SPECIAL GUEST EDITOR SECTION

Inedible Azo Dyes and Their Analytical Methods in Foodstuffs and Beverages

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Edible colorants, as an important part of food additives, can not only enhance the sensorial attributes of foodstuffs, but can also increase one's appetite. They hold a very important position in food processing. In the past decades, the illegal addition of the inedible colorants has become one of the major issues of food safety. Industrial dyes, especially some azo dyes, are illegal additives frequently found in foodstuffs. They cannot provide any nutrients for the human body and even have toxicity, carcinogenic, or mutagenic effects, which may cause serious damage to consumers' health. There is an increasing demand to detect the inedible azo dyes in foodstuffs for health and safety reasons. In this review, the types and the physicochemical properties of the inedible azo dyes adulterated in foodstuffs and beverages are summarized, and the emphases are focused on the sample pretreatment methods and analytical techniques for monitoring of these dyes in foodstuffs and beverages.

Color is one part of the important sensory attributes of foodstuffs. It has been thousands of years since colorants were employed to improve food organoleptic characteristics. According to the U.S. Food and Drug Administration, food colorants in a broad sense are any dyes, pigments, or substances that, when added or applied to a food, drug, or cosmetic, or to the human body, are capable (alone or through reactions with other substances) of imparting color. Most food colorants added in foodstuffs can increase one's appetite and incite their consumption of these products. As one important category of food additives, food colorants are subdivided into natural and synthetic colorants. Natural colorants, extracted from animals, plants, and microorganisms, have limited application in food industries because of the disadvantages of high cost, poor coloring ability, and ease of discoloration. In contrast, synthetic colorants are brightly colored and stable, so they are extensively employed in food industries. Different countries and regions have strict legislations for types, application scopes, and limit standards of the synthetic colorants approved for consumption.

Because of the few variety and relatively high price of the approved synthetic food colorants, industrial dyes with the features of strong coloring ability and low price have been illegally added in food products in recent years by some vile food manufacturers. Industrial dyes often have high toxicity and adverse effects, including teratogenic, carcinogenic, and mutagenic effects. Thus, ingestion of the foodstuffs containing the illegal dyes poses great risk to consumers' health. In the past decades, a large number of food safety incidents caused by the illegal addition of inedible dyes have occurred all over the world and have received increasing attention as one of the major issues of food safety.

There are three major categories of industrial dyes that tend to be illegally added in food products, i.e., azo dyes, triphenylmethane dyes, and xanthene dyes (1, 2). Among them, azo dyes are the largest group of synthetic dyes (3). Up to now, there were more than 3000 azo dyes in the world, and they accounted for approximately 65% of the commercial dye market in the food industry (4, 5). Since the second half of the 19th century, azo dyes have been widely used for coloring various materials in multitudinous industries, such as clothes, leather, food products, plastics, toys, and cosmetics, owing to their wide color spectra, colorfastness, and low price. These dyes, synthesized through nitrogen-coupling reaction of aromatic compounds such as benzene and toluene, are characterized by aryl and one or more azo groups (R-N=N-R'). The number of azo groups determines the chromaticity of the azo dyes, and along with the increase of the number of azo groups, the color of the dyes gets deeper. Although some azo dyes are nontoxic or low-toxic, most azo dyes are harmful to human health. Chemical analysts have developed a variety of methods to quantify the azo dyes illegally added in food for monitoring the quality and safety of food products.

The Chemical Properties of Commonly Inedible Azo Dyes in Foodstuffs and Beverages

According to the function groups, the inedible azo dyes frequently detected in food can be classified into lipophilic azo dyes, acidic azo dyes, and basic azo dyes.

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Lipophilic azo dyes illegally added in food mainly include some Sudan dyes (Sudans I–IV, Sudan red B, Sudan red 7B, Sudan red G, Sudan orange G, methyl yellow, and para red). Sudan I is a monoazo dye and Sudan II is a dimethyl derivative of Sudan I. Sudan III and Sudan IV are diazo dyes. Para red is chemically similar to Sudan I (6).

Acidic azo dyes illegally added in food mainly include orange II, orange G, acid yellow II, and metanil yellow.

Basic azo dyes frequently detected in food mainly include basic orange 2, basic orange 21, and basic orange 22.

Table 1 summarizes the chemical structures and physicochemical properties of inedible azo dyes commonly detected in foodstuffs and beverages.

Illegal Addition of Inedible Azo Dyes in Foodstuffs and Beverages

The above-mentioned azo dyes are widely used as coloring agents in the chemical industries including oils, waxes, petrol, textile, leather, printing floor polishing, spirit varnishing, etc. (7). Because of their intense red-orange color, wide availability, low cost, and chemical stability, these compounds are illegally added to food products in spite of their prohibition as food colorants. Their use in foodstuffs and beverages, at any level, is forbidden by the European Community and many other countries and organizations (8). Butter yellow was approved for use as a food additive in 1918, but was withdrawn 6 months later because of contact dermatitis from occupational exposure [International Agency for Research on Cancer (IARC) 1975; 9]. Basic orange 2, one of the cationic dyes, is prohibited from being applied in food products in China (10) and other countries (11).

Sauces and condiments.—According to the Rapid Alert System for Food and Feed (RASFF; European Regulation 2002/178/EC), there were 390 notifications in the period from 2003 to 2004 about the fraudulent use of dyes in foods, mainly involving chili or curry powder contaminated with either Sudan I or IV or both (RASFF Annual Report, 2006). In most cases, India was identified as the country of origin of the adulterated products, followed by Turkey (7, 12–14).

Chili powders and chili tomato sauces are the condiments that tend to be adulterated with Sudan dyes. Sudan I and IV were found simultaneously in some products, mostly in different types of chili and curry products (powders, sauces, etc.) as well as in seasonings and spice mixtures. These types of products originated mostly from Turkey, India, and the Russian Federation (7, 14). Since 2003, there have been around 20% cases reported by RASFF in which Sudan dyes have been found in palm oils. Palm oils from many African countries were contaminated mostly with Sudan IV (less often with Sudan I; 12, 15).Butter yellow was added illegally at the level of 0.65 to 174 μg/g to spices such as curry powder, chili powder, barbeque kebab powder, and spice mixture imported from India, Ghana, and China (16). In 2005, Para red was found in 35 products, mainly cooking sauces, though some were spices (17).

Beverage products.—The color of beverages is often associated with certain flavors, such as various fruit flavors, thereby influencing the perception of the flavor from milks to wines. Based on this, different inedible azo dyes may be illegally added in a variety of commercial beverages in order to simulate or maintain the "natural" hue preferred by the consumers. Sudan dyes are widely applied in flavored beverages, which caused a lot of food safety problems (18). In addition, Sudan I may exist in drinks containing Sunset yellow because it is probably produced in Sunset yellow synthesis (19).

Other foods.—In China and many other countries, egg yolks with an intense yellow-orange or red hue are the most desired by consumers as it is thought that the nutrition and freshness is generally related with the color. The inedible dyes including Sudan dyes are illegally added into feedstuffs for poultry such as ducks and hens. Consequently, the dyes are enriched in egg yolks, leading to the red-yolk eggs produced (20). In addition, butter yellow was reported to be added in dried tofu and spicy bean curd.

Most basic orange dyes were detected in some yellow fish, fried tofu skin, and spiced corned eggs in China (21, 22). Among them, dried beancurd stick, a favorite traditional bean product, was adulterated with basic orange 21 and basic orange 22.

