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REVIEW OF RECENT SAMPLE PREPARATION METHODS FOR THE ANALYSIS OF AZO DYES IN FOODS AND DRINKS: A COMPARISON OF SOLID-PHASE EXTRACTION AND QuEChERS

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ABSTRACT

Azo dyes, characterized by the presence of the azo functional group (-N=N-), are among the most widely used synthetic colourants in the food and beverage industry because of their low cost, chemical stability, and vibrant coloration. However, a number of studies notably have linked dyes such as Sudan I–IV, Tartrazine, and Sunset Yellow to genotoxicity, carcinogenesis, and allergic reactions, raising serious public health concerns. Therefore, it is essential to develop trustworthy analytical techniques for identifying and measuring azo dyes in intricate food matrices. This study compares Solid-Phase Extraction (SPE) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), two commonly used sample preparation methods in food analysis. The methods were evaluated based on analytical efficiency, recovery performance, solvent use, and operational cost. Literature data indicate that SPE yields recoveries of 80–95%, while QuEChERS achieves 85–120%, with reduced solvent usage and time. Although conventional SPE can be labour-intensive, modified forms such as dispersive SPE (d-SPE) have improved extraction speed and reproducibility. Both methods face limitations from matrix effects in high-fat or protein-rich foods. Future trends include green analytical modifications, such as bio-sorbent-based SPE and miniaturized QuEChERS formats, to enhance environmental sustainability. Consequently, because of its exceptional effectiveness, ease of use, and environmental advantages, QuEChERS is suggested for further study and regular analytical applications in food safety and quality control.

Keywords: Azo dyes, Solid-Phase Extraction (SPE), QuEChERS, Food Analysis, Sample Preparation, Recovery rate, Green Analytical Chemistry, Food Safety

INTRODUCTION

Azo dyes contain the azo functional group (-N=N-), which links two aromatic rings and gives rise to vivid coloration. They are widely used as synthetic colorants due to their stability, low cost, and strong tinting strength, finding applications in foods, beverages, cosmetics, and pharmaceuticals (Barciela *et al.*, 2023). Despite these advantages, several studies have raised concerns about their potential health risks, including carcinogenicity, genotoxicity, and allergic reactions (Vieira Rubio et al. 2025). While azo dyes dominate the textile sector—accounting for about 60–70% of global dye usage (Azo Dyes and Pigments, 2014)—their use in food products presents unique safety challenges. This dual significance underscores the need for accurate analytical detection and regulation across industries. However, in more recent times the range of longer wavelength absorbing azo dyes has been extended, leading to the emergence of significant numbers of commercially important blue azo dyes. Perhaps the prime reason for the commercial importance of azo colorants is that they are the most cost-effective of all the chemical classes of organic dyes and pigments. It is conceivable that azo dyes may assume even greater importance in the future as some of the other chemical types, notably anthraquinones, become progressively less economic. Azo dyes represent the largest production volume of dye chemistry today, and their relative importance may even increase in the future. They play a crucial role in the governance of the dye and printing market. These dyes are synthesized from a simple method of diazotization and coupling. Different routes and modifications are made to obtain the desired color properties, yield and particle size of the dye for improved dispersibility (Benkhaya, M'rabet, and El Harfi 2020).

MATERIALS AND METHODS

Commonly Used Azo Dyes in Foods and Drinks

Azo dyes, including Tartrazine, Sunset Yellow, Sudan I-IV and Carmoisine, are added to foods to provide color, but they have no value with regard to nutrition, food preservation, or health benefits. Because of their availability, affordability, stability, and low cost, and because they provide intense coloration to the product without contributing unwanted flavours, the food industry often prefers to use synthetic azo dyes rather than natural colorants (Barciela and Prieto 2023). Colour is one of the most valued qualities, when consumers evaluate food products, and colorants, both natural and synthetic, are often used to enhance attractiveness. Colorant use can also help to preserve the original colour of a food product, which otherwise may be lost during processing (Monisha et al. 2023)(Barciela & Prieto, 2023). Some dyes are extracted from plant or animal sources, but the most commonly used food dyes are synthetic organic compounds (Lipskikh et al. 2018). The use of natural dyes is limited by factors such as lower colouring strength, degradation during food processing, and, in some cases, undesirable flavours. Dyes can also be classified as cationic, anionic, or non-ionic. Most anionic and non-ionic dyes contain anthraquinone or azo chromophores. Azo dyes contain one or more R^1-N– –N-R^2 bonds; these bonds may be reduced enzymatically, yielding aromatic amines. They may also have amphoteric properties due to the presence of carboxyl, hydroxy, amino, or sulfonyl functional groups (Benkhaya et al. 2020).

Tartrazine (TTZ) and sunset yellow (SY) are widely used in food processing; they are often used in combination, to impart yellow colour (Okeke et al. 2022). Both dyes are approved by European Food Safety Authority (EFSA) and Food and Drug Administration (FDA). Allowable Daily Intake (ADI) values for SY and TTZ have been established as 4.0 and 7.5 mg/kg bw/day, respectively (Dey and Nagababu 2022).

Chemical Properties of Azo Dyes

Azo dyes are organic compounds containing one or more azo (-N=N-) linkages between aromatic groups, often substituted with sulfonate, hydroxyl, or amino functional groups that enhance water solubility and reactivity. They are typically synthesized via diazotization and coupling reactions, producing diverse shades depending on substituents (Benkhaya *et al.*, 2020). In foods and drinks, common azo dyes include Tartrazine (E102), Sunset Yellow (E110), Carmoisine (E122), and the Sudan I–IV series (Okeke *et al.*, 2022; Barciela & Prieto, 2023). Sudan dyes, although formerly used, are now banned by the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) because of their confirmed carcinogenic potential.

