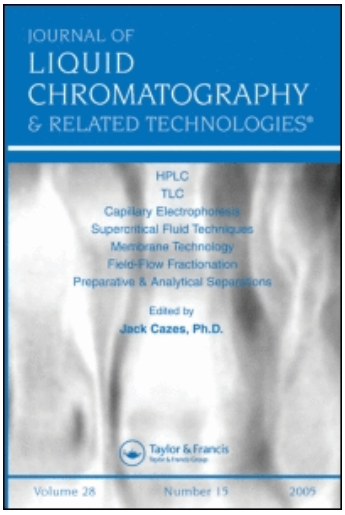

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Kandler, H. , Bleisch, M. , Widmer, V. and Reich, E.(2009) 'A Validated HPTLC Method for the Determination of Illegal Dyes in Spices and Spice Mixtures', Journal of Liquid Chromatography & Related Technologies, 32: 9, 1273 — 1288
To link to this Article: DOI: 10.1080/10826070902858293
URL: http://dx.doi.org/10.1080/10826070902858293

Journal of Liquid Chromatography & Related Technologies®, 32: 1273–1288, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070902858293

A Validated HPTLC Method for the Determination of Illegal Dyes in Spices and Spice Mixtures

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Abstract: For screening large numbers of samples of spice and spice mixtures for the presence of illegal dyes a rapid high performance thin layer chromatography (HPTLC) method was developed and validated. From paprika, chili, or curry powder, dyes are extracted with acetonitrile. An aliquot of the raw extract is filtered and treated with iron(III) chloride to oxidize the natural dyes. After evaporation to dryness, the residue is taken up with basic dichloromethane, cleaned through a solid phase extraction cartridge containing silica gel, and evaporated again. The purified extract is dissolved in acetonitrile, separated on a reversed phase (RP18) HPTLC plate, and then evaluated visually and by scanning densitometry. The screening method was validated regarding precision and recovery. It has been routinely used by the Official Food Control Authority of the Canton of Zurich since 2007.

Keywords: Densitometry, HPTLC, Illegal food colors, Spices, Sudan dyes

INTRODUCTION

Food dyes are used to offset discoloration and color changes of food during processing and storage, or to generate a more appealing appearance. Therefore, food dyes contribute significantly to the selection and acceptance of food. There are natural and synthetic food dyes. Synthetic dyes used as food color include azo compounds, triarylmethane dyes,

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indigo dyes, xanthene dyes, and quinoline compounds. They are usually water soluble and have been widely used for many years. According to current scientific knowledge, certain dyes may pose a health risk if not used properly. Therefore, in most countries the use of food dyes is strictly regulated. Any substance not listed in the corresponding guidance documents[1,2] is considered illegal.

In 2003, several member states of the European Union detected the illegal dyes Sudan I-IV in chili powder.[3] In 2004 alone, the European Rapid Alert System for Food and Feed (RASFF) registered 282 cases of Sudan I, not only in chili, but also in spice mixtures and sauces.[4] The fat soluble azo dyes, Sudan I, Sudan II, Sudan III, and Sudan IV, are classified as class 3 carcinogens and there is no tolerable daily intake determined.[5,6]

In order for state agencies to ensure food safety, it is essential to have suitable methods in place that allow detection of these illegal dyes in