Toxicity and Metabolism

According to IARC, the chemicals with carcinogenicity can be divided into four categories, including human carcinogens, possible human carcinogens, animal carcinogens, and noncarcinogens. The inedible azo dyes' toxicity depends on their metabolism and/or reduction. Sudan I, II, III, IV, 7B, basic orange 2, and orange G have been found to have carcinogenic effects, belonging to Group 3, namely animal carcinogens. Sudan I can produce benzenediazonium ion during cytochrome P450 catalyzed metabolism, which is considered the possible mechanism of Sudan I activation to an ultimate carcinogen (23). Sudan III used in cosmetics has been reported to yield 4-aminoazobenzene and aniline through potential metabolic cleavage by skin bacteria (24). There is evidence that some Sudan dyes have genotoxic effects (25). In addition, some inedible azo dyes can be reduced to potentially toxic and carcinogenic aromatic amines by the microbiota of human skin and the gastrointestinal tract through cleavage of the azo group (26). The aromatic amines can be metabolically activated to mutagenic and carcinogenic DNA-binding intermediates.

Sample Preparation

Appropriate sample pretreatment strategies are necessary for the accurate quantification of inedible azo dyes in food samples. There are often matrix interferences such as fat, protein, and carbohydrates in foodstuffs. In addition, it is reported that illegal azo dyes are usually found in various food matrices at low levels of milligrams per kilogram (27). Until now, there were no general standard sample pretreatment methods for inedible azo dyes in food samples reported. However, most analytical methods involve the procedure of extraction, cleanup, and concentration of target azo dyes. At present, the most methods reported for extraction of inedible azo dyes from different food products are solvent extraction, solid phase extraction (SPE), matrix solid-phase dispersion extraction (MSPDE), gel permeation chromatography (GPC), and molecularly imprinted technique (MIT). In addition, ultrasound-assisted extraction (UAE) and cloud point extraction (CPE) have been reported as well.

Solvent Extraction

Most of the extractions of the inedible azo dyes described in the literature are solvent extraction. In solvent extraction, the extraction efficiency of the analytes from food samples

Table 1. The chemical structures and physicochemical properties of common inedible azo dyes[a]

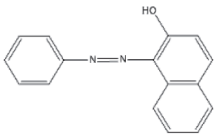
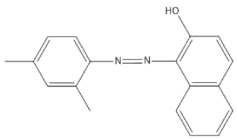
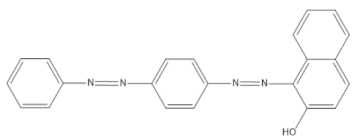
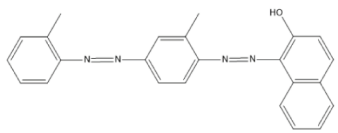
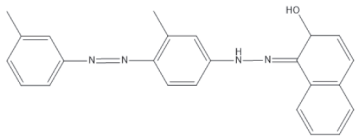
Azo dyes	CAS No.	Chemical structure	Molecular formula	Molecular weight, g/mol	Physical description	Chemical and physicochemical properties	Toxicity
Sudan I	842-07-9		$C_{16}H_{12}N_2O$	248.285	Dark reddish-yellow leaflets or orange powder; brick-red crystals (leaflets from ethanol); slight odor.	*(1)* bp: 333–444°C. *(2)* mp: 129–134°C. *(3)* Soluble in ethanol, acetone, ether, benzene, petroleum ether, carbon disulfide, concn hydrochloric acid; 0.674 mg/L in water at 25°C *(4)* LogP: 3.31–4.46	Carcinogen: Group 3; Genotoxic both in vitro and in vivo. The subcutaneous administration in mice resulted in liver tumors. LD50 in rabbits: <500 mg/kg bw[b]
Sudan II	3118-97-6		$C_{16}H_{16}N_2O$	276.339	Brown-red crystals; red needles	*(1)* bp: 334–477°C *(2)* mp: 166°C *(3)* Soluble in ethanol, acetone, ether, and benzene; 0.055 mg/L in water at 25°C *(4)* LogP: 3.90–5.37	Carcinogen: Group 3; genotoxic in vitro; a possible carcinogen as the increase in the incidence of bladder cancer was reported in mice; potential skin sensitizers; the maximum tolerate dose: 40 mg
Sudan III	85-86-9		$C_{22}H_{16}N_4O$	352.397	Reddish-brown or yellowish-red crystals; brown crystals with green, metallic glimmer (crystallized from glacial acetic acid)	*(1)* bp: 361–585°C. *(2)* mp: 199–200°C. *(3)* Soluble in chloroform, glacial acetic acid; moderately soluble in ethanol (3% at room temperature), ether, acetone, petrol ether, oils, and waxes; very soluble in benzene; $7.366.79 \times 10^{-9}$ ~ $7.396.79 \times 10^{-6}$ mol/L in water *(4)* LogP: 3.83–7.63	Carcinogen: Group 3; LD_{50} in rats: 0.5g/kg bw
Sudan IV	85-83-6		$C_{24}H_{20}N_4O$	380.451	Reddish-brown crystals	*(1)* bp: 361–619°C *(2)* mp: 186–233° *(3)* Solubility: $5.346.79 \times 10^{-10}$–$2.656.79 \times 10^{-6}$ mol/L in water. *(4)* LogP: 4.41–8.72	Carcinogen: Group 3; potentially genotoxic and possibly carcinogenic
Sudan red B	3176-79-2		$C_{24}H_{20}N_4O$	380.451	Reddish powder	*(1)* mp: 173–175°C *(2)* bp: 582.3°C	

Table 1. (*continued*)

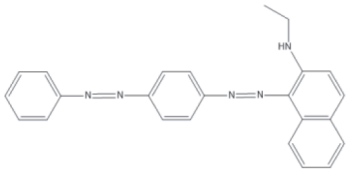
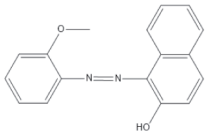
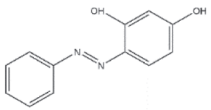
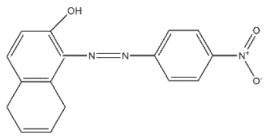
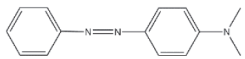
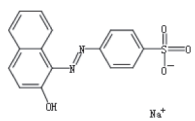
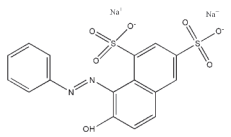
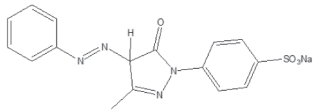
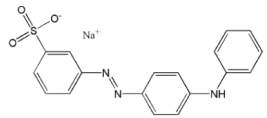
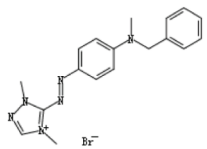
Azo dyes	CAS No.	Chemical structure	Molecular formula	Molecular weight, g/mol	Physical description	Chemical and physicochemical properties	Toxicity
Sudan red 7B	6368-72-5		$C_{24}H_{21}N_5$	379.467	Bordeaux crystalline powder	*(1)* bp: 341–607°C *(2)* mp: 161–215°C *(3)* Soluble in ethanol; very soluble in acetone and benzene; 6.79×10^{-10} ~ 2.77×10^{-6} mol/L in water *(4)* LogP: 3.93–7.93	Carcinogen: Group 3
Sudan red G	1229-55-6		$C_{17}H_{14}N_2O_2$	278.311	Odorless reddish-orange powder	*(1)* bp: 334–491°C *(2)* mp: 183°C *(3)* 9.62×10^{-7} ~ 2.42×10^{-5} mol/L in water *(4)* LogP: 2.90–5.59	LD50: >2000 mg/kg bw for rabbit (skin); >5000 mg/kg bw for rat (oral)
Sudan orange G	2051-85-6		$C_{12}H_{10}N_2O_2$	214.224	Red-orange powder	*(1)* bp: 327–408°C *(2)* mp: 170°C *(3)* 9.33×10^{-4} mol/L in water *(4)* LogP: 1.81–3.85	LD50: 600 mg/kg bw for rats (intraperitoneal)
Para red	6410-10-2		$C_{16}H_{11}N_3O_2$	293.281	Red solid	*(1)* bp: 330–535°C *(2)* mp: 175–219°C *(3)* Solubility: 2.23×10^{-7} ~ 9.56×10^{-5} in water *(4)* LogP: 3.47–5.90	NF[C]
Butter yellow	60-11-7		$C_{14}H_{15}N_3$	225.295	Yellow crystalline leaflets	*(1)* bp: 306–371°C *(2)* mp: 110–114°C *(3)* Soluble in alcohol, benzene, chloroform; ether, petroleum ether, mineral acids, oils; 1.02×10^{-6} in water. 0.23 mg/L in water at 25°C. *(4)* LogP: 4.58	Carcinogen: Group 2B; LD50: 230 mg/kg bw (intraperitoneal) and 300 mg/kg bw (oral) for mice; 230 mg/kg bw (intraperitoneal) and 200 mg/kg bw (oral) for rats.
Orange II	633-96-5		$C_{16}H_{11}N_2NaO_4S$	350.324	Orange to red powder	*(1)* bp: 466°C *(2)* mp: 246°C *(3)* Solubility: (at 25°C) 1.21×10^{-2} mol/L in water *(4)* *(4)* LogP: 1.92	Skin irritation (Category 2) Eye irritation (Category 2A)