Health Risks and Regulatory Considerations

Excessive intake of synthetic azo dyes has been linked to allergic responses, hyperactivity in children, and potential DNA damage (Vieira Rubio *et al.*, 2025). Sunset Yellow (E110), a monoazo dye, is polar, water-soluble, and used in soft drinks, desserts, and confectionery. Studies suggest it may induce chromosomal aberrations and gastrointestinal effects in experimental models, although long-term human data remain inconclusive (Rovira and Domingo 2019). In contrast, regulatory agencies like EFSA and FDA have established Acceptable Daily Intakes (ADIs)—7.5 mg/kg bw/day for Tartrazine and 4.0 mg/kg bw/day for Sunset Yellow—to ensure safe consumption levels.

Sunset Yellow SY (E110)

Is a synthetic azo dye commonly used as a food colorant; it is also known as Cl Food Yellow Cl 3, Orange Yellow S, and Yellow 6 (Ali et al. 2019) SY is polar, water soluble, and poorly soluble in ethanol. Aqueous solutions are orange-yellow, becoming red-brown in alkaline and neutral solutions. SY can be found in a wide variety of food products, such as aromatized and fermented beverages, ice cream, condiments, confectionery, chewing gum, jams, jellies, desserts, soups, fish roe, fish paste, and crustaceans (Silva, Reboredo, and Lidon 2022). SY may be teratogenic and may cause gastric upset, diarrhea, and vomiting; allergic reaction, intolerances, and behavioral disorders in children; or sleep disturbances. Dwivedi and Kumar, 2015, reported the effects of SY on chromosomal aberrations (genotoxic and cytotoxic effects) in the plant species *Brassica campestris* L (Dwivedi and Kumar 2015). However, no consensus has been reached with regard to possible adverse effects of SY in humans. EFSA and FDA have noted that SY has never shown carcinogenic or genotoxic effects in long-term studies in mice and rats, either *in vitro* or *in vivo* (Rovina *et al.*, 2017b).

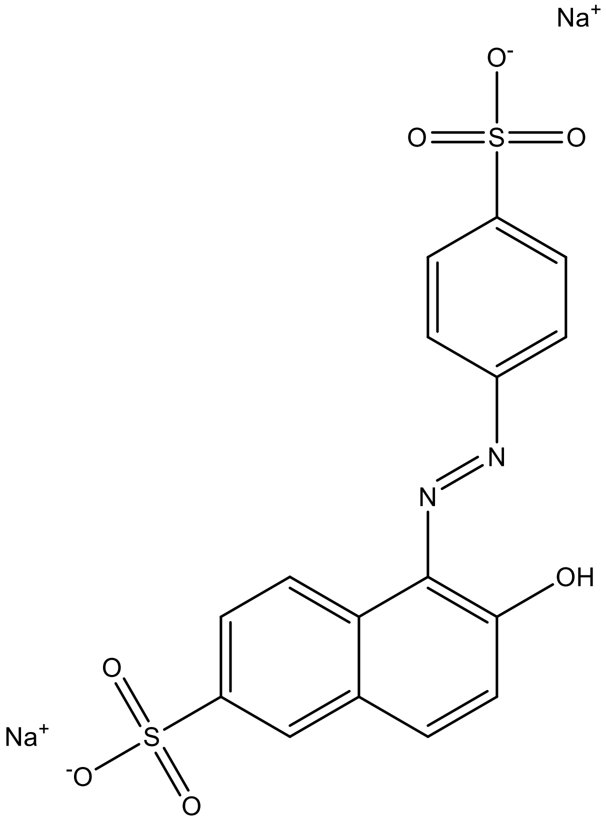

Figure 1: Chemical Structure of Sunset Yellow (E110)

Tartrazine (TTZ; E102)

Also known as Cl Food Yellow 4 and FD & C Yellow 5, provides a lemon-yellow color; it is soluble in water and poorly soluble in ethanol (Silva *et al.*, 2022). TTZ synthesis can be accomplished by condensation of phenylhydrazine-*p*-sulfonic acid with oxaloacetic ester; the product is combined with diazotized sulfanilic acid, giving rise to an ester which is then hydrolyzed with NaOH. An alternative method is the condensation of 2 mol of phenylhydrazine-*p*-sulfonic acid with 1 mol of dihydroxytartaric acid.

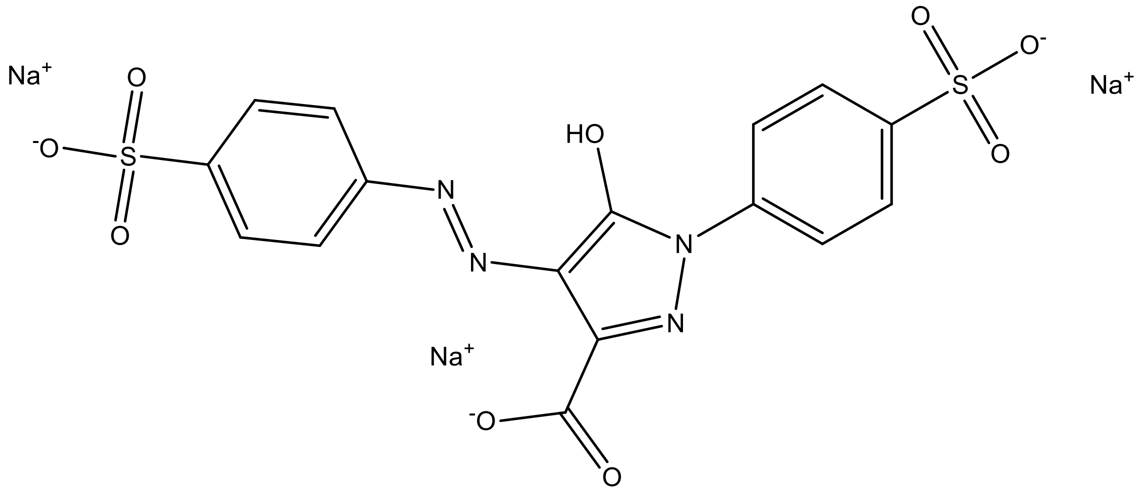

Figure 2: Chemical Structure of Tartrazine (TTZ; E102)

