FOOD COMPOSITION AND ADDITIVES

A Simple 2-Directional High-Performance Thin-Layer Chromatographic Method for the Simultaneous Determination of Curcumin, Metanil Yellow, and Sudan Dyes in Turmeric, Chili, and Curry Powders

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A method using simple extraction and 2-directional high-performance thin-layer chromatography (HPTLC) was developed for the simultaneous determination of curcumin, metanil yellow, and sudan dyes in turmeric, chili, and various mixed curry powder formulations. The method offers resolution (Rf) of turmeric pigments, namely, curcumin (0.77), demethoxycurcumin (0.69), bis(demethoxy)curcumin (0.61), and the synthetic dye metanil yellow (0.05) by the first-directional mobile phase, chloroform–methanol (9 + 1, v/v). The resolution (Rf) of sudan I (0.30) and sudan IV (0.23) was achieved by the second-directional mobile phase, toluene–hexane–acetic acid (50 + 50 + 1, v/v/v). Natural pigments of both turmeric and chili showed no interference in the detection and quantification of synthetic colors. The limit of detection and limit of quantification values for curcumin, metanil yellow, sudan I, and sudan IV were 17.39, 42.90, 15.45, and 7.01 and 52.71, 130.0, 46.80, and 21.24 ng/spot, respectively. Analysis of a few market samples showed the presence of metanil yellow (1.5–4.6 mg/g), sudan I (4.8–12.1 mg/g), and sudan IV (0.9–2.0 mg/g) in loose turmeric and chili samples, whereas the curcumin content in turmeric and mixed curry powder samples ranged from 6.5 to 36.4 and from 0.3 to 1.9 mg/g, respectively. The method is relatively simple, offers reasonable sensitivity, and can be used to screen a large number of samples.

Spices have been part of Indian culinary art since ancient times. Both turmeric and red chili are used in everyday household food preparation, are part of nearly all mixed curry powder formulations available in the marketplace, and are known to offer a specific aroma to a particular dish. The Prevention of Food Adulteration Act (PFA) of India has prescribed quality standards for spices and requires that chili, turmeric, and mixed curry powders be free from extraneously added artificial colors (1). However, the addition of colors like sudan dyes in chili powder and metanil yellow in turmeric powder has been reported occasionally (2–6) and may constitute a risk to consumer health. In the area of legal malpractice for economic considerations, turmeric powder has also been found to be admixed with foreign starches and may contain a low level of curcumin, depending on the extent of substitution. Thus, monitoring results for curcumin content may partly reflect the authenticity of turmeric powders. Surprisingly, although the Bureau of Indian Standards suggests a minimum of 3% for the curcumin content in powdered turmeric (7), PFA rules are silent about this criterion, but they do prescribe a maximum starch content of 60% (1).

Various methods to measure curcumin have been described (8–12), but the simultaneous determination of curcumin and metanil yellow in powdered turmeric has not been reported. Similarly, methods to monitor sudan dyes in powdered chili have been described (6, 13, 14), but no method has been developed to determine sudan dyes along with curcumin and metanil yellow in mixed curry powders containing both turmeric and chili powders. In this investigation, a simple method using extraction and 2-directional high-performance thin-layer chromatography (HPTLC) for resolution was developed for the simultaneous determination of curcumin, metanil yellow, and sudan dyes in turmeric and chili and in curry powder formulation. The method is fast and sensitive and has been tested in the screening of spice powder samples from the marketplace.

Experimental

Reagents

Curcumin (technical) was purchased from Aldrich Chemical Co., Milwaukee, WI, and sudan I and sudan IV were from Harleco, Hartman Leddon Co., Philadelphia, PA. Precoated HPTLC aluminium Silica Gel $60F_{254}$ sheets for

Received June 10, 2008. Accepted by SG July 22, 2008.
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nano-TLC (Art. 5548) were purchased from E. Merck, Darmstadt, Germany. Metanil yellow was from Lobachemie, Indoaustranal Co., Mumbai, India. GR grade chloroform and methanol were purchased from Merck Ltd, Mumbai, India. ExcelaR-quality hexane and toluene were procured from Qualigen Fine Chemicals, Mumbai, India. Other chemicals used were of the highest purity available commercially.

HPTLC Instrumentation

The HPTLC system used was from M/s DESAGA GmbH, Weisloch, Germany, and consisted of an applicator (AS-30), a chromatographic chamber (DC-MAT), and a densitometer (CD 60) with ProQuant software, Version 1.00.005.