Table 1. (*continued*)

Azo dyes	CAS No.	Chemical structure	Molecular formula	Molecular weight, g/mol	Physical description	Chemical and physicochemical properties	Toxicity
Orange G	1936-15-8		$C_{16}H_{10}N_2O_7S_2Na_2$	452.374	Yellowish-red crystals or leaflets	*(1)* bp: 282–361°C *(2)* mp: 192–252°C *(3)* Solubility: [at 25°C water 80 mg/mL, ethanol 3 mg/mL, methyl Cellosolve (monomethyl ether of ethylene glycol)] 40 mg/ml *(4)* LogP: –0.461~ 1.76	Carcinogen: Group 3
Acid yellow II	6359-82-6		$C_{16}H_{13}N_4NaO_4S$	380.354	NF	*(1)* bp: 231°C *(2)* mp: 231°C *(3)* Solubility: soluble in water (3.49e-03 mol/L), ethanol, acetone, soluble fiber element, ethanol yellow, slightly soluble in benzene, insoluble in other organic solvents *(4)* LogP: –0.549 ~ 3.29	NF
Metanil yellow	587-98-4		$C_{18}H_{14}N_3N_3O_3SNa$	375.378	NF	*(1)* bp: 315–741°C *(2)* mp: 195–325°C *(3)* Solubility: water 6.05e-05 to 1.72e-03 mol/L. *(4)* LogP: –0.111~ 4.40	LD_{50}: 1000 mg/kg bw (intraperitoneal) and 200 mg/kg bw (intravenous) for mice; 5000 mg/kg bw rats (oral). Harmful if swallowed, inhaled, and in contact with skin. May cause an allergic skin reaction, serious eye damage, and irritation. Toxic and harmful to aquatic life with long lasting effects
Basic Red 46	12221-69-1		$C_{18}H_{21}BrN_6$	401.312	Dark red powder	*(1)* bp: 326°C *(2)* mp: 124°C *(3)* Solubility: 1.47×10^{-5} mol/L in water *(4)* LogP: 3.72	Contact dermatitis, serious eye damage, eye irritation; harmful to aquatic life with long-lasting effects

[a] From Pubchem, ChemIDplus, Chemistry Dashboard, and HSDB.

[b] bw = Body weight.

[c] NF = Not found.

strongly depend on the solvents. For fat-soluble Sudan dyes, such solvents as acetonitrile (28–31), ethanol (32, 33), acetone (12), ethyl acetate (34), and tetrahydrofuran (35) are often employed. Acetonitrile has been mostly chosen because of its good extraction efficiency, lower fat solubility, and satisfactory protein precipitation (1). In addition, the mixtures of water with methanol or acetonitrile (36), acetonitrile–ethyl acetate (34), petroleum ether–ethanol (37), or acetone–dichlorometane–methanol (38) have also been reported as extraction solvents for these dyes. For water-soluble dyes, such as sulphonated azo dyes and basic orange dyes, ammonia is commonly added in the extraction solvent (22). T. Zou et al. (39) developed an HPLC diode-array detector (DAD) and tandem mass (MS/MS) method for the determination of seven synthetic dyes including orange II in animal feeds and meat. Ethanol–ammonia–water (80+1+19, v/v/v) solution served as the extract solvent. The recoveries of these seven dyes in animal feed and chicken meat were between 71 and 97%.

The traditional solvent extraction has the defects of requiring a large amount of hazardous organic solvents and much time consumption. Therefore, new techniques based on the traditional solvent extraction with modifications or improvements have been developed and applied in the analysis of the inedible azo dyes, such as liquid–liquid microextraction and automatic online liquid–liquid extraction (40).

SPE

SPE is the most frequently used procedure in extraction and cleanup of the inedible azo dyes from food and beverage samples. Selection of suitable absorbents is essential for the extraction of the target compounds in SPE. In most literature involving the analysis of fat-soluble Sudan dyes, neutral alumina serves as the absorbent in SPE cartridges (41). Nonpolar SPE column, such as C_{18} (30), and Oasis Hydrophilic–Lipophilic Balanced phases have been reported as well. The typical procedure of SPE is as follows: *(1)* sample loading, in which the liquid food sample or extracted solution is loaded onto SPE cartridge; *(2)* washing, during which the cartridge is cleaned up with appropriate solvents to remove the interference substances; and *(3)* elution, in which the analytes adsorbed are eluted with a suitable solvent or solvent mixture. The sorbents of C_{18} can also be chosen for extraction of acidic and basic azo dyes because of their chemical structures with benzene rings. Pérez-Urquiza et al. employed a C_{18} SPE cartridge for cleanup of seven sulphonated azo dyes in water (42). Bonan et al. developed an HPLC method for the simultaneous determination of synthetic dyes in foodstuffs, including orange II and metanil yellow. The solid food samples were extracted with a water–alcohol mixture, cleaned up by using a polyamide SPE cartridge, and eluted with basic methanol solution (36). Additionally, the cation exchange column, such as the Oasis MCX, can also be employed in the pretreatment of basic azo dyes because these compounds have positive charges from the chemical structures of amino groups, imino groups, or quaternary ammonium cation (22).

In order to improve selectivity of SPE, a large number of new materials are introduced as SPE adsorbents for the detection of inedible azo dyes in foods, such as molecularly imprinted polymers (MIPs) and magnetic nanoparticles (43–45). Yu and coworkers (46) reported a magnetic solid-phase extraction method with the nanocomposites of polystyrene-coated magnetic nanoparticles as the adsorbent for the extraction of four Sudan dyes (I–IV) in red wines, juices, and mature vinegars. The prepared magnetic nanoparticles exhibited highly hydrophobic properties and showed excellent adsorption capacity for these lipophilic Sudan dyes.

