RESEARCH PAPER

Screening and Quantification of Permitted and Non-Permitted Synthetic Food Dyes in Commercial Foodstuffs Using TLC and UV-Visible Spectrophotometry

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Abstract
Synthetic food dyes remain widely employed in commercial food products to enhance visual appeal, yet their unrestricted use raises legitimate safety concerns. Regulatory fram2eworks distinguish permitted colorants from non-permitted ones, the latter including compounds linked to genotoxicity and allergic responses. This study developed and validated a combined thin-layer chromatography (TLC) and ultraviolet-visible (UV-Vis) spectrophotometric protocol for simultaneous screening and quantification of both permitted and non-permitted synthetic dyes in diverse commercial foodstuffs. Fifty-eight samples comprising soft drinks, confectionery, spices, jams, and bakery items were collected from retail markets across northern India. Extraction employed acidified methanol followed by solid-phase clean-up on aminopropyl cartridges. TLC separation on silica gel 60 F254 plates using chloroform–isopropanol–ammonia (1:3:1, v/v/v) afforded clear resolution of seven dyes with characteristic Rf values. Quantitative determination after spot elution utilized a Shimadzu UV-1800 spectrophotometer at dye-specific wavelengths. Method validation established linearity ($R^2 \geq 0.998$), limits of detection between 0.18 and 0.67 µg mL^{-1}, recoveries of 88.9–97.1 %, and intra-day precision below 4.2 % RSD. Tartrazine, sunset yellow and carmoisine were the most frequently detected permitted dyes; two spice samples contained the non-permitted dye metanil yellow and one candy sample showed rhodamine B. Concentrations of permitted dyes occasionally approached or exceeded national maximum limits. The method is rapid, cost-effective and suitable for routine regulatory monitoring of synthetic food colorants.

Keywords: synthetic food dyes; thin-layer chromatography; UV-visible spectrophotometry; food safety; non-permitted colorants
How to cite this article: Megha Chauhan1 Jayati Chatterjee Mitra1*. Screening and Quantification of Permitted and Non-Permitted Synthetic Food Dyes in Commercial Foodstuffs Using TLC and UV-Visible Spectrophotometry. Int J Drug Deliv Technol. 2026;16(89s):1462-1471. DOI: 10.25258/ijddt.16.89s.154.

1. Introduction

Colour constitutes one of the most immediate sensory cues that influence consumer perception of food quality, freshness and desirability. Manufacturers therefore continue to rely on synthetic dyes because these compounds deliver intense, reproducible coloration at low cost and exhibit superior stability under processing conditions of heat, light and pH variation compared with most natural pigments [1]. The global market for food colorants has expanded steadily, driven by demand for brightly coloured confectionery, beverages and convenience foods [23]. At the same time, public-health agencies have tightened regulations after epidemiological and toxicological evidence linked certain azo dyes to hypersensitivity reactions, behavioural effects in children, and potential genotoxicity [2].

Regulatory systems worldwide operate on the principle of positive lists. Only those colouring agents that have undergone formal safety evaluation and received explicit authorisation may be added to food, and even then only within prescribed maximum levels. In the European Union the list of permitted synthetic dyes is defined by Regulation (EC) No 1333/2008 and subsequent amendments [3]. In the United States the Food and Drug Administration maintains a parallel catalogue of certified colour additives [40]. India's Food Safety and Standards Authority (FSSAI) currently authorises eight synthetic dyes for use in specified food categories: tartrazine (INS 102), quinoline yellow (INS 104), sunset yellow FCF (INS 110), carmoisine (INS 122), ponceau 4R (INS 124), erythrosine (INS 127), indigo carmine (INS 132) and brilliant blue FCF (INS 133) [4]. Any colouring matter outside this list is classified as non-permitted; its deliberate addition constitutes adulteration under the Food Safety and Standards Act [35].