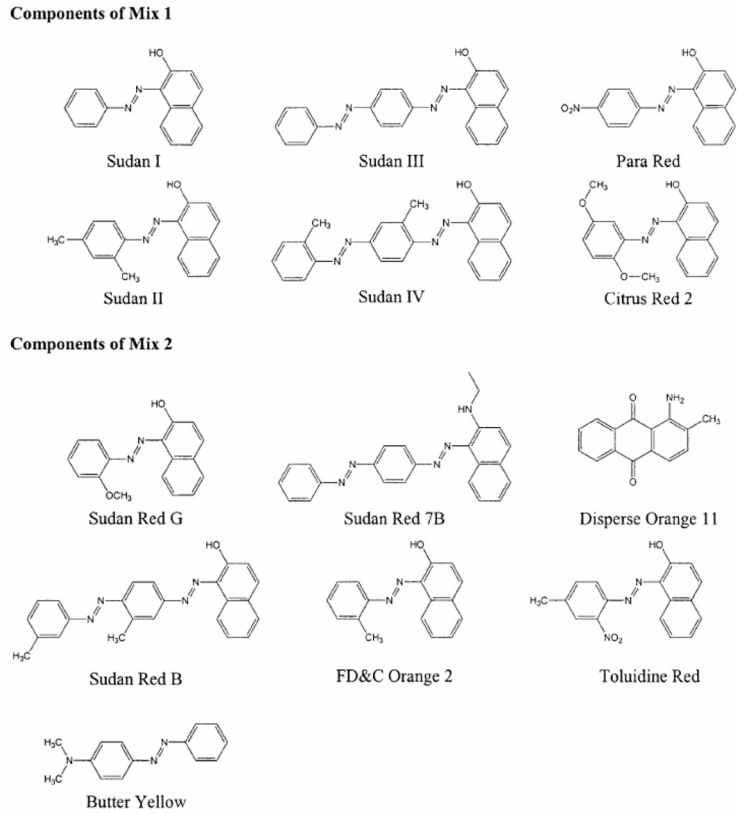


Figure 1. Structures of the investigated dyes.

various matrices. For testing spice, spice mixes, and spice preparations, we developed and validated a rapid HPTLC screening method for routine investigation of large numbers of samples. We aimed at visual evaluation of the chromatograms to determine the presence of either, Sudan I, II, III, IV, Sudan Red B, Sudan Red 7B, Sudan Red G, as well as Para Red, FD&C Orange 2, Butter Yellow, Citrus Red 2, Toluidine Red, and Disperse Orange 11 (Figure 1) in paprika, chili, and curry. Visual detection under white light allows simple and rapid screening of different samples. After screening, the presence or absence of a given dye can be confirmed and/or quantified by scanning densitometry.

EXPERIMENTAL

Materials

All dyes (except Citrus red, synthesized by azo coupling from 2-naphthol and 2,5-dimethoxyaniline at the lab of the Official Food Control Authority of the Canton of Zurich[7]) and solvents were of analytical grade and were purchased from Acros (Ghent, Belgium), Sigma Aldrich (Buchs, Switzerland), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany). Paprika, chili, curry, as well as two spice mixes, were obtained from the local market.

Basic dichloromethane was always freshly prepared by shaking 250 mL of dichloromethane in a separatory funnel with 10 mL of ammonia, 25%. After separation of the phases the dichloromethane was transferred into an Erlenmeyer flask with glass stopper.

An iron(III) chloride solution (5 mg/mL) was prepared in a 50 mL volumetric flask by dissolving 250 mg of $FeCl_3$ in acetonitrile and adjusting the final volume to the mark.

For sample preparation, the following instruments were used: knife mill Grindomix 200 (Schieritz & Hauenstein AG, Switzerland), turbomixer Polytron PT 3100 (Kinematica AG, Switzerland), or multiple stirrer Variomag Poly15 (VWR International AG, Switzerland), and evaporator Syncore® Polyvap (Büchi, Switzerland) consisting of Rack 24-ASE, vacuum pump V700, vacuum controller V855, and chiller B-740. Silica gel cartridges Chromabond SiOH 6 mL/1000 mg were purchased from Macherey Nagel (Oensingen, Switzerland).

HPTLC plates RP 18 F_{254s} 10×10 cm and HPTLC plates RP18 20×10 cm from Merck were used without pretreatment. Chromatography was performed with CAMAG equipment: Linomat 5 for sample application, Twin Trough Chambers (10×10 cm, 20×10 cm) for chromatogram development, DigiStore 2 for documentation, TLC Scanner 3 for densitometry, winCATS 1.4.3 software for instrument control and evaluation.

Preparation of Stock Solutions (ca. 200 µg/mL)

Of each dye, 20 mg were exactly weighed and transferred into separate 100 mL volumetric flasks. Para Red, Sudan III, Sudan IV, Toluidine Red, and Sudan Red 7B were dissolved in acetone and filled up to the mark. Sudan I, Sudan II, Citrus Red, Butter Yellow, Sudan Red B, FD&C Orange 2, Sudan Red G, and Disperse Orange 11 were dissolved in acetonitrile and filled up to the mark. The individual stock solutions can be stored in the dark at 5°C for six months.

Preparation of Standard Solutions (ca. 100 µg/mL)

Mix 1: 5.0 mL of each of the stock solutions Sudan I, Sudan II, Sudan III, Sudan IV, Citrus Red, and Para Red (ca. 200 µg/mL) were transferred into a 100 mL heart shaped flask and evaporated under vacuum (50°C, 120 mbar) to dryness. The residue was dissolved with exactly 10.0 mL of acetonitrile.