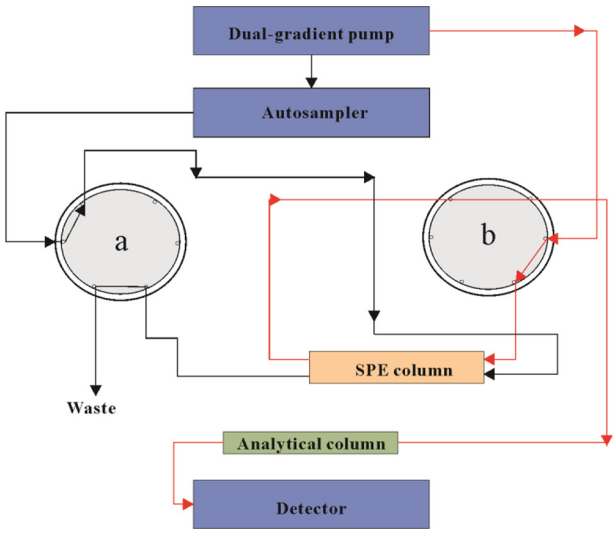


Figure 1. The schematic of online SPE-UHPLC system. Valve position (a) online extraction; valve position (b) separation of extracted dyes.

The aforementioned offline SPE methods consume of large amount of organic solvents and are relatively time-consuming and tedious. Online SPE is an effective way to overcome these disadvantages. Khalikova et al. (47) presented a simple, fast, and effective online SPE-ultra-HPLC-UV/Vis method using fused core particle columns for the extraction, separation, and quantitative analysis of nine illegal dyes in chili-containing spices, including Sudans I–IV, Sudan red 7B, Sudan red G, Sudan orange G, para red, and methyl red. Their method is fast (taking only 9 min including the online SPE step) and more sensitive than conventional offline SPE methods (Figure 1; 41).

MSPDE

MSPDE is a good sample preparation technique for complex solid, semisolid, or viscous samples. It simultaneously performs homogenization, extraction, cleanup, and even concentration in one single process (1). It has the advantages of a wide range of samples, high extraction efficiency, and less sample and solvent consumption. MPSDE was first proposed in 1989 by Barker et al. and was applied in isolation of drug residues in mammalian tissues. In recent years, the application of MSPDE has been extended greatly, and there have been many reports on the application of the extraction of inedible azo dyes from different foodstuffs (48, 49). Hou et al. (50) used MSPDE with alumina N as adsorbent and a mixture of n-hexane–acetone as the eluent for the extraction of para red and Sudan red I–IV from egg yolk. The extracts were analyzed by ultra-performance LC-MS/MS with the mean recoveries of 63.2 to 98.6% for the five dyes. Enríquez-Gabeiras et al. (51) developed a fast, simple, and effective MSPDE method for the simultaneous cleanup and quantitative extraction of Sudan red I–IV from different sauces and condiments. The method is based on the retention of the Sudan dyes in a polar sorbent of Florisil, elimination of the

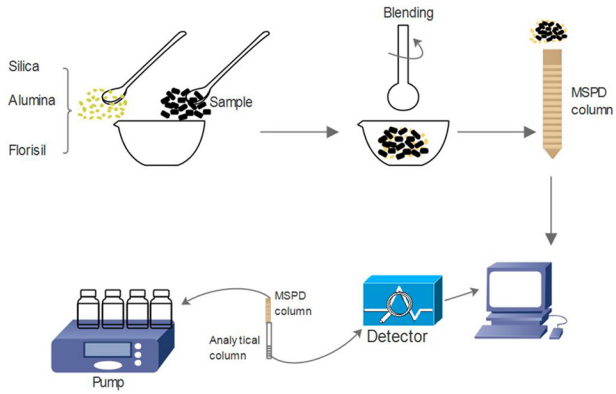


Figure 2. The schematic of online MMSPDE process.

less-polar interferences such as fat with n-hexane, and selective elution of the analytes with acetonitrile. Li et al. (52) employed MSPDE for multiresidue analysis of 25 synthetic colorants in meat matrices, including acid orange 2, acid orange 3, basic orange 2, basic orange 21, and basic orange 22. The homogenized meat samples were blended with C_{18} materials (10–40 µm) and eluted with methanol–formic acid (99+1, v/v) and methanol–ammonia (95+5, v/v), respectively. Furthermore, new selective materials such as molecularly imprinted polymers have also been used in MSPDE for extraction of inedible azo dyes (53).

Over the last few years, MPSDE underwent modification based on miniaturization and automation. Rajabi et al. (54) developed an online micro MPSDE coupled with HPLC for simultaneous analysis of Sudan red I–IV, Sudan orange G, Sudan black B, and Sudan red G in different spices. In this method, a MSPDE column with a suitable mixture of polar sorbents including florisil, silica, and alumina was inserted in the pathway of mobile phase (methanol–acetonitrile, 20+80, v/v), and then the analytes were eluted and entered into the analytical column (Figure 2).

GPC

GPC has proven to be a universal cleanup technique that has emerged in recent years, which utilizes porous gel (such as silica, agarose, polyacrylamide, etc.) as stationary phase, and the target compounds are separated from coextracted interferences according to the difference of their molecular sizes. Most literature have been focused on cleanup of Sudan dyes from chili-containing foodstuffs (16, 37). Among them, commercial GPC systems with glass columns are often employed, and dichloromethane or the mixture of cyclohexane–ethyl acetate serves as GPC mobile phase.

Sun et al. (34) adopted a GPC method to remove the coextracted interferences for the simultaneous determination of 10 banned azo dyes in hot chili and its products, including Sudan I–IV, Sudan orange G, Sudan red B, Sudan red G, Sudan red 7B, butter yellow, and para red. After extraction using ethyl acetate, the target dyes in samples were cleaned up by a GPC system equipped with express glass column (250 × 15 mm id) packed with 22 g 200–400 mesh Bio-Breads S-X3 resin and eluted out with a mixture of ethyl acetate–cyclohexane (50+50, v/v). Zhu et al. (55) utilized the same GPC system for analysis of 14 fat-soluble dyes in chili products, including Sudan dyes and other dyes, and obtained the recoveries ranging from 73.4 to 103.5%. Pardo et al. (13) developed an automated and sensitive procedure for the determination of seven inedible azo dyes in chili and hot chili products by pressurized solvent extraction, GPC cleanup, and LC-MS/MS analysis. The GPC cleanup procedure avoided some interference and the matrix effect during the MS/MS detection. Internal standard was used for correcting the loss of analytes. The estimated LODs and LOQs were in the range of 0.02–0.12 ng/g and 0.05–0.36 ng/g, respectively, and the recoveries ranged from 94 to 105%.

MIT

MIT is a technique to create template-shaped cavities in polymer matrices with memory of the template molecules to be used in molecular recognition. The polymer is called MIP, which is a synthetic crosslinked polymer possessing specific cavities designed for a target molecule (the template) and can selectively bind with the target analyte (56, 57). A schematic of the molecular imprinting process is shown in Figure 3. In the past decade, MIT has attracted increasing attention because of its feature of special target recognition ability, easy preparation, and high thermal and chemical stability. Over the years, a number of MITs for analysis of inedible azo dyes in foods have been developed (53). For example, a molecularly imprinted amino-functionalized silica gel polymer was prepared by combining a surface-imprinting technique with a sol-gel process and applied as sorbent for the determination of trace Sudan I in chili powder by online SPE-HPLC (58). The successful preparation of MIPs depends on the monomer, the crosslinker, and the appropriate polymerization condition for a specific analyte. Zhao et al. (44) synthesized an MIP with Sudan I as template molecule, 2-vinylpyridine as functional monomer, and ethylene glycol dimethacrylate as the crosslinking agent, respectively, which was used for the determination of Sudan dyes by online SPE and HPLC. Zhan et al. (59) synthesized a novel MIP by using acid orange II as the template, 3-(triethoxysilyl)propyl isocyanate as the functional monomer, and tetraethoxysilane as the crosslinker.