Calibration Curves of Curcumin, Metanil Yellow, Sudan I, and Sudan IV

Stock solutions (1 mg/mL) of curcumin, metanil yellow, and sudan I were prepared in methanol, and that of sudan IV was prepared in chloroform. Different concentrations of working solutions from 2 to 100 μg/mL were then prepared in methanol, and 5 μL of each was applied in duplicate on HPTLC plates by using a microliter syringe (Hamilton, Reno, NV) to obtain concentrations ranging from 10 to 500 ng/spot. The spots were developed by using chloroform–methanol (90 + 10, v/v) as the developing solvent (A) for curcumin and metanil yellow, and by using toluene–hexane–acetic acid (50 + 50 + 1, v/v/v) as the developing solvent (B) for sudan I and sudan IV. Densitometric scanning was performed in the absorbance mode at 420 nm for curcumin and metanil yellow, 491 nm for sudan I, and 520 nm for sudan IV. The slit dimension was kept at 10 × 2 mm, and the source of radiation used was deuterium and a tungsten lamp. The data for peak area versus concentration were evaluated for linear regression.

Precision

Repeatability values for sample application and peak area measurement were determined by using 3 replicates of the same analyte. The intra- and interday variations for the determination were evaluated at 2 concentration levels: 100 and 200 ng/spot for curcumin and metanil yellow; 25 and 50 ng/spot for sudan I; and 10 and 25 ng/spot for sudan IV. The intraday precision was evaluated by using triplicates at 2 different concentrations of the analyte on the same day. Interday precision was evaluated by using a standard solution at 2 different concentrations on 3 different days.

Limit of Detection and Limit of Quantification

In order to determine the limit of detection (LOD) and limit of quantitation (LOQ), concentrations of cucumin, metanil yellow, sudan I, and sudan IV in the lower part of the linear range of the calibration curve were used. The LOD and the LOQ were calculated by using the following equations: $LOD = 3.3 (\sigma/S)$ and $LOQ = 10 (\sigma/S)$, respectively, where σ is the standard deviation of the response and S is the slope of the corresponding calibration curve.

Chromatographic Separation and Spectral Characterization

Curcumin and metanil yellow in turmeric and sudan I and sudan IV in chili powders were quantified by 1-directional chromatography. Aliquots of 5 μL turmeric and chili powder extracts (500 ng/spot), along with standard metanil yellow and curcumin (200 ng/spot) and sudan I and sudan IV (25 ng/spot) were applied on a precoated HPTLC plate and resolved by using solvent systems A and B, respectively.

For the simultaneous determination of curcumin, metanil yellow, sudan I, and sudan IV in mixed curry powders, 2-directional chromatography was used. All of the spots were applied at a distance of 10 mm from the *Y*-axis. The first spot of 5 μL extract was applied at a distance of 15 mm from the *X*-axis, followed by a second spot of curcumin (200 ng/spot) at 80 mm and a third spot of metanil yellow (200 ng/spot) at 90 mm. The plate was developed in chloroform–methanol (9 + 1, v/v; solvent system A) for a distance of 45 mm from the point of application. After the first development, the plate was dried at room temperature and the spots for curcumin and metanil yellow were scanned densitometrically at 420 nm. The plate was then turned at an angle of 90°, and spots of sudan I and sudan IV containing each dye at 25 ng/spot were applied at distances of 10 and 20 mm from the *X*-axis. The plate was developed in toluene–hexane–acetic acid (50 + 50 + 1, v/v/v; solvent system B) for a distance of 80 mm from the point of application. After the plate was dried at room temperature, the spots for sudan I and sudan IV were read in the densitometer against the respective λ_{max} values of 491 and 520 nm. The optimized chamber saturation time for both mobile phases was 10 min at room temperature.

The spectra of spots of different colors were recorded from 200 to 700 nm by using a densitometer with a slit width of 4 mm and equipped with ProQuant software in the spectra mode.

Recovery Studies

Recovery studies were performed in duplicate by spiking turmeric powder with known concentrations of standard curcumin and metanil yellow, and chili powder with known concentrations of sudan I and sudan IV. Metanil yellow at concentrations of 500, 1000, and 1500 μg was added to 500 mg portions of turmeric powder, and each portion was extracted 5 times with 5 mL methanol each time. The extracted fractions were pooled, and 5 μL was applied to HPTLC plates. Similarly, sudan I and sudan IV at concentrations of 100, 200, and 300 μg were added to 500 mg portions of chili powder. The samples were extracted 4 times with 5 mL methanol each time. The extracted fractions were pooled, and 5 μL was applied to the HPTLC plate.

Because of the endogenous presence of curcumin in turmeric, standard synthetic curcumin at concentrations of 80, 120, and 160 μg was added to 5 mg portions of turmeric powder, and each portion was extracted 4 times with 1 mL methanol each time. The extracted fractions were pooled, and 5 μL was applied to the HPTLC plate.