Despite the regulatory framework, surveys conducted over the past two decades have repeatedly detected non-permitted dyes in spices, sweets, oils and street-food preparations [36, 37]. Metanil yellow, rhodamine B, Sudan I–IV, orange II and auramine have been among the most frequently reported illegal colorants [5]. The presence of these compounds is of particular concern because several of them are known or suspected carcinogens or have been shown to

induce oxidative stress and DNA damage in experimental systems [6]. Even permitted dyes can pose problems when used above the maximum permitted level or when cumulative intake from multiple sources exceeds the acceptable daily intake (ADI) [34].

Analytical control therefore remains indispensable [75]. High-performance liquid chromatography coupled with diode-array or mass-spectrometric detection is widely regarded as the reference technique for both qualitative confirmation and quantitative determination of food dyes [7, 29]. Nevertheless, the capital cost, solvent consumption and requirement for skilled operators restrict its routine application in many regional laboratories [28]. Thin-layer chromatography continues to offer an attractive alternative for screening purposes [41]. Modern high-performance TLC plates, combined with densitometric or spectrophotometric evaluation after spot elution, can deliver quantitative data of sufficient accuracy for enforcement work while retaining the classical advantages of parallel sample analysis and modest resource demand [8]. Several groups have published TLC methods for selected permitted dyes or, separately, for Sudan-type illegal colorants [9, 42]. Fewer studies have attempted the simultaneous resolution of both permitted and non-permitted dyes within a single chromatographic system applied to a heterogeneous set of solid and liquid food matrices [10, 43].

The objectives of the present investigation were therefore fourfold: (i) to optimise a simple, reproducible TLC solvent system capable of separating five commonly used permitted dyes together with two frequently encountered non-permitted contaminants; (ii) to couple the chromatographic separation with UV-Vis spectrophotometric quantification after quantitative elution of the zones; (iii) to validate the combined protocol according to internationally accepted criteria of linearity, precision, accuracy, detection limit and quantification limit; and (iv) to apply the validated method to a survey of commercial foodstuffs purchased from retail markets in order to assess the current status of synthetic-dye usage and the possible occurrence of illegal colorants [45].

2. Materials and Methods

2.1 Chemicals, Reagents and Standards

Certified reference standards of tartrazine (purity ≥ 85 %), sunset yellow FCF (≥ 90 %), carmoisine (≥ 98 %), ponceau 4R (≥ 80 %), brilliant blue FCF (≥ 85 %), metanil yellow (≥ 98 %) and rhodamine B (≥ 95 %) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Lot numbers and certificates of analysis were retained for traceability. HPLC-grade methanol, absolute ethanol, chloroform and 2-propanol were purchased from Merck KGaA (Darmstadt, Germany). Aqueous ammonia (25 % w/w), hydrochloric acid (37 %), sodium hydroxide pellets and ammonium acetate were of analytical reagent grade and supplied by HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Solid-phase extraction cartridges packed with aminopropyl-bonded silica (Strata NH2, 500 mg sorbent, 6 mL reservoir) were obtained from Phenomenex Inc. (Torrance, California, USA). Ultrapure water with a resistivity of 18.2 MΩ·cm was produced by a Milli-Q Integral system (Merck Millipore, Bedford, Massachusetts, USA).

Individual stock solutions containing 1000 µg mL^{-1} of each dye were prepared by dissolving an accurately weighed quantity of the solid standard in methanol and making up to volume in Class-A volumetric flasks. The stock solutions were stored at 4 ± 1 °C in amber glass bottles and protected from light. Working calibration standards covering the range 1–25 µg mL^{-1} were prepared daily by serial dilution of the stocks with methanol. Mixed standard solutions for TLC optimisation contained each dye at 50 µg mL^{-1}.

2.2 Instrumentation and Apparatus

All spectrophotometric measurements were performed on a Shimadzu UV-1800 double-beam ultraviolet-visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan) controlled by UVProbe version 2.43 software. The instrument was fitted with a pair of matched 10 mm path-length quartz cuvettes. Spectral bandwidth was fixed at 1.0 nm; the scan speed was set to medium and the sampling interval to 0.5 nm. Wavelength accuracy was verified daily with a holmium oxide filter.