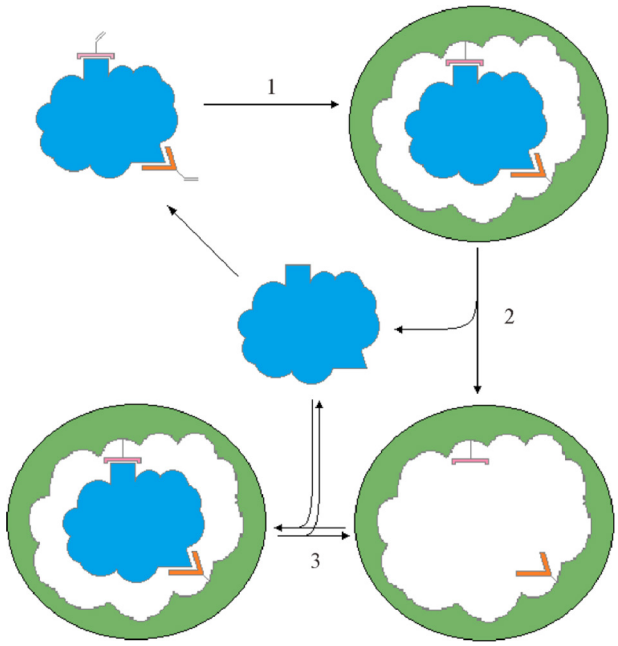


Figure 3. Scheme of the molecular imprinting process.

The MIP was used as adsorbent of an online SPE and a magnetic SPE cartridge and showed excellent recognition and outstanding selective abilities for trace amounts of the target molecules.

Other Methods

UAE.—The classical technique of solvent extraction is greatly improved through ultrasound assistance, which can significantly speed up the procedure of sample pretreatment and increase extraction efficiency. Khalikova et al. (35) developed a method of ultra-high-performance supercritical fluid chromatography for the separation and quantitative determination of 11 illegal dyes in chili-containing spices, including Sudan I–IV, Sudan red 7B, Sudan orange G, para red, Sudan G, and butter yellow. In their method, a simple ultrasound-assisted liquid extraction with tetrahydrofuran as the extraction solvent was involved.

CPE.—CPE is a promising environmentally benign extraction technique that is based on phase separation behavior exhibited by aqueous solutions of certain surfactant micelles (Figure 4; 60, 61). Unlike liquid-liquid extraction using toxic organic solvents, it requires small quantities of nontoxic and nonvolatile surfactants. Pourreza et al. (62) proposed a CPE procedure using mixed micelles of cetyltrimethylammonium bromide, Triton X-114, and Triton X-100 for preconcentration of trace amounts of allura red in food samples prior to spectrophotometric determination. Their method showed high enrichment factors and separation efficiencies.

Analytical Methods

Since the food scandal involving Sudan red in 2003, a variety of analytical methods have been reported to determine certain inedible azo dyes in a wide range of food matrices, such as HPLC with UV detection, DAD, MS or MS/MS detection, TLC, capillary electrophoresis (CE), and ELISA, as well as other methods such as spectrophotometry, GC-MS/MS, electrochemical sensor, surface-enhanced Raman scatting (SERS), and reduction methods.

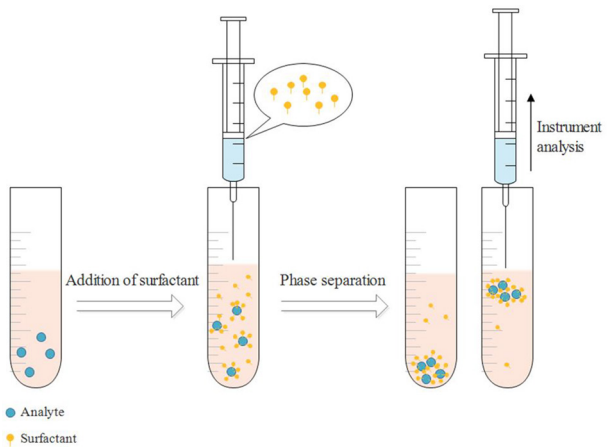


Figure 4. Scheme of the cloud point extraction process.

TLC

TLC is the cheapest and quickest separation method for many classes of chemical compounds (63) because it can analyze multiple samples simultaneously and requires no sophisticated sample preparation procedure. It is also a convenient choice for the analysis of inedible azo dyes in foods. After the target azo dyes in the sample are extracted and purified, the sample solution is loaded on the plate (as stationary phase), then the analytes are separated by a developing solvent or solvent mixture (as mobile phase), and, finally, the colorimetric determination is performed after the desorption of the analytes with the appropriate solvent. A TLC system with aluminium-backed silica gel sheets as stationary phase and benzene–chloroform (10+1, v/v) as the developing solvent for the analysis of Sudan dyes was described by Marshall (64). Hoodless et al. (65) adopted reversed-phase TLC with cellulose-coated plates impregnated with liquid paraffin to separate and identify 10 oil-soluble food colors, including Sudan I, Sudan II, Sudan Red G, and butter yellow. TLC can only be used for qualitative and semiquantitative determination. It is only applicable to the preliminary screening of the target compounds. HPTLC with digital processing of images can obtain rapid quantitative determination for these dyes (63).

HPLC

HPLC-UV/DAD.—HPLC is the most popular method for the determination of inedible azo dyes in foods because of its characteristics of good repeatability, acceptable sensitivity, and wide range of application. Examples of HPLC chromatographic conditions (stationary phase, mobile phase, flow rate, and detector) for the determination of inedible azo dyes in foodstuffs and beverages are listed in Table 2. In HPLC technique, reversed-phase HPLC with C_{18} column is the mostly employed separation mode (66–68). For lipophilic azo dyes such as Sudan dyes, the solvents of methanol or acetonitrile and water or 0.1% (v/v) formic acid aqueous solution often serve as the mobile phase (66–68). However, for ionic azo dyes, the pH of the mobile phase has to be controlled by using ammonia–ammonium acetate or other buffers because it can significantly affect chromatographic separation in several ways, such as selectivity, peak shape, and retention (45).

Because all the illegal azo dyes have absorption in UV-Vis wavelength range, a UV-Vis detector or DAD are often used in their quantitative analysis (66, 69, 70). For the current situation of the illegal addition of multiple azo dyes in foods, there is an increasing demand for the simultaneous determination of these dyes. In ordinary laboratories, cost-intensive LC-MS/MS is often not available. Therefore, the high throughput analytic methods based on HPLC coupled with a UV-Vis detector or DAD still have a more practical application in routine analysis. Besides UV-Vis and DAD detection, chemiluminescence (CL) detection was also reported in the determination of inedible azo dyes. Zhang et al. (32) proposed a method of HPLC with CL detection for the determination of four Sudan dyes in hot chili pepper. The method was based on the enhancement effect of Sudan dyes on the CL reaction between luminol and BrO^-. The LODs ranged from 4 to 8 mg/kg, and the LOQs ranged from 13 to 27 mg/kg.