Mix 2: 5.0 mL of each of the stock solutions Sudan Red B, Sudan Red 7B, Disperse Orange 11, Butter Yellow, Toluidine Red, FD & C Orange 2, and Sudan Red G (ca. 200 µg/mL) were transferred into a 100 mL heart shaped flask and evaporated under vacuum (50°C, 120 mbar) to dryness. The residue was dissolved with exactly 10.0 mL of acetonitrile.

The standard solutions can be stored in the dark at 5°C for three months.

Sample Preparation

In a 100 mL Erlenmeyer flask, 5.0 g of homogenized sample were extracted with 50.0 mL of acetonitrile by stirring (10 min) or by using a turbomixer (1 min). The mixture was filtered through a fluted filter. Of the filtrate, 10.0 mL were transferred into a 60 mL ASE vial. $FeCl_3$ solution (5 mg/mL in acetonitrile) was added drop wise until the color changed from red to greenish (ca. 0.3–0.8 mL). The solution was evaporated to dryness and the residue was taken up in 1 mL of basic dichloromethane.

For clean up, a solid phase extraction (SPE) cartridge with silica gel (Chromabond) was conditioned with 10 mL of basic dichloromethane. Then, the complete sample residue (dissolved in 1 mL of basic dichloromethane) was transferred onto the cartridge. The sample was eluted from the cartridge by adding additional 10 mL of basic dichloromethane. The whole eluate (11–13 mL) was collected in an ASE vial and evaporated to dryness. The residue was taken up with 1.0 mL of acetonitrile.

For screening purposes, fourteen samples were prepared in parallel. For quantitative determinations six aliquots of the sample were prepared in parallel.

Preparation of Standard Solutions for Matrix Calibration (10–120 mg/kg)

In 100 mL Erlenmeyer flasks, 5.0 g of a suitable not contaminated sample were spiked in duplicate with 500, 1500, 3000, 4500, and 6000 μL of Mix 1 and Mix 2 standard solution (100 μg/mL), respectively. After spiking, the samples were made up to exactly 50.0 mL with acetonitrile and prepared as described above.

HPTLC

Samples were applied as 8 mm bands using the spray-on technique (110 nL/s). Distances from the left and right edge of the plate were 15 mm and from the lower edge of plate 8 mm. The distance between tracks was ≥2 mm. Eight or eighteen samples can be applied onto a 10 × 10 cm or 20 × 10 cm plate, respectively. For routine screening, the pattern shown in Table 1 was applied.

Table 1. Application scheme for routine screening of spices

	Track	Vol (μL)	Code
—	1	10	S1
—	2	10	P1
—	3	10	P2
—	4	10	P3
—	5	10	P4
—	6	10	P5
—	7	10	P6
—	8	10	P7
—	9	10	Bs
—	10	10	Bs
—	11	10	P8
—	12	10	P9
—	13	10	P10
—	14	10	P11
—	15	10	P12
—	16	10	P13
—	17	10	P14
—	18	10	S1′

S1, S1′ = Standard-Mix 1 (Mix 2); Bs = Blank spiked (10 ppm); P1–14 = samples.

Table 2. Application scheme for quantitative determination of dyes in spices

	Track	Vol (μL)	Code
—	1	10	S1
—	2	10	S2
—	3	10	S3
—	4	10	S4
—	5	10	S5
—	6	10	B
—	7	10	P1
—	8	10	P2
—	9	10	P3
—	10	10	P4
—	11	10	P5
—	12	10	P6
—	13	10	B′
—	14	10	S1′
—	15	10	S2′
—	16	10	S3′
—	17	10	S4′
—	18	10	S5′

S1–S5, S1′–S5′ = Standards for matrix calibration Mix 1 (Mix 2); B = Blank uncontaminated; P1–P6 = replicates of sample.

Table 3. Absorption maxima of the investigated dyes

Dye	CAS No.	Color index	λ_{max}
Sudan I	842-07-9	12055	495 nm
Sudan II	3118-97-6	12140	508 nm
Sudan III	85-86-9	26100	523 nm
Sudan IV	85-83-6	26105	534 nm
Para Red	6410-10-2	12070	498 nm
Citrus Red 2	6358-53-8	12156	529 nm
Sudan Red 7B	6368-72-5	26050	551 nm
Sudan Red G	1229-55-6	12150	514 nm
Sudan Red B	3176-79-2	26110	533 nm
FD & C Orange 2 (Orange OT)	2646-17-5	12100	502 nm
Butter Yellow	60-11-7	11020	453 nm
Toluidine Red	2425-85-6	12120	522 nm
Disperse Orange 11	82-28-0	60700	488 nm

For quantitative determination of contamination, standards for matrix calibration (10–120 ppm) were used as shown in Table 2.

