JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Simultaneous Determination of Banned Acid Orange Dyes and Basic Orange Dyes in Foodstuffs by Liquid Chromatography−Tandem Electrospray Ionization Mass Spectrometry via Negative/Positive Ion Switching Mode

Guozhen Fang, Yu Wu, Xiaomeng Dong, Cuicui Liu, Shaoyuan He, and Shuo Wang*

Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, Tianjin 300457, China

ABSTRACT: Simultaneous detection of two classes of dyes possessing different chemical properties is difficult. In this study, through negative/positive ion switching mode, simultaneous determination of four typical acid orange dyes and three typical basic orange dyes was achieved by a single high-performance liquid chromatography−tandem mass spectrometry (LC-MS/MS) method, and the analytical efficiency of multiresidues identification was greatly improved. To enhance detection sensitivity, the sample pretreatment conditions and HPLC-MS/MS determining conditions were carefully optimized. Under optimal conditions, good linearity was obtained over the range of 5−500 μg L^{-1} with a correlation coefficient (R^2) >0.9998. Limits of detection (LODs) and limits of quantification (LOQs) of the seven dyes were 0.5−3.0 and 2.0−6.0 μg kg^{-1}, respectively. The recoveries of the seven dyes in soybean products and marinated eggs were 74−126% with relative standard deviations (RSDs) of 2.22−25.4%, suggesting the developed method is promising for the accurate quantification of the seven dyes at trace levels in foods.

KEYWORDS: *acid orange dyes, basic orange dyes, foodstuffs, LC-MS/MS, negative/positive ion switching mode*

INTRODUCTION

Acid orange dyes are a class of synthetic azo dyes that are widely used as coloring agents in paper, leather, textile, food, and so on. When these azo dyes are ingested by the human body over the long term, they will be deoxidated into carcinogenic aromatic amines by metabolites of human bodies. It has been proved that most of the azo dyes, precursors, intermediates, and degradation products are genetic toxic, carcinogenic, and mutagenic.[1−3] Basic orange dyes are cationic dyes that are mainly used for fiber, leather, textile fabric, and wood product dyeing. They are high intensity, bright in color, light-fast, and resistant to degradation. These cationic dyes can cause acute and chronic poisoning via ingestion, inhalation, and absorption through the skin.[4,5] Therefore, it was forbidden to add these synthetic colorants into foods in our country according to the Using Standard of Food Additives.[6] Nevertheless, because these colorants are cheaper and more stable than natural food colors, many peddlers add these colors into food illegally. For the assurance of food safety, scientists have paid considerable attention to the detection of forbidden pigments in different kinds of food matrices and established many analytical methods. However, acid orange dyes and basic orange dyes added in food matrices illegally have received relatively a little attention. In 2002, Fuh and Chia[7] detected sulfonated azo dyes in food by ion-pair liquid chromatography with photodiode array and electrospray mass spectrometry detection (LC-DAD-MS), and A7 and A20 have been detected in this paper. In 2009, Wen[8] developed a LC-DAD method for the detection of B2, B21, and B22. In 2010, Ferrer Amate et al.[9] developed a LC-MS/MS method for the simultaneous

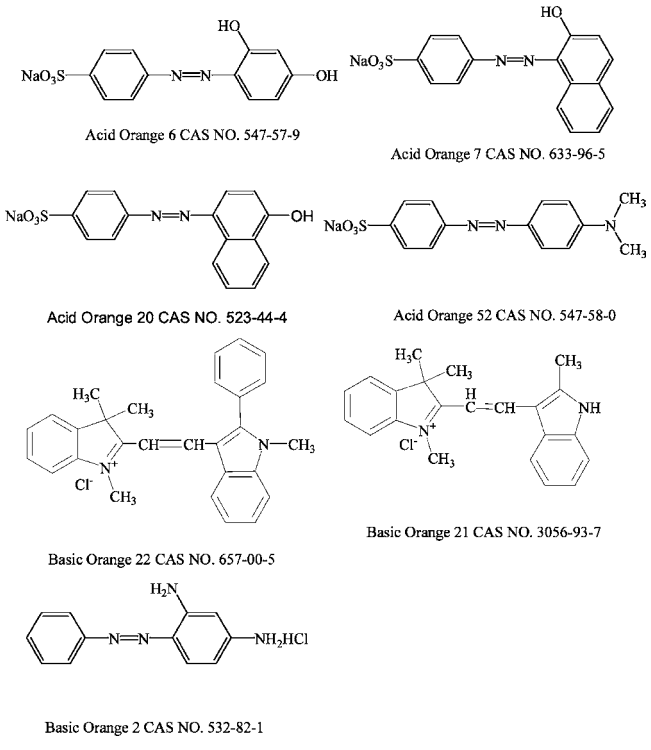

Figure 1. Structures of four acid orange dyes and three basic orange dyes.