Table 2. HPLC methods for the determination of inedible azo dyes in food samples

Analytes	Matrix	Sample pretreatment	Analytical conditions	Method performances	Refs.
Sudan (I–IV), Sudan orange G, Sudan red B, Sudan red G, Sudan red 7B, butter yellow, and para red	Hot chili products	Liquid–liquid extraction and gel permeation chromatography cleanup	Column: Inertsil ODS-3 column (250 × 2.1 mm id, 5 μm); mobile phase: 0.1% formic acid and methanol; detector: MS/MS	Quantification method: Standard solution calibration curves; R^2 = 0.9984–0.9997; LOD and LOQ were in the ranges of 0.1–1.8 and 0.4–5.0 μg/kg depending on matrices, respectively; recoveries = 81.7%–92.9%	(34)
Para red Sudan I, Sudan II, Sudan III, Sudan IV, canthaxanthin, and astaxanthin	Animal feeds	Extracted by using acetonitrile and cleaned up on a C_{18} SPE column	Column: cquity UPLC BEH C_{18} column (1.7 μm, 2.1 × 50 mm); mobile phase consisted of (A) acetonitrile–formic acid (100+0.1, v/v) and (B) water. Initial condition was 60% A and 40% B followed by linear gradient to 70% for A in 3 min and linear gradient to 90% for A in 1 min; detector: DAD/500 nm	Quantification method: external standard curves; R^2: 0.9962–0.9994; LODs = 0.006–0.02 mg/kg; intraday RSD = 2.6–10.4%; interday RSD = 4.0–13.2%; recoveries = 62.7–91.0%	(71)
Sudan dye (I, II, III, and IV)	Sausage products	Molecularly imprinted solid-phase extraction (MISPE) combined with ultrasound-assisted dispersive liquid–liquid microextraction (DLLME)	Column: Agilent C_{18} column (5 μm, 150 × 4.6 mm id); mobile phase: methanol–methanoic acid (99.7+0.3, v/v); flow rate: 1.0 mL/min; detector: UV/475 nm	Quantification method: external standard calibration curves using spiked sample; R^2 = 0.9993, 0.9996, 0.9991, and 0.9991 for Sudan I–IV; LOQs = 0.001, 0.001, 0.002, and 0.005 μg/g for Sudan I–IV; RSD < 5.2%; recoveries = 86.3–107.5%	(53)
Basic orange 2, basic orange 21, basic orange 22, acid orange 6 and acid orange 7, acid orange 20, and acid orange 52	Dried beancurd sticks, oil tofu skins and spiced eggs	SPE	Column: Zorbax Eclipse plus C_{18} column (2.1 × 150 mm, 3.5 μm); mobile phase: water (pH= 9 adjusted with ammonia) (A) and methanol (B) with gradient elution; injection volume: 1 μL; detector: MS/MS	Quantification method: external standard calibration curves; R^2 > 0.9998; LODs = 0.5–3.0 μg/kg; LOQs = 2.0–6.0 μg/kg; RSD = 2.22–25.4%; recoveries = 74–126%	(22)
Sudan dyes II, III, and red 7B	Saffron and urine	SPE	Column: Capital C8 column (250 × 4.5 mm, 5 μm); mobile phase: methanol–water (70+30, v/v); flow rate: 1 mL/min; column temperature: room temperature; injection volume: 20 μL; detector: UV/507 nm	Quantification method: external standard calibration curves; R^2 = 0.9983–0.9995; LODs = 4.6–6.6 μg/L; LOQs = 13.0–19.8 μg/L; RSD = 2.3–3.6%; recoveries = 92.5–113.4%	(72)
Auramine O, rhodamine B, basic orange 21, and basic orange 22	Chili sauce, soybean paste, and tomato sauce	Mixed hemimicelles solid-phase extraction (MHSPE)	Column: Agilent XDB-C_{18} column (4.6 × 150 mm, 5 μm) with a C_{18} guard column (4.6 × 12.5 mm, 5 μm); mobile phase A: 10 mmol/L ammonium acetate (pH 4.5 adjusted by formic acid) and mobile phase B: acetonitrile. Gradient elution; detector: DAD/450 nm for auramine O, basic orange 21, and basic orange 22; 554 nm for rhodamine B	Quantification method: external standard calibration curves; r = 0.99963–0.99996; LODs = 0.2–0.9 μg/kg; LOQs = 0.7–3 μg/kg; RSD < 15%; recoveries = 70.1–104.5%	(45)
Sudan I–IV	Chili tomato sauce, chili tomato cheese sauce	Liquid–liquid extraction	Column: Luna C_{18} narrow-bore column (250 × 2.1 mm, 5 μm); mobile phase: formic acid in methanol–aqueous formic acid 0.1% (97+3, v:v); detector: MS/MS	Quantification method: external standard method; R^2 = 0.990–0.999; LOD = 3–24 μg/kg; LOQ = 5–48 μg/kg; RSD = 2–13%; recoveries = 85–103%	(12)

Table 2. (*continued*)