TTZ is added to ices, candies, jellies, jams, potato chips, cakes, ice cream, sauces, cereals, etc. (Kobun, Siddiquee, and Shaarani 2017). TTZ is occasionally used as a substitute for saffron. Among all azo food dyes, TTZ is suspected to trigger the most serious allergic and intolerance reactions, as well as hyperactivity, and it has been reported that TTZ preparations may contain residues of aromatic amine carcinogens (Rovina, Siddiquee, and Shaarani 2017)(Silva et al. 2022). Children are the most vulnerable sector of the population, particularly since they are the main consumers of brightly colored processed foods (Atlı Şekeroğlu et al. 2017). Asif Ahmed et al. evaluated the intake of fruit juice and drinks, ice cream, and cakes by children aged 6– 17 years; TTZ was present in 42.3% of the products (Asif Ahmed et al. 2021).

Sudan I (also Known as CI Solvent Yellow 14 or Solvent Orange R)

Sudan I is an industrial dye used to color oils, waxes, and polishes. But sometimes it is also added to foodstuffs and cosmetics for the color enhancement. Sudan I is classified as a carcinogenic and mutagenic compound by the International Agency for Research on Cancer. In the year of 2003, a French lab detected for the first time Sudan I in chilli products imported from India. After this, in 2005, the United Kingdom recalled more than 470 food products contaminated with Sudan I (Patra et al. 2017). A large Sudan I adulteration incident spread worldwide in the same year. This created a tremendous panic and concern over food safety. This led to the need for the development of a rapid Sudan I sensing system. HPLC–mass spectrometry (HPLC–MS) has been widely used for direct determination of Sudan dyes. But the application of this method becomes somewhat limited as it is a time-consuming and expensive technique. Further, some other techniques have also been applied for the detection of Sudan dyes. Wang et al. reported nanocolloidal gold-based immuno-dip strip assay for rapid detection this dye in chilli powder and tomato sauce (Wang et al. 2013). In this process, a semiquantitative dip strip assay was prepared by using nanocolloidal gold-labeled monoclonal antibody (8A10) for the rapid detection of Sudan red I in food samples. The protein–Sudan red I conjugate was coated onto a nitrocellulose membrane strip in a defined test line. The time required to perform this test was about 10 minutes and the method had a visual LOD of 10.0 ng/g in tomato sauce and chilli powder samples.

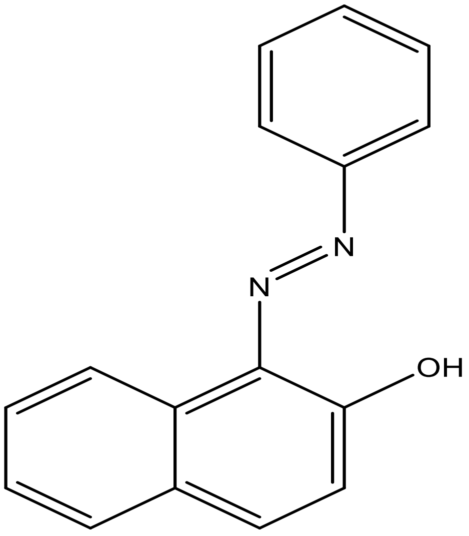

Figure 3: Chemical Structure of Sudan I (1-(Phenyldiazenyl) Naphthalen-2-ol)

Sudan II (1-(2,4-dimethylphenylazo)-2-naphthol)
is an orange-red, fat-soluble azo dye with the molecular formula **$C_{18}H_{16}N_2O$** and a molar mass of **276.34 g/mol**. Its high lipophilicity allows for strong solubility in fats and oils but poor solubility in water. Originally used in industrial processes such as coloring oils, waxes, and plastics, Sudan II has been banned in food products due to carcinogenic potential. Nevertheless, its illicit use in spices (e.g., chili powder, palm oil) continues in some developing regions (Yamjala *et al.*, 2019). Sudan II is metabolized into aromatic amines, which are associated with genotoxicity and potential carcinogenicity. Animal studies have shown liver toxicity, oxidative stress, and DNA damage upon chronic exposure.

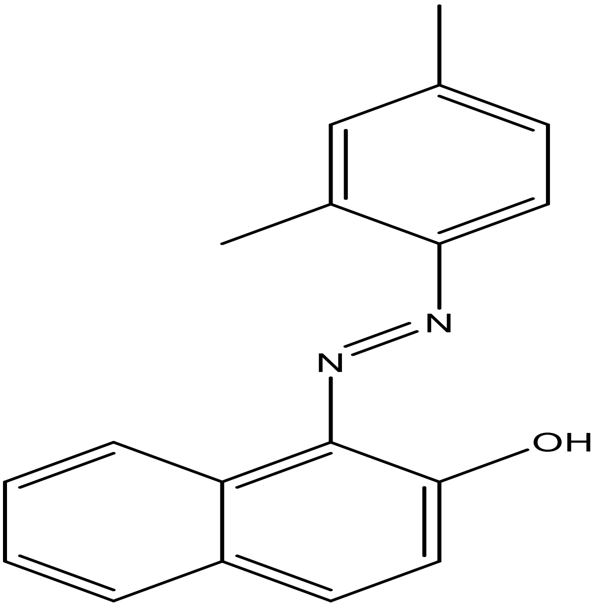

Figure 4: Chemical Structure of Sudan II (*1-(2,4-dimethylphenylazo)-2-naphthol*)

Sudan III (1-(4-(phenyldiazenyl)phenylazo)-2-naphthol)
Is a reddish-orange azo dye with the molecular formula $C_{22}H_{16}N_4O$ and a molar mass of 352.39 g/mol. Its structure includes two azo groups and a naphthol ring, enhancing color stability. It is used in staining biological specimens, plastics, and hydrocarbon fuels. Illicit use in food products, such as processed meats and dairy fats, has been reported, despite its toxicity and regulatory ban. Sudan III's toxicity stems from DNA adduct formation following metabolic conversion. In vitro studies have shown cytotoxic effects in human liver cells, with evidence of oxidative stress and apoptosis. It has also been linked to tumor formation in rodent models (Mohamed et al. 2016). Sudan III is listed among banned dyes in food by the WHO, EFSA, and FDA. The Codex Alimentarius maintains zero tolerance levels, and violators are subject to food seizures and fines.