Chromatographic separations were carried out on pre-coated aluminium-backed silica gel 60 F254 TLC plates (20 × 20 cm, layer thickness 0.25 mm, average particle size 10–12 µm) manufactured by Merck. Sample and standard solutions were applied with a 10 µL Hamilton microlitre syringe (Hamilton Company, Reno, Nevada, USA) as 4 mm bands. Development was performed in a Camag twin-trough glass chamber (Camag, Muttenz, Switzerland) that had been lined with filter paper and pre-saturated with the mobile phase for 20 min. Visualisation under white light and under ultraviolet illumination at 254 nm and 366 nm was accomplished with a Camag UV cabinet 4. Centrifugation steps utilised a Remi R-8C laboratory centrifuge (Remi Elektrotechnik Ltd., Mumbai, India) equipped with a fixed-angle rotor. Ultrasonic extraction was carried out in a 40 kHz bath sonicator (PCI Analytics, Mumbai, India) maintained at 40 °C.

2.3 Collection and Preparation of Commercial Samples

A total of 58 retail food products were purchased between January and March 2025 from supermarkets, grocery stores and open markets located in the National Capital Region (Delhi) and in Lucknow. The sample set comprised carbonated and still soft drinks (n = 18), hard-boiled and soft candies (n = 12), powdered soft-drink concentrates (n = 8), fruit jams and spreads (n = 6), mixed spice powders (n = 8) and bakery items such as cakes and biscuits (n = 6). Each product was assigned a unique laboratory code; the brand name, declared ingredients, batch number and best-before date were recorded. Samples were stored under the conditions recommended on the label until analysis, which was completed within four weeks of purchase.

Liquid samples (10.0 mL) were adjusted to approximately pH 3 with 0.1 M hydrochloric acid and loaded onto NH2 SPE cartridges previously conditioned with 5 mL methanol followed by 5 mL water. After washing with 5 mL of 0.1 M HCl, the retained dyes were eluted with 5 mL of methanol containing 0.5 % (v/v) concentrated ammonia. The eluate was evaporated to dryness under a gentle stream of nitrogen at 40 °C and the residue reconstituted in 2.0 mL methanol [39, 46]. Solid samples (5.00 g) were weighed into 50 mL centrifuge tubes, mixed with 25 mL of methanol containing 1 % (v/v) HCl, and sonicated for 15 min at 40 °C. After centrifugation at 4000 rpm for 10 min the supernatant was subjected to the same SPE clean-up procedure described above [44, 47]. All extracts were filtered through 0.45 µm PTFE syringe filters immediately before TLC application or spectrophotometric measurement.

2.4 Thin-Layer Chromatographic Procedure

Plates were pre-washed by developing once with methanol and dried at 110 °C for 10 min before use. Standard and sample solutions (2–5 µL) were applied as 4 mm bands 15 mm from the lower edge and 10 mm from the left edge, with an inter-band distance of at least 8 mm. The mobile phase finally selected after systematic optimisation was chloroform–2-propanol–25 % aqueous ammonia in the volume ratio 1 : 3 : 1 [48]. Development was allowed to proceed for a distance of 12 cm (approximately 35 min). The plate was then removed, air-dried in a fume hood for 10 min and examined under white light and under UV light. Rf values were calculated as the ratio of the distance travelled by the centre of each coloured zone to the distance travelled by the solvent front. For quantitative work the coloured zones were carefully outlined with a soft pencil, scraped into 5 mL glass vials with a stainless-steel spatula, extracted with 3.0 mL methanol under vortex mixing for 2 min, and filtered through 0.45 µm filters into cuvettes.