Analytes	Matrix	Sample pretreatment	Analytical conditions	Method performances	Refs.
Sudan I–IV	Chili tomato sauce, hot chili pepper	Liquid–liquid extraction	Column: Nucleosil C_{18} column (id, 250 × 4.6 mm, 5 µm); mobile phase: methanol and 0.2% aqueous formic acid (90+10, v/v); detector: chemiluminescence	Quantification method: external standard method; R^2 = 0.9989–0.9995; LOD = 4–8 µg/kg; LOQ = 13–25 µg kg; RSD = 2.5–4.2%; recoveries = 94–105%	(32)
Sudan I–IV	Yellow River water, tomato sauce, sausage	Online MIP-SPE	Column: C_{18} column (Dikma Technologies, 250 × 4.6 mm); mobile phase: 10% ultrapure water and 90% acetonitrile-acetone mixture (90+10, v/v); detector: UV/500 nm	Quantification method: external standard method; LOD = 0.01–3.0 ng/g; LOQ = 0.02–8.0 ng/g; RSD = 3.0%; recoveries > 85.5%	(44)
Sudan I, Sudan II, Sudan III, Sudan IV, Sudan G, Sudan 7B	Chilli powder, sausage	Hydrazone covalent organic polymer-based micro-solid phase extraction	Column: Diamonsil C_{18} column (250 × 4.6 mm id, 5 µm); mobile phase: Acetonitrile and 0.1% (v/v) formic acid aqueous solution; detector: UV-Vis detector	Quantification method: external standard method; R^2 = 0.9954–0.9997; LOD = 0.03–0.15 µg/L; RSD = 1.2–9.4%; recoveries = 73.8–112.6%	(68)
Methyl Red, para red, Sudan red G, Sudan I, Sudan II, Sudan III, Sudan IV, Sudan red 7B, Sudan orange G	Chili sauce, Feferony, Mojo	Online SPE	Analytic column: Ascentis Express RP-Amide (100 × 4.6 mm, 2.7 µm); Mobile phase: Acetonitrile used as mobile phase B and water as mobile phase A; detector: UV-Vis detector	Quantification method: external standard method; R^2 = 0.9990–0.9999; LOD = 3.3–10.3 µg/L; LOQ = 11.0–34.6 µg/L; RSD = 1–6%; recoveries = 90–108%	(47)
Sudan I–IV	Pale pink candy, pink candy, red candy, red drink, pink drink	Ionic liquid/anionic surfactant aqueous two-phase extraction	Column: C_{18} guard column (7.5 × 2.1 mm id, 5 µm); mobile phase: methanol and water (99+1, v/v); detector: UV detector	Quantification method: external standard method; R^2 = 0.9993–0.9998; LOD = 3.68–5.45 µg/kg; LOQ = 12.3–18.2 µg/kg; RSD < 7.41%; recoveries = 82.3–112.1%	(67)
Sudan I–IV	Chili pepper powder	Online solid-phase extraction with a butyl methacrylate monolithic column	Column: C_{18} column (100 × 4.6 mm, 3 µm); mobile phase: Acetonitrile–formic acid (0.5%) solution (10+90, v/v); detector: MS/MS	Quantification method: Isotope dilution of internal standard; R^2 > 0.992; LOD = 0.3 µg/kg; LOQ = 1.0 µg/kg; Intraday RSD = 3.37–7.01%; interday RSD = 5.01–7.68%; recoveries = 94.8–100.9%	(73)
Sudan I	Chili powder and in chili-containing food products (snacks, Italian pasta)	Soxhlet extraction coupled with GPC cleanup	Column: Varian RP-C_{18} (5 µm, 15 cm × 4.6 mm) mobile phase: water–acetonitrile (v/v, 30:70); detector: UV-Vis/480 nm	Quantification method: a multilevel external standard procedure; R^2 = 0.99922; LOD = 6 µg/kg; LOQ = 13 µg/kg; RSD = 3.8%; recoveries = 93.4%	(37)
Sudan I–IV	Spices and chili containing foodstuffs	liquid−liquid extraction	Column: Acquity UPLC BEH C_{18} column (1.7 µm; 2.1 × 100 mm); Mobile phase: acetonitrile with 0.1% formic acid (phase A) and water with 0.1% formic acid (phase B); detector: MS/MS	Quantification method: internal standard curve method; R^2 > 0.9933; LOD = 0.5–100 µg/kg; LOQ = 2.5–200 µg/kg; RSD = 3–19%; recoveries = 81–105%	(29)
Sudan I–IV	Chili powder	Online solid-phase extraction with an imprinted functionalized silica gel sorbent column	Column: Shimadzu VP-ODS column (4.6 × 150 mm); gradient elution: A was 0.1% formic acid water solvent (v/v)–acetonitrile (85+15, v/v); mobile phase B was 0.1% formic acid acetonitrile solvent (v/v)–acetone (80+20, v/v); detector: UV/480 nm	LOD = 1.2 ng/L (Sudan I); RSD = 3.66%; recoveries = 80.31–94.02%	(58)

Table 2. (*continued*)

Analytes	Matrix	Sample pretreatment	Analytical conditions	Method performances	Refs.
Sudan I	Dried hot chili (both ground and powder), turmeric, paprika, and Oven-baked product "taralli"	Solvent extraction (96% ethanol) assisted with shaken and ultrasonic	Column: RP-Amide (C16) column (4.6 × 250 mm, 5 µm, Supelco, Bellefonte, PA, code 505064); mobile phase: acetonitrile (A) and methanol (B); detector: DAD/481 nm; MS/MS	Quantification method: external standard method; R^2 = 0.9980 (HPLC-DAD), R^2 = 0.9983 (HPLC/APCI-MS) HPLC-DAD: LOD: 80 µg/kg; 5 mg/kg; LOQ: 240 µg/kg; 15 mg/kg; HPLC/APCI-MS: LOD: 60 µg/kg; 3 mg/kg; LOQ: 180 µg/kg; 9 mg/kg; RSD = 4–12%; recoveries = 98–105%	(33)
Sudan I–III and Sudan B	Chili oil	Ultrasonic-assisted extraction: acetonitrile	Column: ZORBAX SB C_{18} (150 × 3.0 mm, 3.5 µm); mobile phase: acetonitrile–water with a gradient program; detector: fluorescence detector/λEx = 289 (I); 337 (II); 314 (III); 320 (B) λEm = 340 (I); 375 (II); 369 (III); 373 (B)	Quantification method: external standard method; R^2 = 0.9963–0.9998; LOD = 0.009–0.054 mg/kg; LOQ = 0.030–0.181 mg/kg; RSD = 2.6–3.8%; recoveries = 81.3–100.4%	(83)
Sudan I–IV and canthaxanthin	Salted duck egg yolk	Ultrasonic-assisted extraction: acetonitrile–methanol–chloroform (1:0.5:0.5; v/v/v)	Analytic column: XDB C_{18} (250 × 4.6 mm); mobile phase: acetonitrile–water (95+5; v/v); detector: UV/0~9 min: 478 nm; 9~25 min: 520 nm; 25~35 min: 471 nm	Quantification method: external standard method; R^2 = 0.99986–0.99994; LOD = 4; 7; 10; 12; 15 µg/kg; RSD = 2.7–5.1%; recoveries = 89.56–97.34%	(66)
Sudan I, II, III, and IV	Chili- and curry-based sauces and powdered spices	Acetonitrile extraction	Column: Varian Microsorb-MV RP column (150 × 4.6 mm 100^{-5} C_{18}); mobile phase: acetonitrile–water (80+20; v/v); detector: DAD/478 (I); 496 (II); 510 (III); 520 (IV)	Quantification method: external standard method (matrix-matched calibration standard curves); R^2 > 0.995; LOD = 0.2–0.5 mg/kg (sauces) and 1.5–2 mg/kg (spices); LOQ = 0.4–1 mg/kg (sauces) and 3–4 mg/kg (spices); RSD < 15%; recoveries = 51–86% (sauces); 89–100% (powdered spices)	(69)
Sudan I, II, III, and IV	Hot chili pepper, hot chili tomato sauce, sausage, tomato sauce, hard-boiled egg yolk	Molecularly imprinted solid phase extraction (MISPE)	Column: Onyx Monolithic RP C_{18} (100 × 4.6 mm); mobile phase: acetonitrile–water (80+20; v/v); detector: UV-Vis/476 nm	Quantification method: external standard method; LOD = 0.75 µg/g recoveries = 85–101%	(43)
Sudan I, II, III, and IV	Tomato sauce, chili oil, chili sauce, and natural waters	Dispersive liquid-phase microextraction with solidification of floating organic drop (SFO-DLPME)	Column: Hypersil ODS (150 × 4.6 mm,5 µm); mobile phase: methanol; detector: UV-Vis/506 nm	Quantification method: external standard method; R^2 = 0.9921–0.9990; LOD = 0.10–0.20 ng/g (food) and 0.03 µg/L (water); RSD < 4.8%; recoveries = 92.6–106.6% (food) and 91.1–108.6% (water)	(74)
Sudan I–IV, Sudan red G, Sudan red 7B	Chicken muscle, duck muscle, duck egg	Acetonitrile extraction followed by SPE	Column: Zorbax SB-C_{18} (150 × 4.6 mm, 5 µm); mobile phase: water–0.1% (v/v) formic acid in methanol with a gradient program; detector: DAD/510 nm	Quantification method: external standard method; r^2 > 0.9999; decision limit (CCα) = 7.7–9.0 µg/kg; detection capacity (CCβ) = 9.1–10.3 µg/kg; LOQ = 12.8–15.0 µg/kg; RSD = 2.3–14.9%; recoveries = 77.2–98.0%	(30)

However, when applied in the simultaneous determination of multiple inedible azo dyes, HPLC methods often suffered from time consumption. Recently, ultra-performance LC, as an alternative to HPLC, has been applied for the analysis of multiple inedible azo dyes in food samples, which significantly reduced the separation time without compromising the separation efficiency (29, 71). Traditional HPLC methods are now often applied for screening, and the suspected samples have to be confirmed by HPLC coupled with MS/MS.