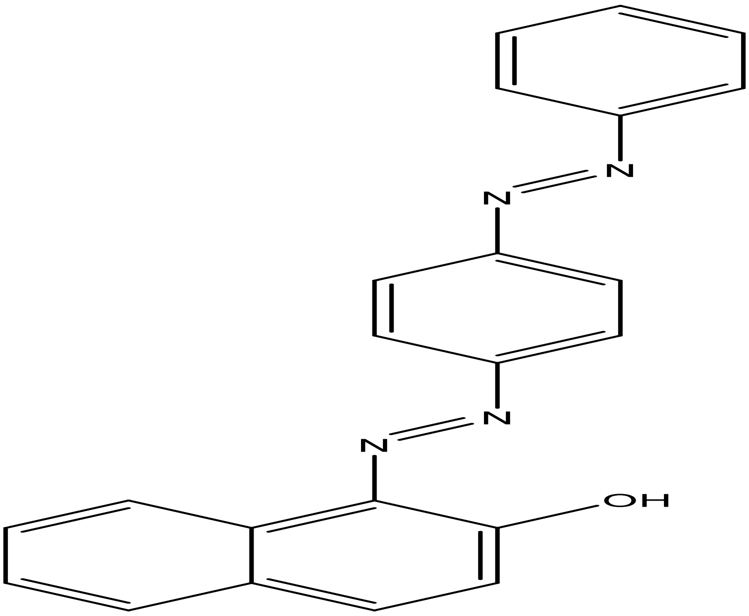

Figure 5: Chemical Structure of Sudan III (1-(4-(phenyldiazenyl)phenylazo)-2-naphthol)

Sudan IV (1-(2,5-dimethylphenylazo)-2-naphthol)

Is a deep red, oil-soluble dye with a molecular formula of $C_{24}H_{20}N_4O$ and molar mass 380.44 g/mol. It is structurally similar to Sudan III but with additional methyl groups enhancing hydrophobicity. Commonly used in petroleum industry and biological staining, Sudan IV has been illegally used in red palm oil, sauces, and condiments to enhance color. Its presence in such foods poses significant health risks. Sudan IV is among the most potent of the Sudan dyes in terms of toxicity. Long-term exposure has been linked to hepatotoxicity, immune suppression, and potential reproductive toxicity. It also displays high bioaccumulation potential in fatty tissues. Sample preparation via SPE or liquid-liquid extraction is essential due to its strong binding with fats. Sudan IV is strictly banned in food worldwide (Mohamed et al. 2016). Multiple alerts and recalls have been issued by Food and Drug Administration (FDA), European Food Safety Authority (EFSA) and NAFDAC in response to its detection in imported foodstuffs, especially palm oil from Africa and Asia.

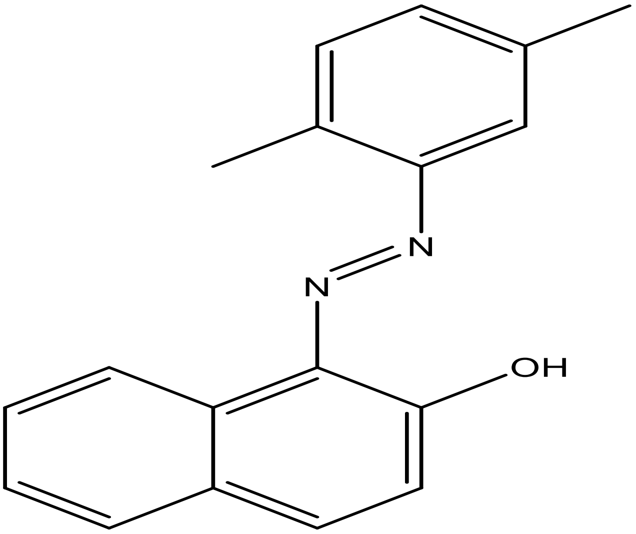

Figure 6: Chemical structure of sudan IV (1-(2,5-dimethylphenylazo)-2-naphthol)

RESULTS AND DISCUSSION

Results

The core objective is to conduct a comparative review of Solid Phase Extraction (SPE) and QuEChERS methods in food and drink sample preparation, to evaluate the recovery rates, detection limits, and reproducibility of the reviewed methods based on recent literature, and to provide recommendations for future research and practical applications in food safety analysis involving azo dyes.

Reviewed Sample Preparation Methods for the Analysis of Azo dyes in Various Food Materials

Sample preparation is critical in the analysis of azo dyes because it removes interfering contaminants and enables accurate quantification. The extraction process used is mostly determined by the food matrix, dye polarity, and analytical goal. The most prevalent extraction procedures include solid-phase extraction (SPE), liquid-liquid extraction (LLE), membrane filtering, and the QuEChERS method. A well-optimized extraction method not only enhances detection accuracy but also contributes to the greenness and cost-effectiveness of the overall analytical process.

Solid-phase extraction (SPE) is among the most widely applied techniques for isolating synthetic colorants from food samples due to its simplicity, reproducibility, and low solvent consumption. It utilizes adsorbent materials such as polyamide, gel permeation chromatography (GPC) columns, and styrene-divinylbenzene polymers to selectively retain target analytes (Yamjala, Nainar, and Ramisetti 2016). Before use, the cartridges must be properly preconditioned with solvents—commonly methanol and acetic acid to activate the sorbent surface (de Andrade et al. 2014). Selecting suitable solvents is essential, as extraction efficiency depends on the solubility and chemical structure of the target dyes.