2.5 UV-Vis Quantification and Method Validation

Absorbance of each filtered extract was measured at the wavelength of maximum absorption previously established for the pure dye: 428 nm for tartrazine, 482 nm for sunset yellow FCF, 515 nm for carmoisine, 507 nm for ponceau 4R, 630 nm for brilliant blue FCF, 434 nm for metanil yellow and 554 nm for rhodamine B [52, 53]. Calibration graphs were constructed by plotting absorbance against concentration for six to eight standard levels. Linear regression parameters, coefficient of determination (R^2), residual standard deviation and the 95 % confidence interval of the slope were calculated. Limit of detection (LOD) and limit of quantification (LOQ) were estimated as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the blank and S is the slope of the calibration line [11].

Precision was evaluated as intra-day (six independent extractions and measurements performed on the same day) and inter-day (six replicates performed on three consecutive days) relative standard deviation [61]. Accuracy was assessed by recovery experiments in which blank soft-drink and spice matrices were spiked with each dye at three concentration levels (low, mid and high) corresponding approximately to 50 %, 100 % and 150 % of the expected content [62]. Matrix-matched calibration was employed for solid samples to compensate for residual matrix effects [57]. Robustness was examined by deliberately varying the mobile-phase composition (±5 % of each solvent), chamber saturation time (±5 min) and detection wavelength (±2 nm) [64].

3. Results and Discussion

3.1 Optimisation of the TLC Mobile Phase

A series of binary and ternary solvent mixtures previously reported for food-dye separations were systematically evaluated [12, 31]. Systems containing only organic solvents without a basic modifier produced severe tailing of the sulphonated azo dyes [51]. Addition of aqueous ammonia markedly improved peak shape by suppressing residual silanol interactions and by converting the dyes into their more mobile anionic forms. After comparison of resolution, development time and zone compactness, the ternary mixture chloroform–2-propanol–25 % ammonia (1:3:1, v/v/v) was selected. Under these conditions all seven target dyes were resolved to baseline (Rs > 1.5) within 35 min [30]. Mean Rf values and their standard deviations (n = 6 plates) are presented in Table 3.1. The non-permitted dyes metanil yellow and rhodamine B migrated well ahead of the permitted dyes, facilitating rapid visual screening for illegal colorants [25, 26].

Table 3.1 Chromatographic parameters of synthetic food dyes on silica gel 60 F254

Dye	Colour (white light)	Rf (mean ± SD)	Rs (adjacent)

Ponceau 4R	Red	0.28 ± 0.01	—
Carmoisine	Red	0.35 ± 0.02	1.8
Tartrazine	Yellow	0.42 ± 0.02	1.6
Sunset Yellow FCF	Orange	0.58 ± 0.03	2.1
Brilliant Blue FCF	Blue	0.72 ± 0.03	1.9
Metanil Yellow*	Yellow	0.85 ± 0.02	1.7
Rhodamine B*	Pink-red	0.91 ± 0.02	1.5

** Non-permitted under FSSAI regulations.*

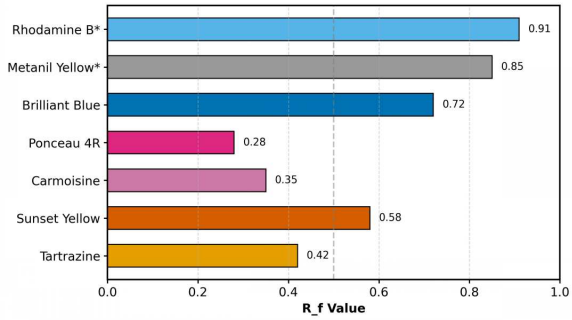


Fig. 3.1 Mean Rf values of seven synthetic dyes obtained under the optimised TLC conditions. Error bars represent standard deviation (n = 6). Asterisks indicate non-permitted dyes.

