samples. Hexane could also be used for the extraction of Sudan dyes; however, the relative content yield is lower than the abovementioned solvents. Therefore, methanol was chosen as the extraction solvent.

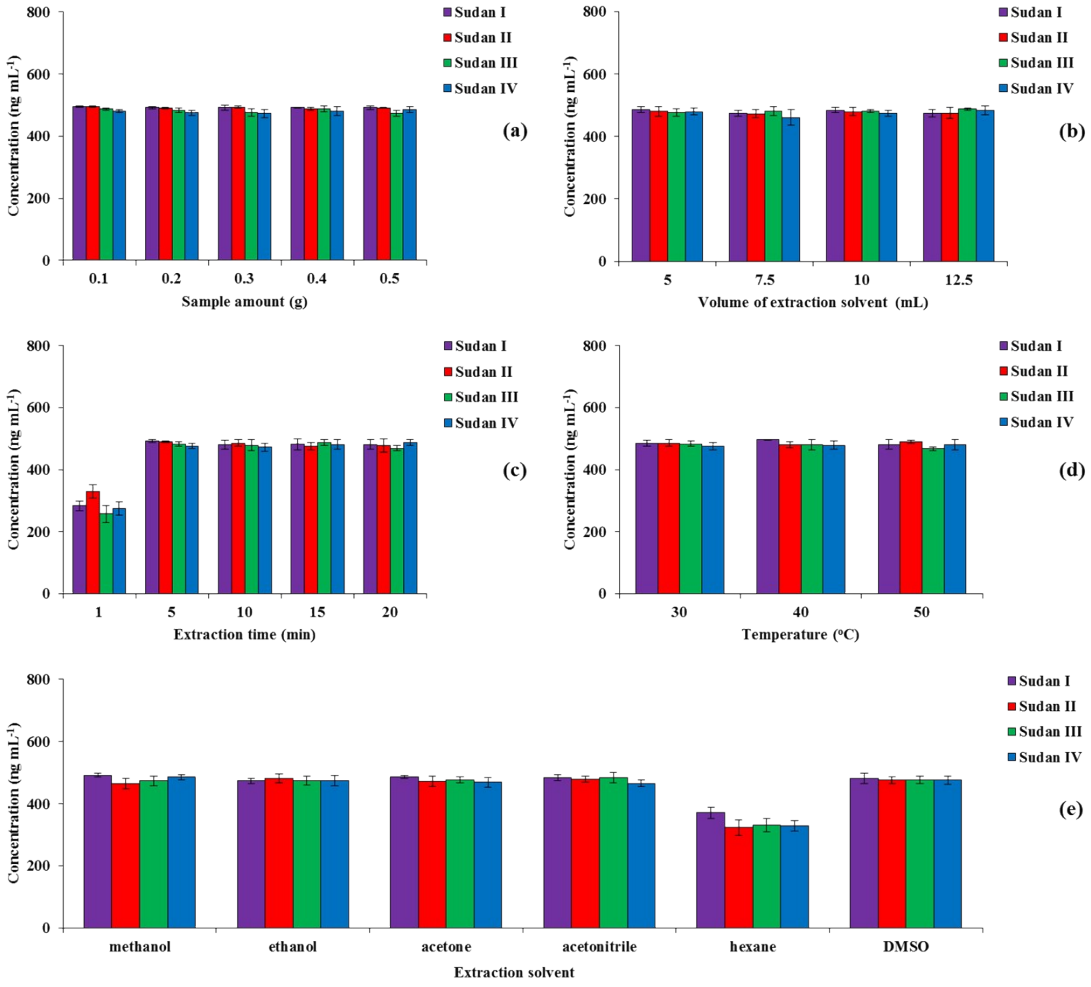


Fig. S1. The optimization conditions using UAE: (a) Effect of sample amount, (b) Effect of volume of extraction solvent, (c) Effect of extraction time, (d) Effect of temperature, and (e) Effect of extraction solvent.