Received: February 7, 2013
Revised: April 6, 2013
Accepted: April 7, 2013
Published: April 8, 2013

determination of aflatoxins, dyes, and pesticides in spices, and only A7 was detected. These papers mainly detected one class of orange dyes, so it was urgent to develop a method to detect acid orange dyes and basic orange dyes simultaneously for the assurance of food safety and human health.

Several methods for the determination of dyes have been reported, including titrimetry,[10] voltammetry,[11] spectrophotometry,[12] thin layer chromatography,[13] and high-performance liquid chromatography (HPLC).[14−16] Titrimetry, voltammetry, spectrophotometry, and thin layer chromatography are simple and time-saving methods, but these methods need a large sample volume and are not suitable for the determination of multiple food color mixtures in the complicated food matrix. Furthermore, these methods are relatively poor in sensitivity and reproducibility. HPLC,[17−20] especially ion-pair HPLC with an ultraviolet−visible spectrophotometry (UV−vis) or diode-array detector (DAD), is a commonly used determination technique for water-soluble dyes. However, it is not sufficient for their structural identification by spectrophotometry because of much spectral interference arising from intermediates, homologues of dyes. These limitations are significantly alleviated by the applications of high-performance liquid chromatography−tandem mass spectrometry (LC-MS/MS), owing to its high selectivity and specificity. In recent years, LC-MS/MS[21−25] that combines the high separation performance with precursor and fragment information has been reported for the multiresidue analysis in food and the environment. Under multiple reaction mode (MRM), it can distinguish and identify targets from background matrix ions, which can increase sensitivity due to the reduction of background signals.[26] Therefore, LC-MS/MS was chosen for the detection of acid orange dyes and basic orange dyes.

In this work, a sensitive and accurate LC-MS/MS method with a solid-phase extraction (SPE) procedure for the simultaneous analysis of four acid orange dyes and three basic orange dyes in a food matrix was developed. The chromatogram of the seven dyes can be divided into seven segments, which means the seven dyes can be detected in negative or positive ion mode, respectively, by a single instrumental method, and the performance of this method was greatly improved. MS parameters were optimized, and the ionization behavior and the MS/MS fragmentation behavior of dyes were researched. The influences of buffer pH, gradient elution step, column temperature, SPE condition, and extract condition were investigated. The structural information of the seven compounds is shown in Figure 1.

MATERIALS AND METHODS

Materials. Chromatographic pure methanol that was purchased from Merk (Darmstadt, Germany) and ultrapure Milli-Q water that was obtained from Millipore were used as mobile phase and for the preparation of solutions. Basic orange 2 (B2) and acid orange 52 (A52) were obtained from Chemservice (West Chester, PA, USA), basic orange 21 (B21) was obtained from Sigma-Aldrich (St. Louis, MO, USA), acid orange 7 (A7) and acid orange 6 (A6) were received from Dr. Ehrenstorfer (Augsburg, Germany), and acid orange 20 (A20) and basic orange 22 (B22) were bought from TCI (Tokyo, Japan).

Disposable SPE Strata-X-AW cartridges (60 mg, 3 mL) were obtained from Phenomenex (Torrance, CA, USA), which were used in the purification step. A 0.22 μm Teflon syringe filter was bought from Whatman (Maidstone, UK).