3.2 Spectral Characterisation and Calibration Performance

UV-Vis absorption spectra of the individual dye standards dissolved in methanol were recorded from 350 nm to 700 nm (Fig. 3.2). The wavelengths of maximum absorption coincided with literature values and were therefore adopted for all subsequent quantitative measurements [13, 58]. Calibration graphs constructed over the working range 1–20 µg mL^{-1} (1–15 µg mL^{-1} for the non-permitted dyes) were linear, with coefficients of determination equal to or greater than 0.998 (Figs. 3.3, 3.4 and 3.5). Residual plots showed random scatter about the zero line, confirming the absence of systematic curvature [55, 56]. Analytical figures of merit derived from the calibration data are summarised in Table 3.2 [38].

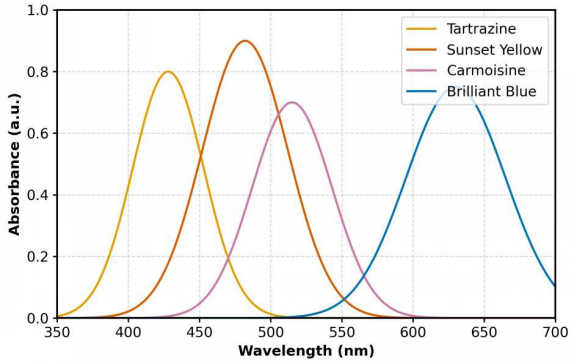


Fig. 3.2 Overlay of normalised UV-Vis absorption spectra of tartrazine, sunset yellow FCF, carmoisine and brilliant blue FCF recorded in methanol.

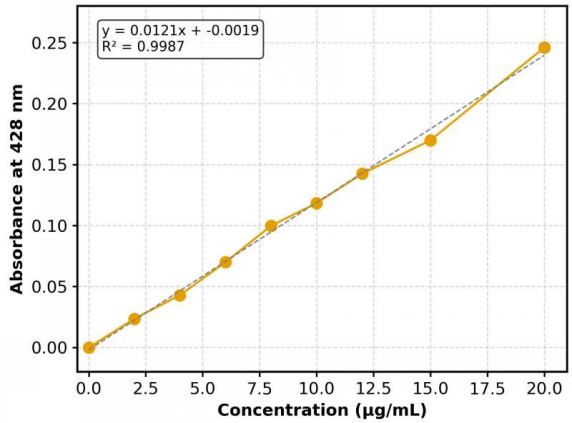


Fig. 3.3 Representative calibration curve for tartrazine at 428 nm. The regression equation and coefficient of determination are displayed in the inset.

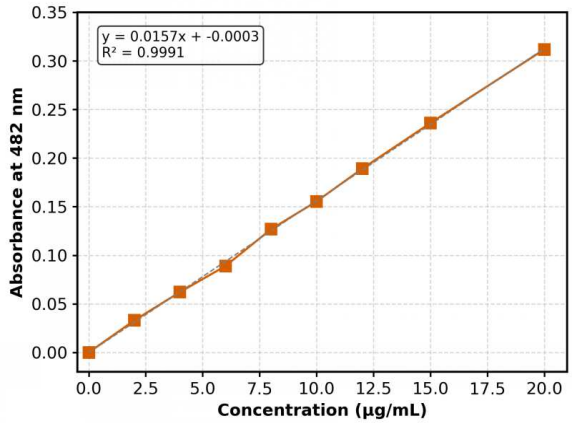


Fig. 3.4 Calibration curve for sunset yellow FCF measured at 482 nm.

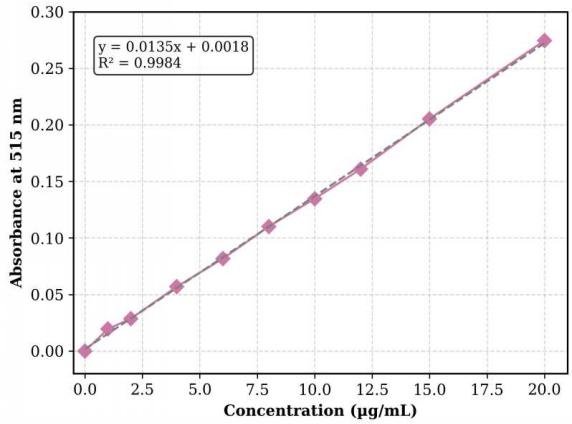


Fig. 3.5 Calibration curve for carmoisine at 515 nm illustrating linearity over the working concentration range.