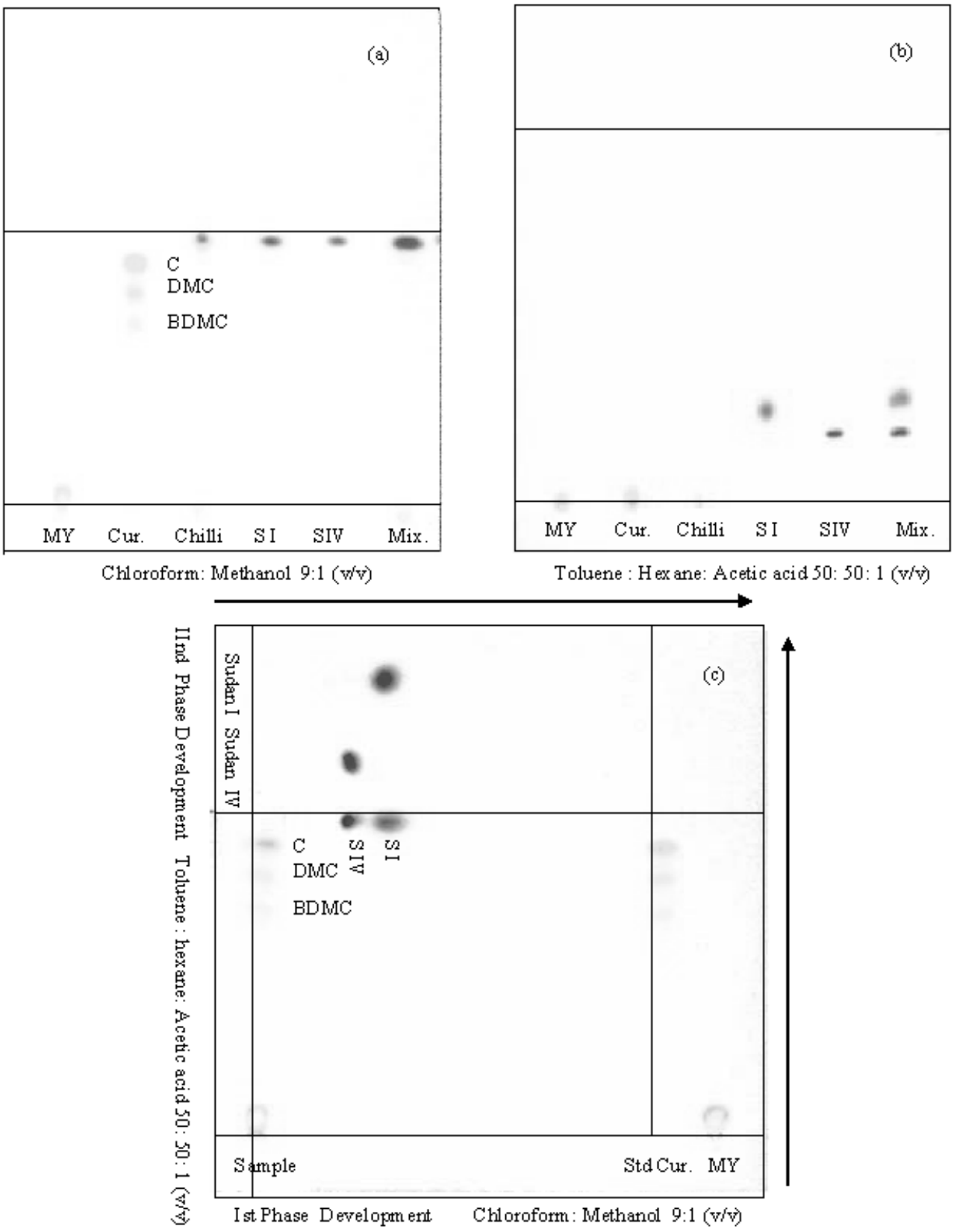

Figure 1. Resolution of (a) curcumin (C), demethoxycurcumin (DMC), bis(demethoxy)curcumin (BDMC), and metanil yellow (MY) in turmeric powder; (b) sudan I and sudan IV in chili powder; and (c) all 4 colors in mixed curry samples.

Table 1. Solvent systems for optimal resolution of metanil yellow, curcumin (C), demethoxycurcumin (DMC), bis(demethoxy)curcumin (BDMC), sudan I, and sudan IV in various samples

	Rf value					
Solvent system	Metanil yellow	C	DMC	BDMC	Sudan I	Sudan IV
Turmeric powder						
Chloroform–methanol (9 + 1, v/v)	0.05	0.77	0.69	0.61	0.91	0.91
Chili powder						
Toluene–hexane–acetic acid (50 + 50 + 1, v/v/v)	—	—	—	—	0.30	0.23
Mixed curry powder						
Chloroform–methanol (9 + 1, v/v)	0.05	0.77	0.69	0.61	0.91	0.91
Toluene–hexane–acetic acid (50 + 50 + 1, v/v/v)	—	—	—	—	0.30	0.23

The plates were developed in the relevant mobile phase(s) as described in *Chromatographic Separation and Spectral Characterization*, and the colors were quantified by densitometric scanning of the spots. Similarly, synthetic colors were also recovered from mixed curry powder samples.