Development was performed in an unsaturated chamber with acetonitrile, ammonia 25% (95:5 v/v) over 6 cm. Development time was 11–12 min.

For visual evaluation the chromatograms were documented under white light in reflectance mode. Densitometry was performed using multiple wavelengths scanning at the absorption maximum of the individual dyes (Table 3). For quantitation, matrix calibration standards are fitted with a Michaelis Menten–2 function.

RESULTS AND DISCUSSION

Method Development

The dyes Sudan I, II, III, IV, Sudan Red B, Sudan Red 7B, Sudan Red G, Para Red, FD & C Orange 2, Butter Yellow, Citrus Red 2, Toluidine Red, and Disperse Orange 11 were selected for the study because of their color, health risks, relevance in reported cases of food contamination, and structural similarity. Based on a comprehensive review of the available literature including official guidance documents and scientific papers, a number of chromatographic systems were evaluated and modified. None of those were able to achieve sufficient separation. Selectivity of chromatography had to be modified (Table 4).

Aiming at sufficient separation of the target compounds, chromatography was performed exclusively on HPTLC plates using a standardized methodology.[13] In general, chromatography on reversed phase gave better separation. Finally method 17 was selected because it provided adequate separation of all standards when grouped in two mixtures (Figure 2). In deviation from our HPTLC standard parameters, chromatography was performed in unsaturated chambers because this mode resulted in less diffuse zones.

In order to detect the target compounds in spices such as paprika, chili, and curry powder, it was important to either selectively extract them from the spice matrix or separate them from naturally occurring colored compounds such as carotenoids, which are extracted simultaneously.

Ethanol, acetone, and acetonitrile were evaluated as extraction solvents. Uncontaminated paprika powder (5.0 g) was extracted with 50 mL of the respective solvent. After filtration, 30 mL of each filtrate were evaporated to dryness, 38, 41, and 20 mg of total matter were thus obtained. Based on these findings, acetonitrile was used for further experiments because it extracted the lowest amount of matrix and provided adequate solubility for most of the target compounds. Nevertheless,

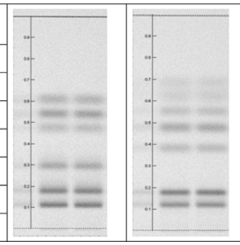
Table 4. Evaluated chromatographic systems for the separation of dyes on HPTLC

Method	Stationary phase	Mobile phase	Source
1	RP18	methanol – acetone – water (7:2:1)	[10]
2	Si60	dichloromethane	[8]
3	Si60	n-hexane–acetone (80:4)	[8]
4	RP8	1st and 2nd development: methanol – water – acetic acid (80:15:5)	[11]
5	RP18	methanol – acetone – water (7:2:0.5)	CAMAG (mod. from [10])
6	Si60	Toluene	CAMAG
7	Si60	n-hexane – ethyl acetate (9:1)	CAMAG
8	Si60	1,2-dichloroethane	CAMAG
9	RP18	Methanol – water (95:5)	CAMAG
10	RP18	acetonitrile – water (95:5)	CAMAG
11	RP18	tetrahydrofuran – water 6:4	CAMAG
12	RP18	tetrahydrofuran – water – formic acid (4:6:0.14)	CAMAG
13	RP18	Methanol – tetrahydrofuran – water (19:12:5)	CAMAG
14	NH_2	Toluene	CAMAG
15	NH_2	methyl-t-butyl ether – heptane (1:2)	CAMAG
16	RP18	acetonitrile–methanol – water–25% ammonia (75:20:5:2)	Draft method [12]
17	RP18	acetonitrile–ammonia 25% (95:5)	Final method [12]

when separated with the selected chromatographic system, most spice extracts showed considerable amounts of colored matrix that could potentially interfere with the target compounds.

Assuming that most of the natural pigments derive their color from extensively conjugated double bonds, in a next step we attempted to

Substances Mix 1	Rf	Relative Rf
Para Red	0.61	1.27
Citrus Red	0.54	1.12
Sudan I	0.48	1.00
Sudan II	0.29	0.60
Sudan III	0.18	0.37
Sudan IV	0.11	0.23
-	-	-



Relative Rf	Rf	Substances Mix 2
1.44	0.69	Disp. Orange
1.31	0.63	Butter Yellow
1.16	0.56	Toluidine Red
1.00	0.48	Sudan Red G
0.81	0.39	FD&C Orange
0.37	0.18	Sudan Red 7B
0.11	0.11	Sudan Red B

Figure 2. Rf-values of investigated dyes.