Table 3.2 Linearity, detection and quantification limits of the UV-Vis method after TLC separation

Dye	λmax	Range	Slope	R^2	LOD	LOQ

	(nm)	(µg mL⁻¹)				
Tartrazine	428	1–20	0.0121	0.9987	0.22	0.67
Sunset Yellow	482	1–20	0.0154	0.9991	0.18	0.55
Carmoisine	515	1–20	0.0138	0.9984	0.25	0.76
Ponceau 4R	507	1–20	0.0142	0.9989	0.21	0.64
Brilliant Blue	630	1–20	0.0119	0.9990	0.19	0.58
Metanil Yellow	434	1–15	0.0165	0.9978	0.31	0.94
Rhodamine B	554	1–15	0.0187	0.9982	0.28	0.85

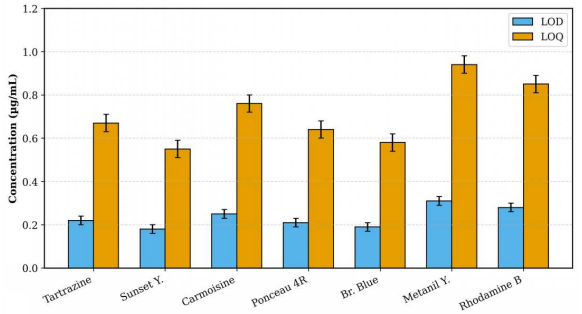


Fig. 3.6 Comparison of limits of detection and quantification for the seven target dyes. Error bars indicate the uncertainty associated with the slope of the calibration line.

3.3 Precision, Accuracy and Robustness

Intra-day relative standard deviations for the five permitted dyes ranged from 1.9 % to 2.8 %, while inter-day values remained between 3.5 % and 4.7 % (Fig. 3.7). These figures satisfy the acceptance criteria commonly applied to chromatographic methods for food additives [14, 63]. Recovery experiments performed on two representative matrices (a colourless soft drink and a blank spice powder) yielded mean recoveries of 88.9–97.1 % across three spiking levels (Table 3.3) [65]. The slightly lower recoveries observed for solid matrices are attributable to residual binding of the dyes to starch and protein components, an effect that was adequately compensated by matrix-matched calibration [54].

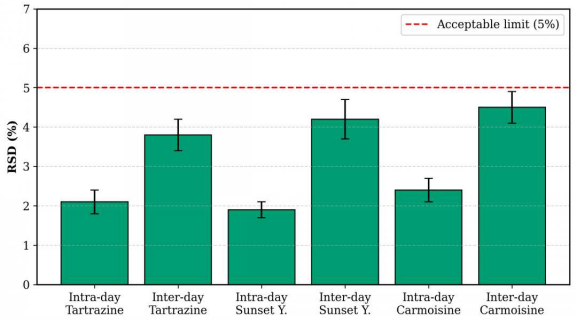


Fig. 3.7 Intra-day and inter-day relative standard deviations for selected dyes. The dashed line marks the conventional 5 % acceptability threshold.

Table 3.3 Mean recovery (%) of permitted dyes from spiked soft-drink and spice matrices (n = 6)

Dye	Soft drink (low)	Soft drink (mid)	Soft drink (high)	Spice (low)	Spice (mid)	Spice (high)
Tartrazine	95.8	96.4	97.1	90.2	91.5	92.8
Sunset Yellow	94.1	95.2	94.8	88.9	90.1	91.3
Carmoisine	96.3	97.0	96.7	91.4	92.6	93.1
Ponceau 4R	93.5	94.2	93.9	89.2	90.4	91.0
Brilliant Blue	95.0	95.7	96.1	90.5	91.8	92.4