Table 1. Scan Segments and the Corresponding Monitoring Fragments

start time (min)	scan type	div valve	ESI	ΔEMV(+)	ΔEMV(−)	compounds
0	MRM	to waste		0	0	
0.5	MRM	to MS	−	0	400	A6
1.3	MRM	to MS	−	0	400	A20, A52
3	MRM	to MS	−	0	400	A7
6.5	MRM	to MS	+	0	0	B2
9	MRM	to MS	+	0	0	B21, B22
22	MRM	to waste		0	0	

Table 2. MS/MS Parameters Used for Quantifications of Seven Dyes

standard	ESI	precursor ion (m/z)	product ion (m/z)	collision energy (V)	fragmentor (V)	dwell time (ms)
B2	+	213.0	121.0[a]	25	120	150
			77.0[b]	15		
B21	+	315.2	285.0[a]	40	135	80
			300.2[b]	20		
B22	+	391.5	219.1[a]	35	160	80
			376.2[b]	30		
A6	−	293.1	156.1[b]	30	135	150
			171.0[a]	15		
A52	−	304.1	80.1[a]	65	155	80
			156.1[b]	25		
A20	−	327.1	247.2[b]	15	135	80
			171.0[a]	15		
A7	−	327.1	156.2[a]	30	135	150
			171.0[b]	15		

[a]Represents confirming ion. [b]Represents quantitation.

Standard stock solutions (1.0 mg mL^{-1}) of dyes were prepared in methanol and then stored at −20 °C in the dark. Working standards were acquired via the dilution of these standard stock solutions daily.

All of the food samples such as dried beancurd sticks, oil tofu skins, and spiced eggs were purchased from TESCO supermarket (Tianjin, China). Dried beancurd sticks and oil tofu skins were ground up by grinder and stored at 4 °C. Spiced eggs were crushed in a mortar and then stored at 4 °C.

Sample Treatment. At first, 1.00 g of homogenized sample and 10 mL of *n*-hexane were mixed by magnetic stirrer for 20 min, and then the *n*-hexane layer was discarded after centrifuging (5000 rpm) for 20 min to eliminate fat. Second, the sample was extracted with 5 mL of acetonitrile/ammonia−water (9:1, v/v) for 30 min in an ultrasonic bath and centrifuged (5000 rpm) for 20 min; this step was repeated twice again. The evaporation residues of merged extracts were redissolved in 10 mL of deionized water as the loading solution of SPE. Third, 10 mL of sample solution was loaded on the Strata-X-AW cartridge before which the cartridge was preconditioned with 10 mL of methanol and 10 mL of water. Five minutes later, the cartridge was rinsed with 5 mL of *n*-hexane and 5 mL of water/methanol (2:8, v/v). After that, the targets were eluted with 18 mL of ethanol that contained 10% (v/v) ammonia−water and evaporated to dryness by rotary evaporator. Finally, the evaporation residue was redissolved in 1