oxidize those compounds to colorless derivatives. Due to their increased polarity, these derivatives could be removed by solid phase extraction on silica gel. The experimental conditions for the oxidation had to be selected in a way that did not affect the structure of the illegal synthetic dyes. The following oxidants were tested: 2% potassium permanganate in acetonitrile, 47% hydrogen bromide in water, 4% iodine in acetonitrile, 10% hydrogen peroxide acidified with 3 drops of hydrochloric acid 37%, 3-chloroperoxybenzoic acid, and 0.5% iron(III) chloride in acetonitrile. It was noted that the degree of oxidation depended on the type, as well as on the amount of oxidant. In most cases, the Sudan dyes were also oxidized to some extent. For further work oxidation with iron(III), chloride was selected because it was convenient to work with, the degree of oxidation could be visually controlled to some extent, and there was only minimal interaction with the synthetic dyes. It was important to add the oxidant slowly and incrementally up to a total of 0.3 to 0.8 mL. In this way, a color change from red to greenish was observed, which indicated the completion of the reaction. The oxidized natural pigments and the remaining iron compounds had to be removed from the mixture because there was still considerable interference with the chromatography of the synthetic dyes. Furthermore, the high sample load caused unfavorable chromatographic conditions. Purification was achieved by first evaporating the mixture to dryness, taking it up again with basic dichloromethane, and then performing a cleanup on silica gel SPE cartridges (see experimental section). Figure 3 shows how oxidation with iron(III) chloride positively affects chromatography.

The complete sample preparation was not only tested on paprika, chili, and curry powder, but also on a commercial spice mix (containing chili 89%, cumin, oregano, and dried garlic) and a very complex spice

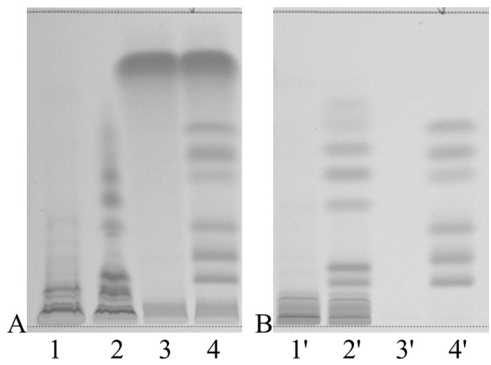


Figure 3. Influence of the oxidation step on chromatography: A: without oxidation; B: oxidation with iron(III) chloride; 1, 1′: paprika blank; 2, 2′: paprika spiked with 50 ppm of Mix 2; 3, 3′: curry blank; 4, 4′: curry spiked with 50 ppm of Mix 1.

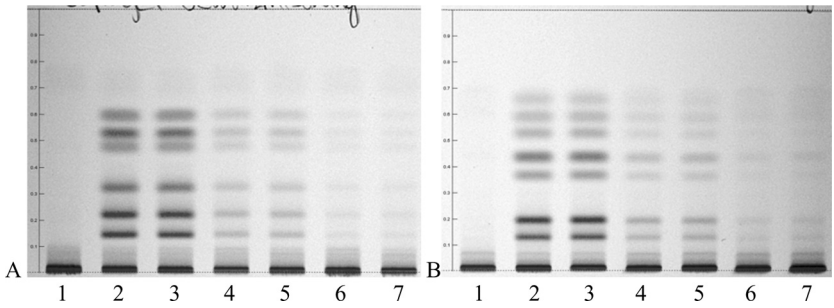


Figure 4. Spiking experiment with "spice preparation for poultry" A: spiked with Mix 1; B spiked with Mix 2. 1: sample not spiked; 2, 3: sample spiked with 50 ppm; 4, 5: sample spiked with 10 ppm; 6, 7: sample spiked with 2 ppm.

preparation for poultry. That commercial spice preparation contained paprika (15%), non-iodized sodium chloride (12%), rosemary, sugar, dried garlic, dried onion, coriander seed, hydrolyzed vegetable protein mixture (hydrolyzed vegetable protein, vegetable oil, acidifier succinic acid), yeast extract, taste enhancer (E635), color (E150a), white pepper, chili powder, mustard seed, cumin, oregano, fennel, marjoram (2%), spices, aroma, star anise, curcuma, basil, celery seed, and color (paprika extract). Figure 4 shows the result of spiking experiments for the spice preparation for poultry with both dye mixtures at different levels in duplicates.