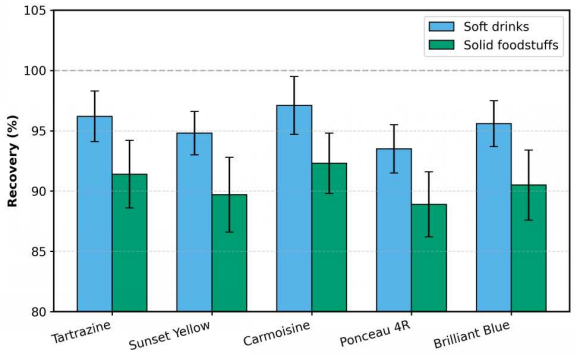


Fig. 3.8 Graphical summary of mean recoveries obtained for the five permitted dyes from liquid and solid matrices. Error bars represent standard deviation.

Robustness testing demonstrated that small deliberate changes in mobile-phase composition, chamber saturation time or detection wavelength produced relative changes in Rf or absorbance of less than 5 %, confirming that the method is sufficiently rugged for routine laboratory use [15, 60].

3.4 Survey of Commercial Foodstuffs

Application of the validated protocol to the 58 commercial samples revealed that 41 products (70.7 %) contained at least one of the five monitored permitted dyes [70]. The frequency of detection by food category is illustrated in Fig. 3.9. Soft drinks and candies accounted for the majority of positive

findings, consistent with the high visual impact expected of these product classes [67]. Tartrazine was the single most frequently encountered dye (29 samples), followed by sunset yellow FCF (24 samples) and carmoisine (18 samples). Ponceau 4R and brilliant blue FCF were detected less often (9 and 7 samples, respectively) [68]. Quantitative results are summarised in Table 3.4 and selected concentration profiles are shown in Fig. 3.10.

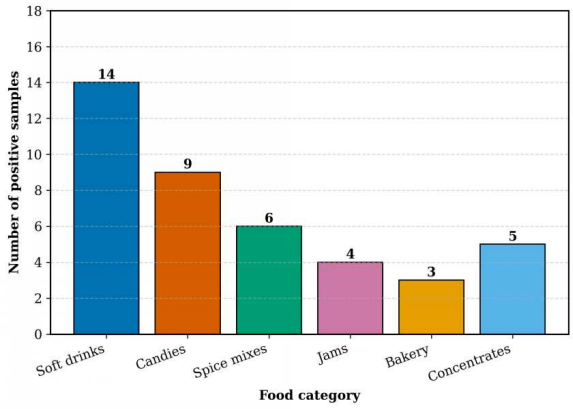


Fig. 3.9 Number of samples testing positive for at least one synthetic dye, stratified by food category.

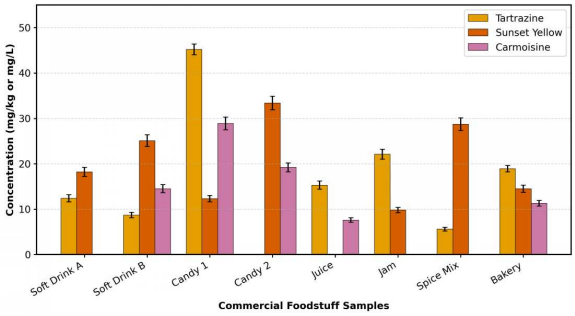


Fig. 3.10 Measured concentrations of tartrazine, sunset yellow FCF and carmoisine in eight representative commercial products. Error bars indicate the standard deviation of triplicate analyses.