dx.doi.org/10.1021/jf400619y | J. Agric. Food Chem. 2013, 61, 3834−3841

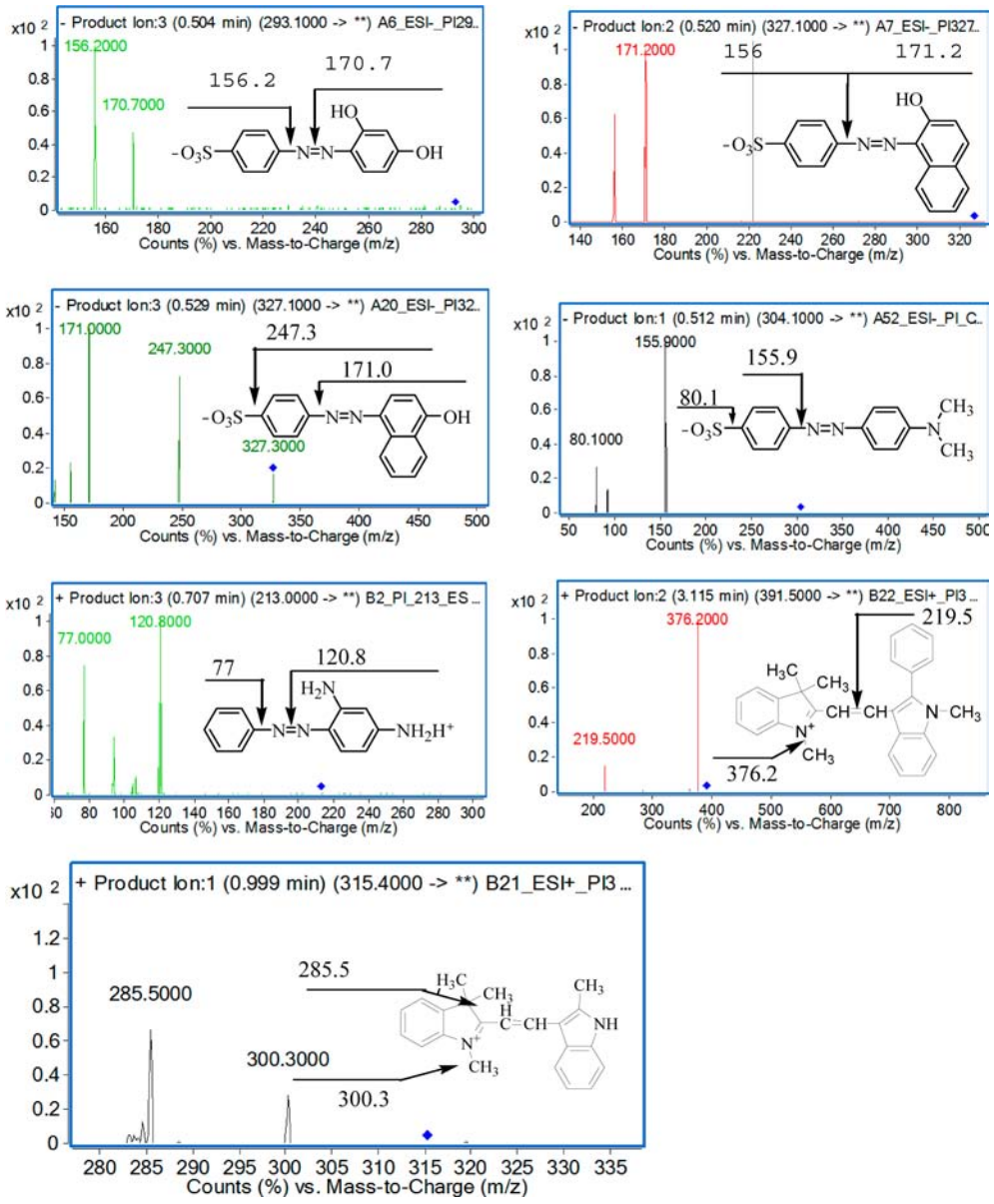

Figure 2. Product ion mass spectra and proposed dissociation mode of seven dyes.

mL of methanol, and then it was filtered through a 0.22 μm Teflon syringe filter for further analysis.

HPLC. A 1200 series LC system, which consists of a vacuum degasser, a binary pump, an autosampler, and a thermostatic column compartment (Agilent, Santa Clara, CA, USA), was used for the separation of dyes. The separation of the seven dyes was achieved by a Zorbax Eclipse plus C_{18} column (2.1 × 150 mm, 3.5 μm; Agilent) at 35 °C. The flow rate of the mobile phase was 0.3 mL min^{-1}, and the injection volume was 1 μL. The mobile phase was water (the pH was adjusted to 9 with ammonia) (A) and methanol (B) with gradient elution steps as follows: 40% of B increased to 95% in 4.5 min and kept at 95% until to 18 min and then returned to the original proportion within 2 min. At last, another 10 min was needed to ensure the stability of baseline for the next injection.

ESI-MS/MS. An Agilent 6410 triple-quadrupole mass spectrometer equipped with electrospray ionization (ESI) was used for dye detection. ESI-MS/MS spectra were acquired via positive and negative ion switching mode with MS/MS detection in MRM. The ESI source temperature was 350 °C. Nitrogen gas was used as both dry gas with a flow rate of 10 L min^{-1} and collision gas. The nebulizer pressure was 40 psi, and the capillary voltage was 4 kV. The analysis was performed in seven time segments (listed in Table 1) to reach the best response for each target. The MS/MS operation parameters of the analytes were optimized by introducing the single standard solution of dyes. The detail parameters of the dyes are listed in Table 2. Data acquisition and processing were carried out via MassHunter workstation software.