Mazdeh *et al.*, (2016), developed a simple, selective, and sensitive solid-phase extraction (SPE) and reversed-phase high-performance liquid chromatography (RP-HPLC) method for detecting eight synthetic food dyes— namely Tartrazine (E102), Sunset Yellow (E110), Quinoline Yellow (E104), Indigo Carmine (E132), Carmoisine (E122), Ponceau 4R (E124), Allura Red (E129), and Brilliant Blue FCF (E133)—in different food matrices like beverages, jelly powders, candies, and toffees. The technique utilized NH_2-aminopropyl-modified silica SPE cartridges and a C18 column, applying a gradient elution of 0.1 M ammonium acetate buffer (pH 7) with methanol–acetonitrile (50:50 v/v), the method achieved detection limits (LOD) between 0.105–1.154 mg/L and quantification limits (LOQ) of 0.318–3.497 mg/L at 250 nm, while recoveries ranged from 94.22% to 106.75% across matrices. Calibration curves were highly linear ($R^2 = 0.999–1.000$) over a concentration range of 5–50 mg/L. Analysis of 30 commercial food samples revealed extensive use of synthetic dyes, with concentrations ranging 1.7–493.8 ppm, particularly high levels of Sunset Yellow (up to 493.84 ppm), Quinoline Yellow (403.39 ppm), and Carmoisine (195.11 ppm), while Tartrazine and Indigo Carmine were not detected. The study demonstrated that the NH_2-SPE method minimized matrix interferences and provided high recovery and reproducibility, making it a reliable tool for routine monitoring of synthetic food colorants in food safety assessments.

Young & Tran (2014) developed a sensitive and reliable analytical method combining solid-phase extraction (SPE) with LC–MS to detect ten Sudan dyes—including Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Orange G, and Sudan Black—in chili oleoresin, a highly complex and oil-rich food matrix. In the procedure, 0.1 g of the sample was dissolved in 1 mL hexane and cleaned up using a Sep-Pak Silica cartridge

(6 cc, 500 mg) preconditioned with 3 mL hexane, followed by washing with 2 mL hexane and elution with 2 mL of acetonitrile/dichloromethane (5:95, v/v). The eluate was evaporated and reconstituted in 150 µL methanol, resulting in nearly sevenfold analyte concentration. The chromatographic separation was carried out using a CORTECS C18 solid-core column (2.7 µm, 2.1 × 100 mm) using a gradient of 0.1% formic acid in water and methanol, with a flow rate of 0.4 mL/min and column temperature of 45°C. Detection was achieved via a Xevo TQD triple quadrupole mass spectrometer using positive electrospray ionization (ESI+) mode, yielding a limit of detection (LOD) in the low ppb (ng/g) range and recoveries between 75–100%, except for Sudan Orange G (~50%) due to strong silica retention. The method showed strong linearity, reproducibility, and robustness over more than 200 injections, with consistent column backpressure (~3100 psi), making it compatible with both HPLC and UPLC systems. This validated approach offers a sensitive, reproducible, and matrix-compatible technique for the trace detection of illegal Sudan dyes in complex food products such as chili oleoresin.

Similarly, Genualdi *et al.,* (2016) employed SPE for the analysis of Sudan dyes in red palm oil and chili spices. By applying LC-Alumina-B SPE cartridges, the method achieved recovery rates ranging from 61–119%, with limits of detection (LOD) between 0.5–0.7 mg/kg and limit of quantification (LOQ) of 1 mg/kg. The use of an internal standard (d_6-Sudan III) enhanced matrix effect correction, leading to improved quantification accuracy during LC-MS/MS analysis. Overall, SPE has been shown to offer high selectivity, shorter analysis time, and compatibility with a wide range of food matrices.

Karatepe *et al.*, (2016), developed and optimized a new solid-phase extraction (SPE) technique using sea sponge (*Demospongiae*) as a natural, and eco-friendly adsorbent for the pre-concentration and determination of two azo dyes—Ponceau 4R and Sudan Orange G—in food samples. The method employed a 10 cm × 1 cm glass column packed with 500 mg of sea sponge, which was pretreated with methanol, nitric acid, sodium hydroxide, acetone, and water. Optimal analytical parameters were achieved at 0.02 M HCl concentration, pH 4.5 acetate buffer eluent (10 mL), and a flow rate of 3 mL/min, yielding quantitative recoveries of 95–100% for Ponceau 4R and 91–99% for Sudan Orange G. Calibration curves were linear with correlation coefficients ($R^2 = 0.999$) across ranges of 8.5×10^{-7}–8.5×10^{-5} mol/L for Ponceau 4R and 2.34×10^{-5}–2.34×10^{-3} mol/L for Sudan Orange G. The method also achieved very low detection limits (0.002 µg/L for Ponceau 4R and 1.9×10^{-4} µg/L for Sudan Orange G) and demonstrated high reproducibility (RSD < 3%). Application to real samples such as powdered drinks, candies, tomato paste, and chili pepper showed Ponceau 4R levels ranging from 7–309 µg/g and Sudan Orange G levels up to 155µg/g, with some exceeding regulatory limits. The study highlighted sea sponge as a low-cost, sustainable, and effectient biosorbent, offering an accurate, rapid, and environmentally friendly alternative for azo dye analysis in food safety monitoring (Karatepe, Akalin, and Soylak 2016).