Quantitative Determination of Contamination

Quantitative measurements were performed by scanning densitometry of the developed plate in absorption mode. Due to the different spectral properties of the individual dyes a multiwavelengths scan was performed taking into account the wavelength of the absorption maximum for each compound. In a first set of experiments calibration curves based on absolute amounts (ng) of all dyes were established at low level (30–150 ng) and high level (100–1200 ng). The obtained data was best fit with a Michaelis-Menten type 2 function (Figures 5 and 6).

The two dye mixtures were also used in a second experiment to establish the detection limit based on visual and densitometric evaluation (Tables 5 and 6). Using densitometry about 5 times lower detection limits were achieved.

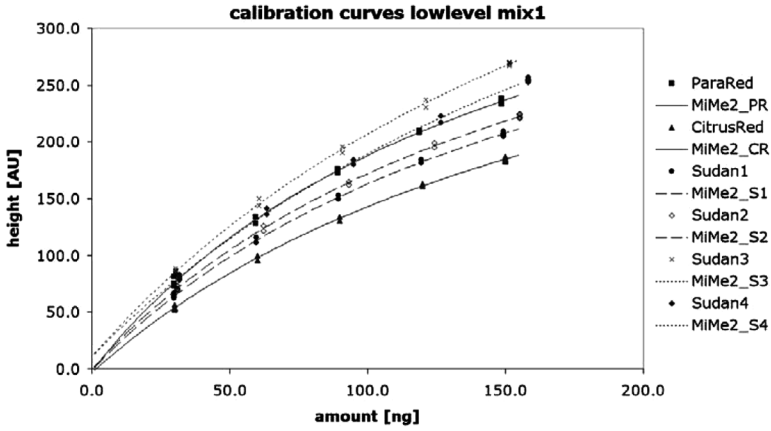
Figure 7 shows an example of the estimation of the limit of detection for Sudan I after densitometric determination. The noise value is determined graphically from the chromatograms.

In a third set of experiments, calibration functions were generated using "matrix calibration" in order to investigate the effects of sample

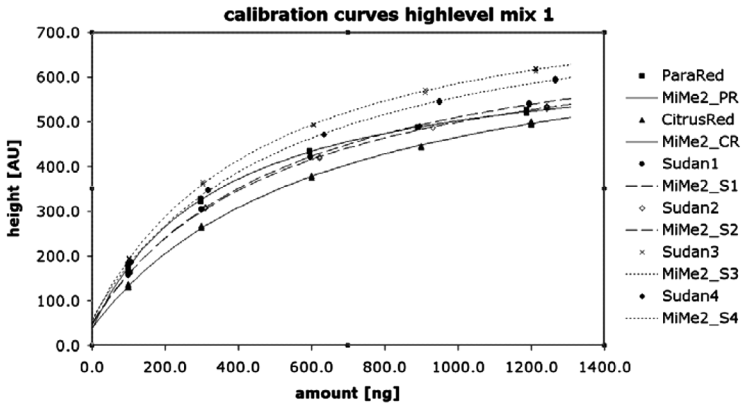
$$Y = \frac{a_1 \times X}{a_2 + X} + a_0$$

Figure 5. Michaelis-Menten type 2 function.

preparation (oxidation, solid phase extraction). The calibration standards were obtained by spiking a contamination free matrix (i.e., curry powder) and performing the complete sample preparation procedure (see experimental section). The graphs for matrix calibration looked generally similar to those obtained in direct calibration. In most cases values



(a)



(b)

Figure 6. (a) Calibration curves for components of Mix 1 at low level; (b) Calibration curves for components of Mix 1 at high level.

Table 5. Limits of detection for visual determination. Estimation of limit of detection (LOD) for the individual dyes; O: zone not visible, +: zone barely visible, ++: zone visible

	Visual determination [ng]*			LOD_{visual}	
Dye	O	+	++	(ng)	(mg/kg)
Para Red	9.9*	19.8	29.7	ca. 20	2
Citrus Red	10.0	20.0	30.0	ca. 20	2
Sudan I	10.0	19.9	29.9	ca. 20	2
Sudan II	10.4	20.7	31.1	ca. 20	2
Sudan III	10.1	20.2	30.3	ca. 20	2
Sudan IV	10.6	21.1	31.7	ca. 20	2
Disperse Orange	39.8	49.8	–	ca. 50	5
Butter yellow	20.7	31.1	41.4	ca. 30	3
Toluidine Red	10.0	20.0	30.0	ca. 20	2
Sudan Red G	10.0	19.9	29.9	ca. 20	2
FD & C Orange 2	9.9	19.7	29.6	ca. 20	2
Sudan Red 7B	10.0	20.0	30.0	ca. 20	2
Sudan Red B	10.1	20.1	30.2	ca. 20	2