Extraction mechanism for the UAE method

It is essential to understand the extraction mechanism to appreciate how UAE works. When methanol comes in contact with the ground sample, it first surrounds the sample particles (which generally have very irregular shapes) to create a solvent layer or film, following which several processes start to occur. Ultrasonic waves facilitate swelling and hydration of the solid particles, leading to cell wall pore enlargement due to cyclic compression and expansion caused by the passage of waves through the fluid. This expansion can create

65 microbubbles or cavities in the fluid,[23,24] as depicted in Fig. 1(a). The improvement of the
66 extraction using ultrasonic waves is attributed to cavitation. Within these imploding
67 microbubbles, the conditions can be dramatic (temperature: 4,500°C; pressure: ≤100 MPa), in
68 turn producing very high shear waves and turbulence in the cavitation zone. In pure liquids,
69 the microbubbles retain their spherical shape during the collapse because of their uniform
70 surroundings. When a microbubble collapses near a surface, deformation leads to an
71 asymmetric collapse, creating high speed solvent jets that are directed toward the solid
72 surface; the jets hit the plant particles at a very high speed (>400 km/h in water).[25] These jets
73 have a hammer-type impact that damages the surface or cell walls. Therefore, the solvent
74 penetration into the cell is enhanced by increasing the contact surface area between solid and
75 liquid phases. This immediately leads to improved diffusion that enhances mass transfer from
76 the plant material to methanol, significantly improving the extraction yield of the target
77 compounds.[26]

78 **Analysis of real samples**

79 The dye extracts from some chili products (chili powder, chili paste, chili sauce, and chili oil)
80 were separated and detected by HPLC-PDA using UAE. The results are tabulated in Table
81 S2. All the four Sudan dyes could not be detected in all the studied samples. However, to
82 verify the accuracy of this extraction method and to check for any matrix interference, the
83 recoveries in the real samples were calculated. Recovery (%) was calculated as follows:[27]

84
$$\%Recovery = \frac{C_{found} - C_{real}}{C_{added}} \times 100$$

85 (1)

86 where C_{found}, C_{real}, and C_{added} are the concentration of the analyte after addition of the known
87 amount of standard in the real sample, concentration of the analyte in the real sample, and
88 concentration of the known amount of the standard spiked in real sample, respectively.

After spiking each sample with 500 ng mL^{-1} of the standard solution of Sudan I–IV, it was subjected to the optimized UAE. The recoveries were expressed as the mean value of three independent determinations ± standard deviation (SD). The recoveries of Sudan I–IV dyes ranged from 99.14 ±3.11% to 108.2 ±6.33%, 94.24 ±7.28% to 104.4 ±2.54%, 90.45 ±3.76% to 98.74 ±2.14%, and 94.57 ±4.44% to 98.14 ±5.26%, respectively (Table 2). The dyes were obtained in good quantity, indicating that the extraction was relatively free of matrix interference. Because UAE yields acceptable recovery values, it can be used for green sample preparation prior to the VA-LPME-SIMB-SFOD procedure.

Table S2 Recovery of Sudan dyes in some chili products by UAE and HPLC-PDA method.

Analyte	**Spiked level**	**Recovery (% ±SD[a], *n* = 3)**			
	(ng mL^{-1})	**Chili powder**	**Chili paste**	**Chili sauce**	**Chili oil**
Sudan I	0	-			
	500	99.14 ±3.11	102.54 ±5.14	108.2 ±6.33	103.11 ±3.34
Sudan II	0	-			
	500	95.18 ±4.24	98.87 ±2.02	104.4 ±2.54	94.24 ±7.28
Sudan III	0	-			
	500	90.45 ±3.76	98.56 ±3.51	98.74 ±2.14	96.53 ±4.58
Sudan IV	0	-			
	500	94.57 ±4.44	97.72 ±2.74	96.41 ±3.32	98.14 ±5.26

[a]Standard deviation