Tran-Lam *et al.,* (2020), developed and validated a highly sensitive method using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) to detect Auramine O (AO), a banned diphenylmethane dye, in 211 food and spice samples collected from Hanoi, Vietnam. The extraction utilized solid-phase extraction (SPE) with methanol, deionized water, and 50 mM ammonium acetate–acetic acid (98:2, v/v) followed by elution using methanol containing 2% NH_4OH. Chromatographic separation was achieved on a CORTECS T3 column (2.1 × 100 mm, 1.6 µm) with a mobile phase of 100 mM ammonium formate (pH 2.9) and acetonitrile at a flow rate of 0.3 mL/min. Detection was done in positive electrospray ionization (ESI) mode using parallel reaction monitoring (PRM). The method showed excellent linearity, precision, and repeatability, with limits of detection (LOD) and quantification (LOQ) of 0.1 µg/kg and 0.5 µg/kg, and recoveries ranging from 80.1–99.4% across food matrices such as sour bamboo shoots, chicken, and curry powder. Relative standard deviations (RSD) ranged from 2.1–6.1%, confirming strong method reliability. Analysis revealed AO presence in 57 out of 211 samples (27%), with concentrations between 3.2–13,024 µg/kg. The highest average contamination was foundin chicken (2,788 µg/kg) and sour bamboo shoots (2,006 µg/kg). The study concluded that AO contamination poses a significant public health risk, highlighting the efficiency of UPLC–MS/MS as a reliable, sensitive, and rapid analytical tool for the monitoring of banned synthetic dyes in food matrices

Ma, Zhang, and Wu (2025) developed a highly sensitive and traceable UPLC-DAD method for the simultaneous determination of 24 water-soluble synthetic colorants in premade cocktails. The optimized chromatographic system used a BEH C18 column (50 × 2.1 mm, 1.7 µm) with a mobile phase of 100 mmol/L ammonium acetate (pH 6.25) and a methanol/acetonitrile mixture (2:8, v/v) under a linear gradient completed within 16 minutes. The method achieved excellent linearity ($R^2 > 0.998$) over the range 0.005–10 µg/mL, with limits of detection (LOD) between 0.66 and 27.78 µg/L and recoveries ranging from 87.8% to 104.5%. Precision was reliable, with relative standard deviation (RSDs) ranging from 0.1–4.9%. Compared to previous HPLC-DAD and LC-MS/MS methods reported between (2014–2024), this approach provided superior sensitivity, a larger number of analytes, and shorter analysis time. Analysis of 100 commercial premade cocktails revealed the presence of seven permitted colorants—such as Allura Red AC, Tartrazine, Sunset Yellow, Brilliant Blue, and Amaranth—within legal concentration ranges (0.04–10.77 µg/mL). The study's findings advance rapid multi-analyte determination, reinforce compliance with GB 2760–2024 and GB 5009.35–2023, and highlight the importance of colorant monitoring in ensuring beverage safety and label accuracy.

However, there have been recent reports on the use of QuEChERS in the analysis of food samples.

QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method provides a greener alternative. It employs acetonitrile-based extraction and salt partitioning, which minimizes solvent use and reduces matrix interference (Rovina *et al.*, 2017).

Perestrelo *et al.*, (2019) provided a comprehensive review of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique as a modern sample-preparation approach widely used in food analysis. The review emphasized QuEChERS flexibility, simplicity, and broad applicability across diverse food matrices. The method involves two main steps: an initial liquid–liquid extraction followed by a dispersive solid-phase extraction (d-SPE) clean-up, which effectively removes matrix interferences and yielding reliable analytical performance across studies in terms of precision, selectivity, and sensitivity. In comparison to traditional extraction procedures like solid-phase extraction (SPE), QuEChERS was reported to be faster, simpler, less labor-intensive, and more environmentally compatible, aligning well with green analytical chemistry principles by reducing organic solvent use and improving automation potential. Furthermore, the authors noted that the method continues to evolve through modifications in sorbents, salts, and operational formats, allowing enhanced applicability in routine food monitoring laboratories, particularly for contaminants such as pesticides, veterinary drugs, colorants, and other chemical hazards 2025/11/6.

Adjei *et al.,* (2020), developed and validated a modified QuEChERS extraction method coupled with UHPLC-UV detection for the simultaneous determination of four Sudan dyes (Sudan I–IV) in chili-based food products in Ghana, demonstrating the method's suitability for routine dye residue monitoring. The procedure involved extracting 2 g of powdered chili with 6 mL acetonitrile/acetone (1:5 v/v) and 2 g QuEChERS salts, followed by vigorous shaking and centrifugation prior to filtration. The optimized chromatographic analysis employed a Shimadzu Prominence UFLC system and UV detection, achieving LOD values of 0.02–0.04 mg/kg and LOQs of 0.05–0.13 mg/kg, while ensuring excellent accuracy with recovery rates ranging from 85.3% to 121.2%. Precision was confirmed with RSDs values <10%, indicating strong reproducibility. Calibration curves showed good linearity using matrix-matched standards, and both fortifed and commercial samples (n = 20) were tested in triplicate to validate real-sample applicability. The study emphasized the efficiency, low solvent consumption, minimal sample preparation, and fast extraction, reinforcing QuEChERS as a cost-effective and green analytical approach for detecting banned azo dyes in food products.

Santana-Mayor et al., (2019), developed a fast and highly sensitive method combining a modified QuEChERS extraction with UPLC–MS/MS (ACQUITY UPLC H-Class with Xevo TQD) to simultaneously detect 11 Sudan and azo dyes in chili, curry, and turmeric powder samples. The procedure involved mixing 2 g of powdered sample with 8 mL water, followed by extraction with 10 mL acetonitrile and QuEChERS CEN salts (4 g $MgSO_4$, 1 g NaCl, 1.5 g sodium citrate), shaking for 1 min, and centrifuging at 4000 rpm for 5 minutes, before analyzing the supernatant. Chromatographic separation was achieved in 12 minutes using an ACQUITY BEH C18 column (2.1 × 100 mm, 1.7 µm) under a mixed solvent gradient (water, methanol, acetonitrile + 0.1% formic acid). The method achieved excellent sensitivity, with reporting limits of 10–50 µg/kg, matrix-matched calibration linearity $r^2 > 0.99$, and recoveries ranging from 60% to 95%, while internal standards were recommended for complex matrices due to observed ion suppression/enhancement effects. Among 14 spice samples analyzed, six tested positive, with concentrations up to 276µg/kg for Sudan orange G and Rhodamine B exceeding the 10µg/kg EU action limit in multiple samples, demonstrating the method's effectiveness for regulatory surveillance. This fast, cost-efficient workflow highlights QuEChERS-UPLC-MS/MS as a robust and high-throughput approach for screening illegal azo dyes in spices.