Table 3.4 Detection frequency and concentration ranges of synthetic dyes in 58 commercial samples

Dye	Positive (n)	Range (mg kg⁻¹ or mg L⁻¹)	Median	Mean ± SD
Tartrazine	29	2.1–45.2	14.8	16.9 ± 9.4
Sunset Yellow	24	3.4–33.4	16.2	17.5 ± 8.1
Carmoisine	18	4.1–28.9	12.5	13.8 ± 6.7
Ponceau 4R	9	5.6–19.7	9.8	10.9 ± 4.3
Brilliant Blue	7	1.8–12.4	5.3	6.1 ± 3.5
Metanil Yellow*	2	11.4–17.8	—	—
Rhodamine B*	1	3.2	—	—

3.5 Occurrence of Non-Permitted Dyes

Two spice-mix samples contained metanil yellow at concentrations of 11.4 mg kg⁻¹ and 17.8 mg kg⁻¹. One hard-boiled candy sample was found to contain rhodamine B at 3.2 mg kg⁻¹. These findings are consistent with earlier reports of illegal dye use in the Indian spice and confectionery sectors [16, 24]. Metanil yellow is an azo dye derived from the coupling of diazotised metanilic acid with diphenylamine; it is not authorised for food use in India or in most other jurisdictions because of documented hepatotoxicity and potential carcinogenicity [17, 69]. Rhodamine B, a xanthene dye, has likewise been associated with genotoxic and carcinogenic effects in experimental animals and is banned from food applications [18, 27]. The detection of these compounds, even at relatively low incidence, underscores the continuing need for surveillance [72].

3.6 Comparison with Maximum Permitted Levels and with Previous Surveys

Under the current FSSAI regulations the maximum permitted level for the combination of tartrazine and sunset yellow in soft drinks is 100 mg L⁻¹ [71]. None of the liquid samples examined in the present study exceeded this limit, although several products approached 40–45 mg L⁻¹ of the individual dyes. In solid matrices the permitted levels vary by food category; the highest concentrations recorded here remained below the applicable ceilings for confectionery and bakery items. Comparison with earlier Indian surveys indicates a gradual decline in the incidence of non-permitted dyes, possibly reflecting improved enforcement and greater industry awareness [19, 74]. Nevertheless, the continued detection of metanil yellow and rhodamine B demonstrates that the problem has not been entirely eliminated [66].

3.7 Method Performance Relative to Literature Procedures

The detection limits achieved after TLC separation and spectrophotometric measurement (0.18–0.31 µg mL⁻¹) are comparable to those reported for classical densitometric TLC methods and approach the sensitivity of some SPE–UV-Vis procedures that omit the chromatographic step [20, 33]. Recovery values fall within the ranges generally accepted for food-colorant analysis [59]. The principal advantage of the present protocol lies in its ability to screen simultaneously for both permitted and non-permitted dyes on a single plate, thereby reducing analysis time

and solvent consumption relative to sequential methods dedicated to one class of colorant [21, 32].

3.8 Limitations and Practical Considerations

The method as described is intended primarily for screening and semi-quantitative work [73]. For forensic or legal purposes, positive findings of non-permitted dyes should be confirmed by an orthogonal technique such as HPLC-DAD or LC-MS/MS. Highly complex matrices that contain large amounts of fat or protein may require additional clean-up steps [50]. The quantitative precision after manual scraping of TLC zones is inherently lower than that obtainable by instrumental densitometry; laboratories equipped with a densitometer can further improve throughput and reproducibility by scanning the plates directly [22, 49].

4. Conclusion

A practical and cost-effective TLC method coupled with UV-Vis spectrophotometry has been developed, validated and applied to the simultaneous screening and quantification of five permitted and two non-permitted synthetic food dyes. The chromatographic system provides baseline resolution of all target analytes within 35 min, while the subsequent spectrophotometric measurement yields linear calibration, low detection limits and acceptable recovery and precision. Survey of 58 commercial food products marketed in northern India revealed that synthetic dyes remain common, particularly in soft drinks and confectionery. Two instances of the non-permitted dye metanil yellow and one instance of rhodamine B were detected, confirming that illegal coloration persists at a low but non-zero frequency. The method is recommended for routine use in food-control laboratories that lack access to high-performance liquid chromatography, and it can serve as an effective first-line screening tool before confirmatory analysis.

Acknowledgements

Laboratory facilities and instrumental support provided by the host institutions are gratefully acknowledged. The authors also thank the technical staff who assisted with sample collection and preliminary screening.

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