Market Samples

Fifteen loosely sold turmeric and chili powder samples were randomly purchased from local markets in Lucknow City, India, and analyzed along with 15 packed, branded counterparts. The loose mixed curry powders were not available, and therefore only packed products were screened. A 1 g portion of each spice powder was taken and analyzed as in the case of the spiked samples.

Results

Development of the Optimum Mobile Phase

Various combinations of the components of the developing solvent were tested for resolution of 3 curcuminoids and metanil yellow in powdered turmeric samples: chloroform–methanol in ratios (v/v) of 9.25:0.75, 9:1, 8:2, 7:3, 6:4, and 5:5. Of these solvents, the combination 9.25:0.75 (v/v) gave good resolution of the 3 curcuminoids, but metanil yellow did not move from the origin. Chloroform–methanol (9 + 1, v/v) proved optimal for the resolution of curcumin (Rf 0.77), demethoxycurcumin (Rf 0.69), bis(demethoxy)curcumin (Rf 0.61), and metanil yellow (Rf 0.05; Figure 1a). However, this solvent system failed to separate the sudan dyes and natural chili pigment, which all moved as a single mixed spot.

In the case of chili powders, usually commercial samples of sudan I are used, which invariably contain traces of sudan IV. Two solvent systems were tested for the resolution of sudan dyes in chili powder. Chloroform–methanol–ammonia (4 + 6 + 3, v/v/v) gave satisfactory separation of sudan I and sudan IV, but natural chili pigment interfered because it moved along with sudan IV. Also, both metanil yellow and curcumin moved along the solvent front. The second solvent system, toluene–hexane–acetic acid (50 + 50 + 1, v/v/v) separated sudan I (Rf 0.30) and sudan IV (Rf 0.23), and the chili pigment did not interfere (Figure 1b), but metanil yellow and curcumin remained unmoved at the point of application. Because of the advantages of the 2 optimal solvent systems for turmeric and chili powders, a 2-directional chromatographic system was contemplated for mixed curry samples containing both turmeric and chili powder. The following 2-directional HPTLC system gave optimal resolution of curcumin (Rf 0.77),

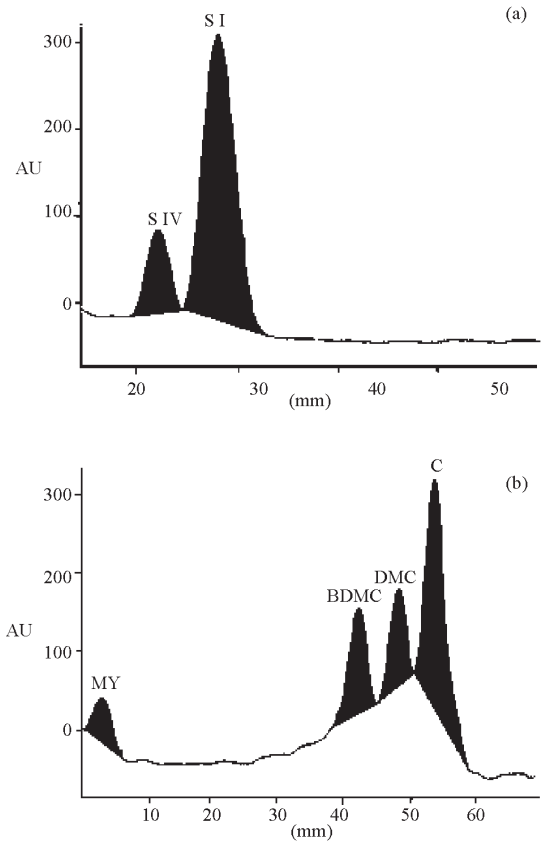

Figure 2. Densitogram showing the resolution of (a) sudan IV (S IV) and sudan I (S I) and (b) metanil yellow (MY), bis(demethoxy)curcumin (BDMC), demethoxycurcumin (DMC), and curcumin (C).