A validated LC-MS/MS method has been developed by Huyen et al. for the simultaneous determination of three illegal azo dyes—chrysoidine G, para red, and pararosaniline—in grilled meat products, employing a QuEChERS-based extraction to manage the lipid- and protein-rich matrix. The method demonstrated strong linearity across concentration ranges of 30–1,000 ng/mL, 5–1,000 ng/mL, and 0.5–1,000 ng/mL for chrysoidine G, para red, and pararosaniline, respectively, with correlation coefficients (R^2) exceeding 0.998. Sensitivity was confirmed by LODs of 10, 1, and 0.2 ng/mL and LOQs of 30, 5, and 0.5 ng/mL for the same dyes, while method reliability was supported by recoveries of 90–98% and repeatability (RSD) between 5.3% and 10.7%. Application to 49 grilled meat samples collected across Hanoi revealed the presence of chrysoidine G in 19 samples, where as para red and pararosaniline were undetected, with dye concentrations reaching as high as several thousand µg/kg in some cases. Notably, certain samples only tested positive when more intensely colored surface portions were selectively analyzed, suggesting uneven dye distribution and highlighting the importance of targeted sampling strategies in fatty meat matrices. This work underscores the effectiveness of LC-MS/MS combined with QuEChERS for detecting trace-level illicit dyes in complex foods and emphasizes the ongoing food-safety risk posed by unauthorized colorant usage (Huyen et al. 2020).

Comparative Performance and Green-Chemistry Considerations

Recent advances in analytical chemistry has led to the development of sensitive and environmentally friendly methods for detecting synthetic azo dyes and related colorants in complex food samples. Mazdeh *et al.,* (2016) optimized an NH_2-SPE coupled RP-HPLC procedure for eight dyes—Tartrazine, Sunset Yellow, Carmoisine, Ponceau 4R, and others—achieving LODs of 0.105–1.154 mg/L, LOQs 0.318–3.497 mg/L, recoveries between 94.22–106.75%, and $R^2 = 0.999–1.000$ in beverages and confectioneries. Young and Tran (2014) improved a silica SPE–LC-MS method for ten Sudan dyes in chili oleoresin with recoveries of 75–100%, low ppb detection limits, strong linearity and minimal solvent use <5 mL, marking early efforts toward miniaturized, low-waste extraction. In a greener development, Karatepe *et al.*, (2016) replaced synthetic sorbents with sea-sponge biosorbent SPE, attaining LOD 0.002 µg/L, LOQ 1.9×10^{-4} µg/L, recoveries 91–100%, and $R^2 = 0.999$, thus demonstrating an environmentally benign alternative for Ponceau 4R and Sudan Orange G analysis. Tran-Lam *et al.*, (2020) enhanced sensitivity through UPLC–MS/MS for the banned dye Auramine O, achieving LOD 0.1 µg/kg, LOQ 0.5 µg/kg, recoveries 80.1–99.4%, and RSD 2.1–7.5% in diverse Vietnamese food samples, highlighting significant contamination levels (up to 13,024 µg/kg). Meanwhile, Adjei *et al.*, (2020) validated a modified QuEChERS with UPLC-UV method for Sudan I-IV dyes in chili-based food products, LOD 0.02 - 0.04 mg/kg, LOQ 0.05 - 0.13 mg/kg, recoveries 85.3–121.1%, confirming the practicality of low-cost, reagent-efficient monitoring. Collectively, these studies demonstrate a progressive shift from solvent-intensive SPE toward greener analytical approaches, emphasizing natural adsorbents, reduced solvent use, and simpler instrumentation meeting the goals of green analytical chemistry by maximizing precision and sensitivity while minimizing environmental impact and resource consumption .

Although both SPE and QuEChERS provide reliable results, QuEChERS methods consistently offers shorter analysis times, reduced organic solvent consumption, and lower waste output, aligning well with principles of green analytical chemistry. SPE, however, offers superior selectivity and ultra-low quantification potential, particularly in complex matrices. Nevertheless, QuEChERS is more practical for routine regulatory monitoring, especially in laboratories with limited resources where cost, speed, and sustainability are key factors.

Table 1: Comparison Between SPE and QuEChERS Techniques

Criterion	SPE Techniques	QuEChERS Techniques
Solvent Consumption	Higher	Lower
Preparation Time	Longer, multi-step	Rapid, simplified
Typical Recoveries	75–106.75%	60–121.2%
Sensitivity (LOD)	Down to 0.002 µg/L	Down to 0.02 mg/kg
Required Expertise	Higher	Moderate
Environmental Impact	Higher waste	Greener workflow
Suitability	Confirmatory analysis	Routine food monitoring

These findings justify ongoing scientific interest in miniaturized, solvent-efficient, and biosorbent supported extraction systems, and provide a foundation for developing improved green protocols for food dye surveillance.

The distinct features of the methods are summarized in Table 1. Evaluation of the data showed that, QuEChERS has a short extraction time, high extraction efficiency, high pre-concentration factor, lower LODs and lower solvent consumption in comparison with SPE Method. Moreover, a high sensitivity, high efficiency, simplicity, rapidity, moderate cost, and less consumption of organic solvent indicate that the extraction based on the QuEChERS can be a promising approach in the field of dyes analysis from solid complicated matrices.