Ion-pair HPLC (IP-HPLC).—Ionic azo dyes show weak retention in reversed-phase chromatographic columns because of the hydrophilicity of these compounds (75). While in normal-phase chromatography, the retention time is too long, and the satisfactory peak shape can hardly be obtained. IP-HPLC with UV detection has been reported to determine various sulphonated azo dyes in water, in which butylamine was used as an ion-interaction reagent (42). However, traditional nonvolatile ion-pair reagents are not compatible with MS detectors because the salt deposition in the ion source would cause instrument failure. Fuh et al. (76) developed an IP-HPLC method with online photodiode-array and electrospray MS detection for determination of 10 sulphonated azo dyes, including orange I, orange II, and metanil yellow in foods. In their study, a reversed-phase C_{18} column with gradient elution was utilized to separate these compounds, and the volatile triethylamine was added in the mobile phase as an ion-pair reagent for chromatographic separation. Good linearities (0.05–10 ppm; $R^2=0.999$) and detection limits (<0.01 ppm) were obtained for this method.

LC-MS

Because the presence of any banned dyes in food is unacceptable at any level, LC-MS–based methods play an increasingly important role in the screening, qualitation, and trace analysis of multiple azo dyes. Currently, they have been successfully applied in the determination of inedible azo dyes in foods. Among them, HPLC triple quadrupole MS is most often used (29, 73, 77). For most azo dyes, including Sudan dyes and basic orange dyes, positive ion mode is the preferred mode because of their protonated nature. However, for acidic subjects such as acid orange dyes, negative ion mode is usually employed because their structures have sulfonic groups with deprotonated property (22). Calbiani et al. (12) developed a LC-electrospray-MS/MS method for the simultaneous determination of Sudan dyes in hot chili products. A C_{18} column with methanol–formic acid aqueous solution as mobile phase was used for separation. MS/MS detection was performed by using triple quadrupole mass spectrometer in positive ion mode. Liu et al. (73) developed a novel online SPE with poly(butyl methacrylate-co-ethylene dimethacrylate) monolithic column for extraction of Sudan dyes in chili pepper powder followed by LC-MS/MS analysis. After online extraction and cleanup, the concentrated analytes were detected by a triple quadrupole mass spectrometer in positive ion mode equipped with a heated electrospray probe. Fang et al. (22) simultaneously determined four acid orange dyes and three basic orange dyes in foodstuffs by LC-MS/MS through negative/positive ion switching mode.

CE

CE is an electrophoretic analysis technique performed inside a capillary for efficient separation of both small and large molecules based on their mobility. It offers the characteristics of high efficiency, rapidness, and consumption of very small samples (nanoliters) compared with the conventional electrophoresis and chromatography and can be used as the supplement of HPLC in the analysis of inedible azo dyes. CE has various separation modes. In the determination of inedible azo dyes in foods and beverages, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are the most commonly used modes. The separation efficiency in CE is influenced by the physicochemical properties of the target dyes (molecular size, the number of charges, etc.), the separation voltage and temperature, and the buffer composition, pH, and concentration (78). Currently, there are only a few pieces of literature on the determination of inedible azo dyes. Chen et al. (70) established a CZE method for the simultaneous determination of nine banned acid azo dyes in beverages and foodstuffs. The method utilized an uncoated fused-silica capillary as separation channel, with 15% acetonitrile-10 mmol/L borate buffer (pH 9.0) as running buffer. Neutral analytes such as lipophilic azo dyes, which fail to be separated by CZE, are readily separated by MEKC. Surfactants are added into the background electrolyte to form micelles in MEKC. A method of MEKC with UV detection was developed for the determination of Sudan dyes (I, II, III, and IV). The separation of the four dyes was achieved in 20 min by using a background electrolyte consisting of 5 mM borate (pH 9.3), 20 mM sodium dodecyl sulfate, and 20% acetonitrile (15).

Immunoassays

Immunoassays are the analytical techniques that rely on the specific recognition between an antibody and corresponding antigen (16). Their most significant advantages over the traditional instrumental methods are low cost, high sensitivity, and capability of screening a large amount of samples in one single test. Immunoassays, especially ELISA, have been reported to detect the inedible azo dyes in foodstuffs (77, 79–87). Among them, indirect competitive ELISA was mostly employed. Han et al. (79) and Wang et al. (88) reported the first polyclonal and monoclonal antibody-based immunoassays for the detection of Sudan I in foodstuffs, respectively. Wang et al. (86) prepared specific antibodies of para red by synthesizing a hapten mimicking para red structure and coupling it to carrier protein to form an immunogen. Based on the antibodies, they developed a sensitive and selective ELISA for the analysis of para red in foods. Oplatowska et al. (16) developed a rapid disequilibrium ELISA for the detection of methyl yellow and Rhodamine B dyes in sauces and spices. Their assays only needed 20 min. Xue et al. (87) adopted an indirect enzyme immunoassay for the quantitative determination of orange II in chili powder, chili oil, and braised pork.

Most papers reporting ELISA method commonly detected one or two of these azo dyes. The development of an ELISA for multianalyte determination is highly desirable. Chang et al. (83) reported an indirect competitive immunoassay for simultaneous detection of Sudan I–IV, Sudan G, and para red in egg yolks, and their ELISA results were confirmed with an HPLC method.

Other Methods

Besides the above-mentioned analytical techniques, other methods such as spectrophotometry (89), GC-MS (20), electrochemical analysis (90–93), and SERS (94) have been

reported for the determination of inedible azo dyes in food products. He et al. (20) determined Sudan dye residues (I, II, III, and IV) in eggs by GC-MS. The suspected egg samples were derivatized with N,O-bis (trimethylsilyl) trifluoro-acetamide and confirmed by GC-MS in the electron impact mode. Ma et al. (95) reported an electrochemical method for the determination of Sudan red I in food samples based on the electrochemical catalytic activity of graphene-modified glassy carbon electrode and the enhancement of electrochemical effect of sodium dodecyl sulphonate. Cheung et al. employed a portable Raman spectrometer by using surface enhanced Raman scattering with multivariate chemometrics for quantitative analysis of Sudan I.

In addition, the inedible azo dyes are also analyzed by reduction methods. The dyes react with reducing agents such as sodium dithionite and tin(II) chloride to form amines, which are collected and determined by using spectrophotometry, TLC, GC, HPLC, or other analytical techniques (4, 94, 96).

Conclusions and Perspectives

The illegal addition of industrial azo dyes into food products can pose a great hazard for consumers' health. For the current situation of the illegal addition of trace amounts of multiple azo dyes, it is necessary to develop the detection techniques with high sensitivity and wide applicability. Although SPE is still the most widely used sample pretreatment method for the extraction of banned azo dyes from food samples, the development of green chemistry has led sample extraction procedures to be miniaturized, efficient, online, and environmental friendly. The analysis of inedible azo dyes tends to use the techniques such as HPLC-MS/MS, which has a strong qualitative ability and high sensitivity, for simultaneously and rapidly analyzing multiple inedible dyes in different food matrices. The rapid detection techniques such as immunology or electrochemical methods have the characteristics of rapid, accurate, and low cost, which can meet the requirements of initial screening and quick determination on the spot. In general, the detection of inedible azo dyes should be developed in a rapid, highly automated, sensitive, and environmentally friendly way.

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