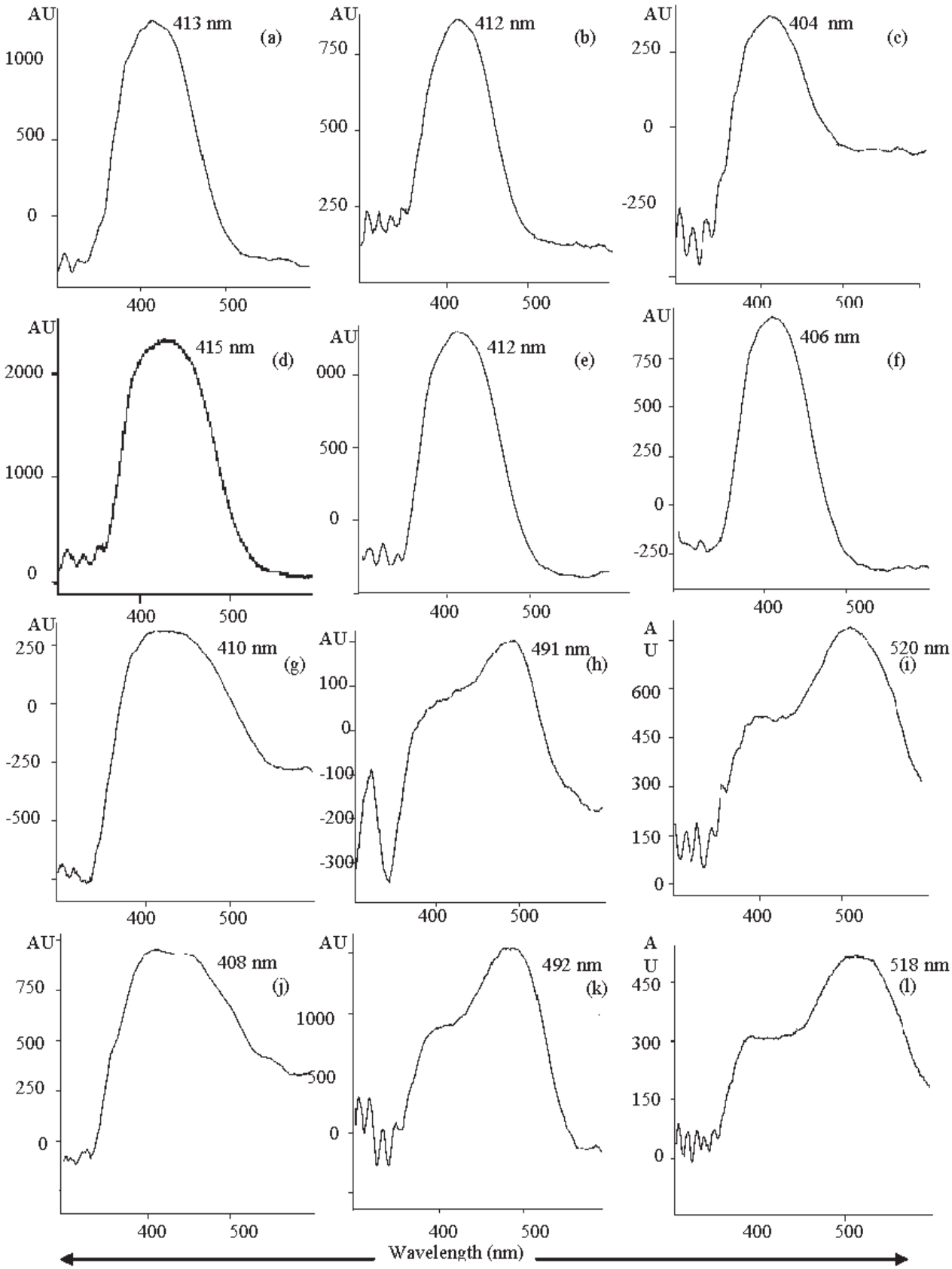

Figure 3. Comparision of spectra of standard curcuminoids, metanil yellow (MY), and sudan dyes with spectra from sample analyses: (a) standard curcumin, (b) standard demethoxycurcumin (DMC), (c) standard bis(demethoxy)curcumin (BDMC), (d) curcumin in sample, (e) DMC in sample, (f) BDMC in sample, (g) standard MY, (h) standard sudan I, (i) standard sudan IV, (j) MY in sample, (k) sudan I in sample, and (l) sudan IV in sample.

Table 2. Validation parameters for the individual colors

Parameter	Curcumin[a]	Metanil yellow[b]	Sudan I[b]	Sudan IV[b]
Linearity range, ng	50–300	100–500	25–100	10–50
Limit of detection (LOD), ng/spot	17.39	42.90	15.45	7.01
Limit of quantitation (LOQ), ng/g	52.71	130.0	46.80	21.24
Regression equation[c]				
Correlation coefficient (r^2)	0.9866	0.9990	0.9951	0.9927
Slope	1.379	0.9917	3.118	3.903
Confidence limit[d] of slope	1.194–1.563	0.9490–1.034	2.815–3.420	3.440–4.367
Y-intercept	–15.77	5.571	6.236	2.714
Confidence limit[d] of intercept	(–)49.02–17.48	(–)7.351–18.49	(–)16.66–29.13	(–)11.32–16.75

[a] $n = 6$.
[b] $n = 5$.
[c] The data for the regression equation were calculated by Graph Pad Prism 3.0 software.
[d] 95% confidence limit.

demethoxycurcumin (Rf 0.69), bis(demethoxy)curcumin (Rf 0.61), sudan I (Rf 0.30), sudan IV (Rf 0.23), and metanil yellow (Rf 0.05; Figure 1c, Table 1): chloroform–methanol (9 + 1, v/v) as the first-directional mobile phase and toluene–hexane–acetic acid (50 + 50 + 1, v/v/v) as the second-directional mobile phase. The HPTLC densitogram of the resolved spots of spiked turmeric and chili powders is shown in Figure 2.