Table 1: The Analytical Performance of SPE and QuEChERS Method for Azo Dyes Analysis in Foods and Soft Drinks

Food Matrix	Target Dyes	Sample Prep Method	LOD	LOQ	Recovery (%)	RSD (%)	Extraction Time	Centrifugation	Analytical Instrument	Reference
Drinks, jelly powder, candies, toffees	Tartrazine, Sunset yellow, Indigo carmine, Carmoisine, Ponceau 4R, Allura red and Brilliant blue	SPE	0.105–1.154 mg/L	0.318–3.497 mg/L	94.22–106.75	NR	~15 min	5000 rpm	RP-HPLC	Mazdeh et al. (2016)
Chili oleoresin, chili oil	10 Sudan dyes	SPE	Low ppb range	NR	75–100	NR	NR	NR	HPLC-MS	Young & Tran (2014)
Red palm oil, chili spices	Sudan I-IV dyes	SPE	0.5–0.7 mg/kg	1 mg/kg	61–119	NR	~5 min	1800 rcf	LC-MS/MS	Genuald et al. (2016)
Candy, tomato paste, drink powders, chili pepper	Ponceau 4R, Sudan Orange G	SPE (Sea sponge adsorbent)	0.002 µg/L	NR	91–100	<3	NR	NR	UV-Vis	Karatepe et al. (2016)
Beverages, sauces, confectionery	Chrysoidine G, Para Red, and Pararosaniline	QuEChERS	0.2 - 10	0.5 -30	90 - 98	5.3 - 10.7	5 min	6000 rpm	LC - MS/MS	Huyen et al. (2020)
Spices, fermented vegetables, meats	Auramine O	SPE	0.1 µg/kg	0.5 µg/kg	80.1–99.4	2.1–6.1	~10 min	9000 rpm	UPLC-MS/MS	Tran-Lam et al. (2020)
Premade cocktails	7 permitted dyes	SPE	0.66–27.78 mg/L	NR	87.8–104.5	0.1–4.9	~5 min	8000 rpm	UPLC-DAD	Ma et al. (2025)
Chili pepper powder	Sudan I–IV	QuEChERS	0.02–0.04 mg/kg	0.05–0.13 mg/kg	85.3–121.2	<10	~5 min	4000 rpm	UHPLC-UV	Adjei et al. (2020)
Curry, chili, turmeric powders	Sudan I-IV, Sudan red G, Sudan red 7B, Sudan black B, Dimethyl yellow and Rhodamine B	QuEChERS	10–50 µg/kg	NR	60–95	NR	~5 min	4000 rpm	UPLC-MS/MS	Santana et al. (2019)

NR = Not reported; SPE = Solid-phase extraction; LOD = Limit of detection; LOQ = Limit of quantification; RSD = Relative standard deviation; QuEChERS = Quick, Easy, Cheap, Effective, Rugged, and Safe

Discussions

The determination of azo dyes in food and beverages has received increasing attention due to their potential toxicological effects, including carcinogenicity, genotoxicity, and allergic responses. As demonstrated in recent literature, the reliability of analytical outcomes is strongly influenced by the extraction technique applied prior to instrumental analysis. Solid-phase extraction (SPE) is frequently selected for its high selectivity and strong ability to eliminate interferences, particularly in highly complex food matrices (Okeke et al. 2022). Nevertheless, SPE remains limited by its operational steps, as it often requires cartridge conditioning, multiple wash cycles, and relatively high solvent consumption. In addition, SPE performance can be hindered by matrix effects, especially when processing lipid-rich or protein-dense foods where fat accumulation on sorbent surfaces can reduce analyte recovery and compromise extraction efficiency.

In comparison, the QuEChERS method has gained prominence as a fast, affordable, and environmentally friendly alternative. It reduces solvent usage, simplifies sample handling, and support high-throughput analysis, making it particularly suitable for regulatory laboratories and routine monitoring. Reports also indicate strong recoveries for dyes like Sudan I–IV, Rhodamine B, and Tartrazine when QuEChERS is coupled with chromatographic detection.

However, a key limitation lies in its performance in greasy or complex matrices, where residual fats and pigments may still co-extract, necessitating additional clean-up steps (Perestrelo *et al.*, 2019b). Although modifications including enhanced sorbent formulations and novel extraction salts have improved selectivity, further validation is needed across diverse food matrices, especially those that are processed or high in fat. However, the QuEChERS technique has remained a vital tool for processing food and environmental samples, with only slight improvements involving new extraction solvents and sorbent materials (Shinkafi *et al.*, 2024).

Looking forward, future trends in azo-dye analysis are expected to emphasize green and sustainable sample-preparation methods, such as bio-sorbent-assisted extraction, micro-SPE, solvent-free techniques, and automated QuEChERS system. Emerging approaches, including magnetic sorbents, ionic-liquid-based extraction, and miniaturized dispersive techniques, show promise for improving matrix clean-up while reducing environmental impact. Furthermore, the use of advanced tools such as chemometrics, machine learning for data interpretation, and high-resolution mass spectrometry could further enhance sensitivity and efficiency. Overall, continuous optimization and method harmonization are essential to support robust dye monitoring programs and uphold food-safety standards, particularly in rapidly expanding food markets where dye adulteration remains a concern.

CONCLUSION

This review has highlighted recent advancements in sample preparation techniques for the determination of azo dyes in food and beverage products. Solid-phase extraction (SPE) remains valued for its high selectivity and effective clean-up in complex samples, its longer processing time and higher solvent demand limit routine use. On the other hand, the QuEChERS method offers faster extraction, lower solvent consumption, and reduced operational cost, making it more suitable for regular screening and high-throughput food-safety monitoring. Advancing extraction techniques particularly those emphasizing green chemistry principles will be essential to enhance analytical performance, promote sustainability, and ensure effective monitoring of synthetic dye residues for regulatory and public health protection.

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