*The figures in the columns correspond to the amount of dyes applied to the plate. The detection limit was established after chromatography.

were lower in accordance with substance loss during the cleanup steps. Figure 8 gives an example. On the other hand, depending on the matrix, compounds such as Butter Yellow or Para Red show higher response for

Table 6. Limits of detection for densitometric determination

Dye	Amount (ng)	Peak height (AU)	Noise (AU)	S/N	LOD (ng)	LOD (mg/kg)
Para Red	4.95	15.76	4	3.9	3.8	ca. 0.4
Citrus Red	8.00	17.21	4	4.3	5.6	ca. 0.6
Sudan I	4.98	13.67	4	3.4	4.4	ca. 0.4
Sudan II	5.18	13.20	4	3.4	4.6	ca. 0.5
Sudan III	5.05	16.06	4	4.0	3.8	ca. 0.4
Sudan IV	5.28	15.36	4	3.8	4.2	ca. 0.4
Disperse Orange 11	21.10	20.49	4	5.1	12.4	ca. 1.2
Butter Yellow	8.28	18.21	4	4.6	5.4	ca. 0.5
Toluidine Red	5.00	13.25	4	3.3	4.5	ca. 0.5
Sudan Red G	4.98	16.45	4	4.1	3.6	ca. 0.4
FD & C Orange 2	4.93	14.09	4	3.5	4.2	ca. 0.4
Sudan Red 7B	5.00	15.16	4	3.8	3.9	ca. 0.4
Sudan Red B	5.03	15.66	4	3.9	3.9	ca. 0.4

Table 7. Calibration functions for dyes of Mix 1 at low level (LL) and high level (HL)

$Y = \frac{a_1 \times X}{a_2 + X} + a_0$	a_0	a_1	a_2	*sdv*
Low level				
Para Red	−1.6	495.8	161.5	1.46
Citrus Red	−3.2	441.1	201.8	1.37
Sudan I	−1.0	461.9	181.8	1.27
Sudan II	−0.3	477.1	177.1	1.30
Sudan III	10.8	663.8	238.3	1.35
Sudan IV	10.8	637.5	256.3	1.33
High level				
Para Red	36.9	629.4	350.4	0.75
Citrus Red	39.2	701.8	642.4	1.08
Sudan I	48.2	711.4	538	0.70
Sudan II	50.8	684.4	524.3	0.58
Sudan III	54.2	777.4	462.5	0.52
Sudan IV	58.5	753.5	515.1	0.56

matrix calibration in comparison with direct calibration. This can be explained by incomplete oxidation and insufficient chromatographic separation of natural colors. For this reason, a suitable matrix calibration should be preferred for quantitative determination.

Validation

The proposed method is intended for semi-quantitative routine screening of red spices for presence of illegal dyes. Validation included determination of quality of the calibration function, accuracy, and precision of the overall process, and detection limits during routine use.

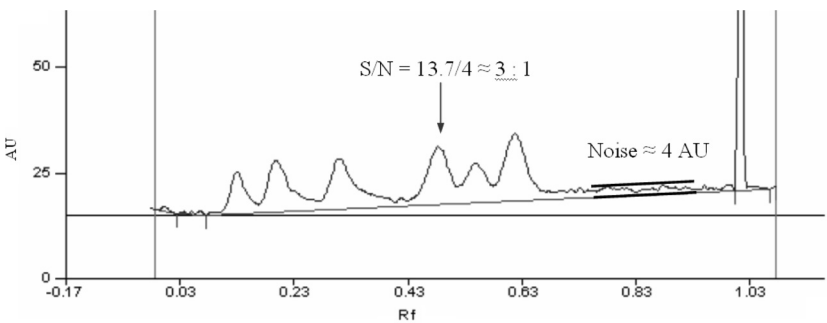


Figure 7. Limit of detection for Sudan I (signal to noise ≈3:1; 4.98 ng) for densitometric determination.