Validation of the Method

Validation of spectral characteristics of standard and spiked samples.—The spectra of standard curcumin, metanil yellow, sudan I, and sudan IV were compared with those of samples containing these colors (Figure 3). The spectrum of standard curcumin showed 3 peaks having λ_{max} values of 413, 412, and 404 nm, representing curcumin, demethoxycurcumin, and bis(demethoxy)curcumin, respectively (Figure 3, a–c) and matching the peaks of the 3 curcuminoids found in turmeric samples (Figure, 3 d–f). Similarly, spectra of standard metanil yellow (410 nm), sudan I (491 nm) and sudan IV (520 nm; Figure 3, g–i) matched those of samples containing these colors (Figure 3, j–l), thereby validating the presence of the colors in the samples.

Calibration and linearity.—The linearity of standard curcumin, metanil yellow, sudan I, and sudan IV was tested and gave a good linear correlation between spot peak area and concentration of the individual colors (Figure 4). Data for the other validation parameters, namely, linearity, LOD, LOQ, and regression equations, are given in Table 2. Metanil yellow and curcumin gave respective linearity ranges of 100–500 and 50–300 ng/spot. In the case of sudan I and sudan IV, the linear ranges were 25–100 and 10–50 ng/spot, respectively. The respective LOD and LOQ values were curcumin, 17.39 and 52.71 ng/spot; metanil yellow, 42.90 and 130.0 ng/spot; sudan I, 15.45 and 46.80 ng/spot; and sudan IV, 7.01 and 21.24 ng/spot. The regression equations, with correlation coefficients in the range of 0.98–0.99, show the high-performance potential of the method (Table 2).

Precision.—The repeatability values and spot peak area measurements of all the 4 colors by the proposed method were expressed in terms of % mean area, % relative standard deviation (RSD), and standard error (SE; Table 3). The intra- and interday precision values of the individual colors at 2 different concentrations showed close correlation and gave satisfactory ranges of RSD values, 2.51–7.96%, and mean areas 96.16–100.6%. The SE values ranged from 1.39 to 4.82, indicating good statistical precision (Table 3).

Recovery studies.—Results of the recovery studies are summarized in Table 4. The recoveries of metanil yellow ranged from 97.1 to 103% with RSD values of 2.37–3.86%, whereas those of curcumin ranged from 86.7 to 98.7% with RSD values of 1.26–3.32%. For sudan I, recoveries ranged from 78.7 to 91.3%, and for sudan IV, they ranged from 68.6 to 74.5%. The respective ranges of the RSD values for sudan I and sudan IV were 1.61–2.92 and 2.04–4.81% (Table 4).

Determination of curcumin, metanil yellow, and sudan dyes in market samples.—The developed method was used to measure curcumin and extraneously added colors in a few branded and loose turmeric, chili, and mixed curry powder samples from the marketplace (Table 5). Among the loose turmeric and chili powders, 26.7 and 66.7% of the samples, respectively, were found to contain artificially added colors (metanil yellow in turmeric and sudan I and traces of sudan IV in chili powder). We found that the concentrations of metanil yellow ranged from 1.5 to 4.6 mg/g in loose turmeric samples. In loose red chili powder sudan I ranged from 4.8 to 12.1 mg/g, whereas sudan IV ranged from 0.9 to 2.0 mg/g. None of the branded turmeric, chili, and mixed curry powders was found to contain any extraneous color. The curcumin content in loose turmeric samples was lower in those with metanil yellow (6.5–10.1 mg/g) than in those without metanil yellow (13.2–20.3 mg/g). Branded turmeric samples had

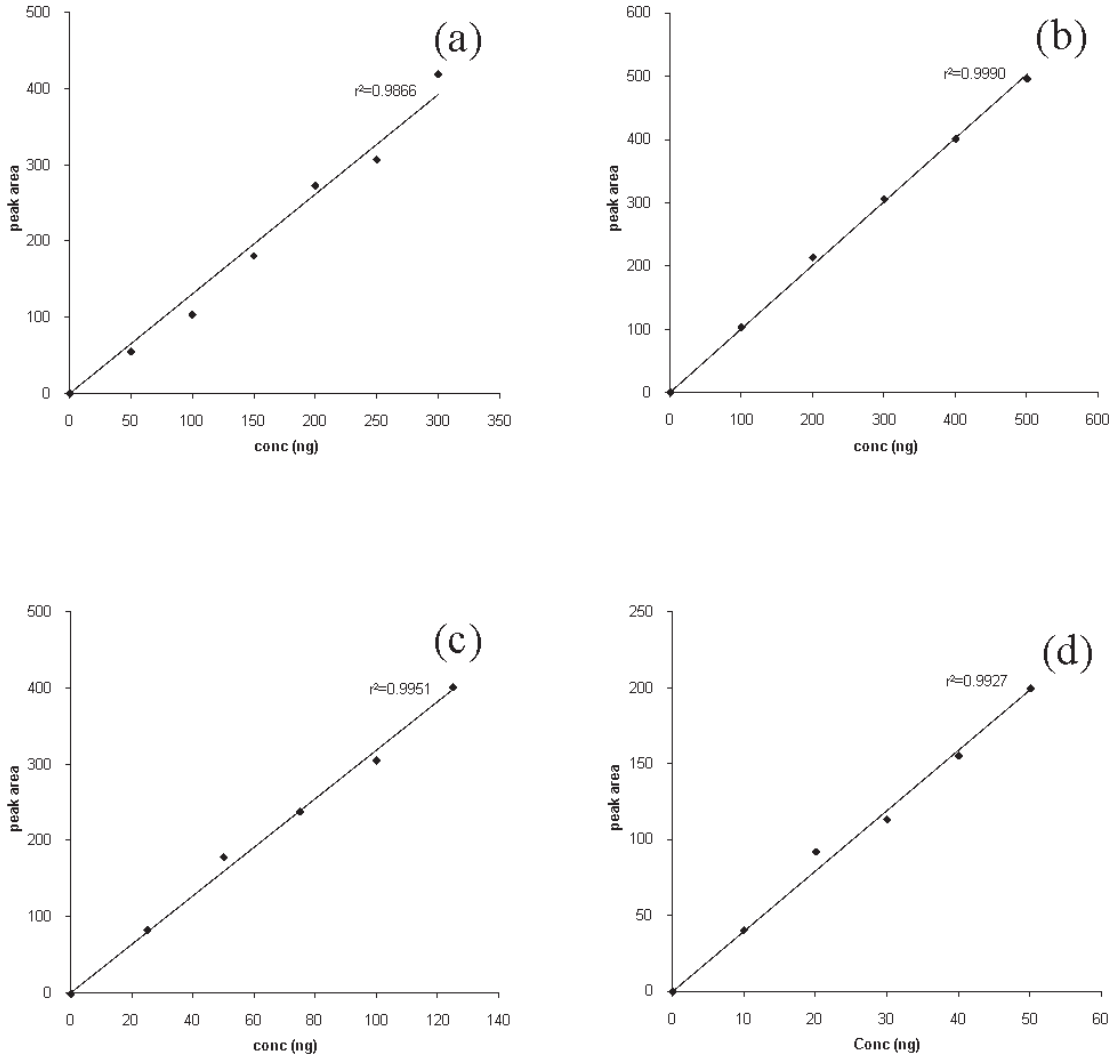

Figure 4. Calibration curves showing the linearity of (a) curcumin, (b) metanil yellow, (c) sudan I, and (d) sudan IV.

relatively higher levels of curcumin that ranged from 21.7 to 36.4 mg/g, whereas branded mixed curry samples contained curcumin levels that ranged from 0.3 to 1.9 mg/g. The identities of the individual sample color spots were confirmed by comparing the spectra recorded for these colors with those of standard colors (data not shown).

Discussion

Turmeric, chili, and various mixed curry powder formulations are popular household commodities in India and are exported to various countries. The quality standards for spices, prescribed under the PFA of India, state that chili, turmeric, and mixed curry powders should be free from extraneous coloring matter (1). However, admixtures of sudan dyes in chili and metanil yellow in turmeric powder have been reported from time to time (2–6, 15), and these findings necessitate continuous monitoring to ensure compliance with the rules and to safeguard the health of consumers. It has also been observed that turmeric powder is equally vulnerable to substitution with extraneous starches and that such samples usually show lower curcumin content (16). Thus, measurement of curcumin content partially serves to check the authenticity of turmeric powder. The Bureau of Indian Standards, the highest standards-setting body in India, suggests a minimum content of 3% curcumin for turmeric powder (7). The present 2-directional HPTLC method is able to quantify curcumin, metanil yellow, sudan I, and sudan IV dyes simultaneously.

Various methods for the quantification of curcumin in turmeric have been reported. The spectrophotometric methods (8, 10, 11), in general, measure the total color equivalent of the curcuminoids, i.e., the mixture of curcumin, demethoxycurcumin, and bis(demethoxy)curcumin.

Table 3. Intra- and interday precision values for determination of the individual colors by the proposed method[a]

		Intraday precision			Interday precision		
Colors	Amount, ng/spot	Mean area, %	RSD, %	SE	Mean area, %	RSD, %	SE
Curcumin	100	100.12	7.92	4.59	100.22	5.71	3.31
	200	100.11	3.06	1.77	99.98	5.82	3.36
Metanil yellow	100	99.99	6.05	3.63	99.98	3.32	1.92
	200	100.15	7.96	4.82	100.13	3.34	1.93
Sudan I	25	96.16	2.51	1.39	100.06	5.71	3.30
	50	100.59	4.84	2.81	100.02	2.61	1.51
Sudan IV	10	100.08	2.74	1.59	100.05	4.20	2.42
	25	100.11	4.11	2.37	100.33	4.43	2.57

[a] $n = 3$.