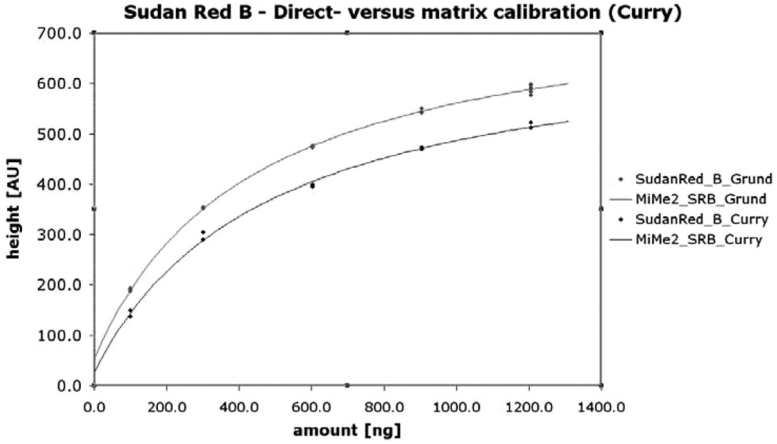


Figure 8. Comparison of direct calibration for Sudan Red B with matrix calibration (curry).

All calibration functions (each dye at low and high level) included five standard levels with six replicates at the lowest and the highest level, while the remaining three levels used duplicates. Michaelis-Menten type 2 functions gave the best fit for the resulting data. Details for the components of Mix 1 are shown in Table 7. The relative standard deviation ($n = 6$) of the lowest calibration point ranged from 2.3 to 6.7% and that of the highest calibration point from 0.5 to 2.9% for the low level calibration. Those values for the high level calibration are 0.7 to 6.4% at the lowest calibration point and 0.3 to 4.3% at the highest calibration point. Relative residual standard deviation of all low level calibration functions ranged from 1.2 to 3.8% with eight out of thirteen dyes achieving $<2.0\%$. Relative residual standard deviation of all high level calibration functions ranged from 0.5 to 4.8% with eight out of thirteen dyes achieving $<2.0\%$.

The accuracy and the precision of the method were evaluated together using paprika and curry as matrix. Six samples of each spice were spiked at the 50 ppm level with Mix 1 and Mix 2, respectively. Precision of the determination ($n = 6$) was determined using matrix calibration. Table 8 summarizes the data. For the determination of individual dyes precision ranged from 0.4 to 7.0%. Recovery data for all dyes were within $+/-$ 10% of 100% as expected for matrix calibration.

The detection limits for all dyes were estimated visually and densitometrically for the matrices paprika and curry. Individual samples were spiked in duplicate with 1 to 17 ppm (in steps of 2 ppm) of Mix 1 and Mix 2, respectively, and then prepared according to the method. Decisions about LOD were based on visibility of the individual zones or signal to

Table 8. Average recovery and relative standard deviation (RSD) of dyes spiked into paprika and curry respectively (n = 6)

	Paprika RSD	Aver. recovery (%)	Curry RSD	Aver. recovery (%)
Mix 1				
Para Red	1.65	106.6	1.56	102.9
Citrus Red	4.30	98.1	0.49	106.2
Sudan I	3.58	104.5	0.42	102.5
Sudan II	3.59	104.3	0.65	103.6
Sudan III	4.04	104.9	0.95	110.8
Sudan IV	5.15	105.0	1.08	107.3
Mix 2				
Disperse Orange 11	1.99	106.8	0.68	105.0
Butter Yellow	5.33	102.6	1.68	101.1
Toluidine Red	3.08	99.6	1.03	99.0
Sudan Red G	3.39	96.7	1.24	96.1
FD & C Orange 2	3.26	98.4	1.03	97.9
Sudan Red 7B	7.01	94.7	1.98	95.9
Sudan Red B	5.05	101.9	2.17	95.0

noise ratio, respectively. Visual detection limits were found at 3 ppm for most dyes in either matrix, 5 ppm for Sudan I, 13 ppm (11.5 ppm) for Disperse Orange, and 7 ppm (5 ppm) for Butter Yellow. The numbers in parentheses refer to curry, the other to paprika. In comparison, densitometric LODs for all dyes were lower by a factor of 2. Values of 1–3 ppm were reached except for Disperse Orange with an LOD of 7 ppm.

For each series of fourteen samples, two contamination free samples were spiked and densitometrically analyzed for control purposes. Recovery data of those control samples over time will generate a quality control chart.

CONCLUSION

Because of the small effect on the color of a spice, contamination with illegal dyes at the low ppm level is usually not an issue. Typical contamination exceeds 100 to 1000 ppm. Therefore, the proposed HPTLC method is a rapid, sensitive, and well suited tool for the screening of spices for the presence of illegal dyes under routine conditions of an official food control laboratory. Positive results of typically contaminated samples can easily be verified by comparing the UV-VIS spectra of separated dyes with those of reference substances. Further confirmation, especially for minimally contaminated samples, by LC-MS is always possible as well.[9]

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Received December 16, 2008
Accepted January 5, 2009
Manuscript 6469D