However, in cases in which extraneous color, namely, metanil yellow, has been added, the same metanil yellow will be measured along with the curcuminoids because of similar λ_{max} values; thus, spectrophotometric methods have a limited scope. Some of the earlier HPLC methods using an amino-bonded stationery phase and either UV or fluorescence detection for resolution of individual curcuminoids were found to give nonreproducible results with tailing peaks and poor resolution (17, 18). This problem was solved in subsequent methods, which successfully resolved the 3 curcuminoids in turmeric (9, 19). However, when these methods were tested, they did not provide simultaneous measurement of metanil yellow and the curcuminoids in turmeric powders. Thus, no method to quantify metanil yellow in powdered turmeric is available, although a qualitative paper strip test to detect the presence of artificial coloring matter is included under Food and Agriculture Organization/World Health Organization (FAO/WHO)-Joint Expert Committee on Food Additives (JECFA) specifications for curcumin (20). Metanil yellow is not included in the permitted list of food colors in India and other countries, and it shows diverse toxic responses (21–23). It is noteworthy that loose turmeric samples containing metanil yellow showed low levels of curcumin (6.5–10.1 mg/g), compared with levels of curcumin in loose turmeric samples without metanil yellow (13.2–20.3 mg/g) or in branded turmeric samples (21.7–36.4 mg/g). This finding indicates the inverse relationship between metanil yellow and curcumin in samples.

The illegal use of sudan dyes in chili powders has been encountered from time to time and is a major problem for the food industries because of its impact on public health. Because sudan I has been classified as a category 3 carcinogen by the International Agency for Research on Cancer (24) and as a category 3 mutagen by the European Council (25), the European Commission now requires that all chili-, curry-, and

Table 4. Results for recovery of the individual colors

Color	Added, ng/spot	Recovered, ng/spot	Recovery, %[a]	RSD, %
Metanil yellow	100	100.7	100.0	3.86
	200	206.9	103.0	2.75
	300	291.3	97.1	2.37
Curcumin	100	98.7	98.7	1.26
	150	130.0	86.7	3.32
	200	185.0	92.5	1.55
Sudan I	25	22.82	91.3	2.92
	50	39.35	78.7	1.77
	75	60.15	80.2	1.61
Sudan IV	25	18.62	74.5	2.04
	50	34.99	70.0	2.33
	75	51.46	68.6	4.81

[a] Each value is the mean of duplicate analyses.

Table 5. Measurement of extraneous colors, metanil yellow, sudan I, and sudan IV in market samples

Spice powder	Total no. of samples	No. of samples with curcumin (range, mg/g)	No. of samples with extraneous colors (range, mg/g)	Adulterated samples, %
Turmeric				
Loose	15	15	4	26.7
With metanil yellow	4	4 (6.5–10.1)	4 (1.5–4.6)	
Without metanil yellow	11	11 (13.2–20.3)	Nil	
Branded	15	15 (21.7–36.4)	Nil	Nil
Chili				
Loose	15	Nil	10 (4.8–12.1)[a] (0.9–2.0)[b]	66.7
Branded	15	Nil	Nil	Nil
Mixed curry powder[c]				
Branded	15	15 (0.3–1.9)	Nil	Nil

[a] Concentration range of sudan I.
[b] Concentration range of sudan IV.
[c] Loose mixed curry powder samples were unavailable in the local market, and thus were not analyzed.

curcuma-containing food products coming into any European Union member state are certified to be free of sudan dyes (26).

Several analytical screening and confirmatory methods for compliance verification have been described (6, 14, 27–29) for the analysis of chili and chili-containing food products for sudan dyes; however, the simultaneous quantification of sudan dyes and curcumin/metanil yellow in mixed curry powders containing both turmeric and chili was not possible, presumably because of the respective water and oil solubility characteristics of metanil yellow and sudan dyes.

In the present method, a simple extraction procedure and developing solvents are used to successfully resolve metanil yellow and the 3 curcuminoids in turmeric powder and sudan I and sudan IV in chili powders. The successive use of solvent systems A (chloroform–methanol, 9 + 1, v/v) and B (toluene–hexane–acetic acid, 50 + 50 + 1, v/v/v) in a 2-directional HPTLC format separated metanil yellow, curcumin, demethoxycurcumin, and bis(demethoxy)curcumin as well as sudan I and sudan IV in mixed curry powders. The developed method is relatively simple, offers reasonable sensitivity, and can be used to screen large numbers of chili, turmeric, and curry powder samples in a short time.

Acknowledgments

The authors are grateful to the director of Indian Institute of Toxicology Research (IITR) for his keen interest in the present study. Subhash K. Khanna is thankful to the Indian Council of Medical Research, New Delhi, India, for the Emeritus Medical Scientist award. The work is supported by CSIR Network Project NWP-17. The manuscript is IITR communication 2675.

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