RESULTS AND DISCUSSION

Optimization of MS/MS Parameters. Positive ion mode is the preferred mode when the subjects contain a secondary amino group or a tertiary amino group due to their protonated nature. Therefore, basic orange dyes with amine groups are detected in positive ion mode. The acidic subjects, especially the subjects that own a chlorine, bromine, or multihydroxy group, are usually determined in negative ion mode due to their deprotonated property. Therefore, acid orange dyes with sulfonic groups were analyzed in negative ion mode. The MS/MS conditions were optimized individually for each subject by injecting 100 μg mL^{-1} standard solution into MS/MS with a mobile phase of acetonitrile and water (50:50, v/v) at a flow rate of 0.3 mL min^{-1}. The precursor ions were found in scan mode individually. The results showed that acid orange dyes'

dx.doi.org/10.1021/jf400619y | J. Agric. Food Chem. 2013, 61, 3834−3841

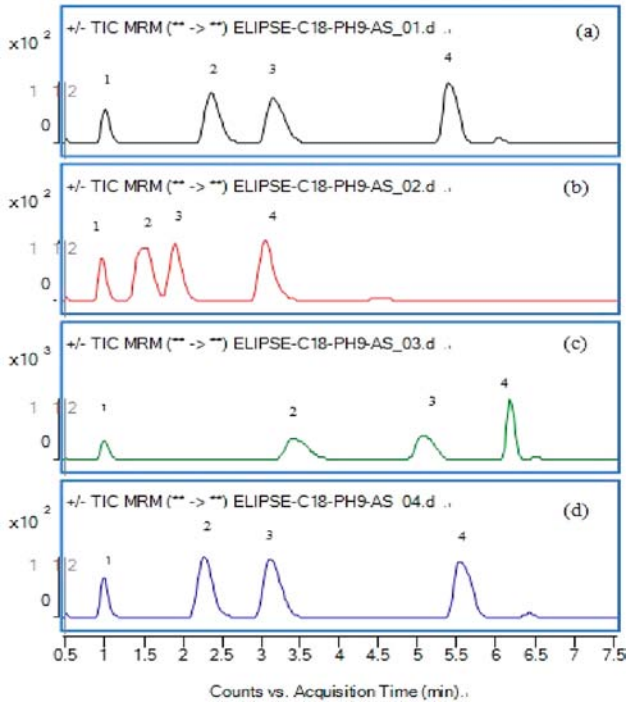

Figure 3. TIC of acid orange dyes with different gradient elution programs. The peak order is (1) A6, (2) A20, (3) A52, and (4) A7. The gradient elution programs were (a) 0 min, 40% B, 4.5 min, 95% B, 8 min, 95% B, 10 min, 40% B; (b) 0 min, 45% B, 4.5 min, 95% B, 8 min, 95% B, 10 min, 45% B; (c) 0 min, 35% B, 4.5 min, 95% B, 8 min, 95% B, 10 min, 35% B; and (d) 0 min, 40% B, 4.5 min, 80% B, 8 min, 80% B, 10 min, 40% B.

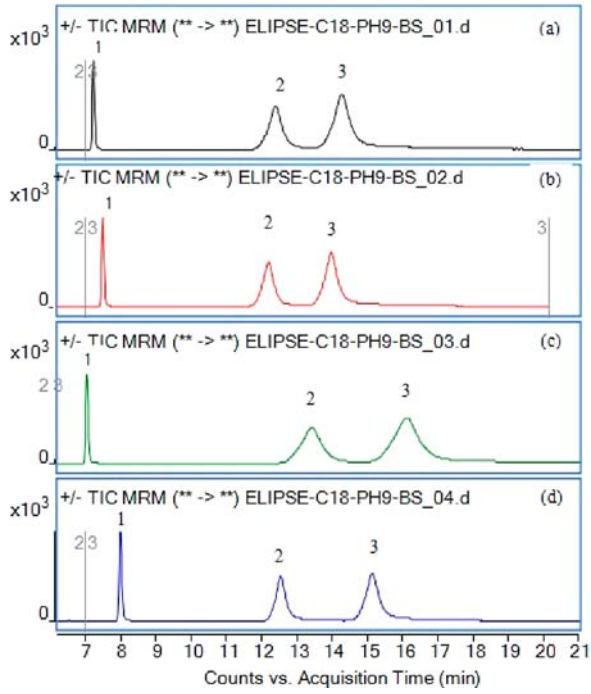

Figure 4. TIC of basic orange dyes with different gradient elution programs. The peak order is (1) B2, (2) B21, and (3) B22. The gradient elution programs were (a) 0 min, 40% B, 4.5 min, 95% B, 18 min, 95% B, 20 min, 40% B; (b) 0 min, 40% B, 4.5 min, 90% B, 18 min, 90% B, 20 min, 40% B; (c) 0 min, 40% B, 4.5 min, 100% B, 18 min, 100% B, 20 min, 40% B; and (d) 0 min, 40% B, 4.5 min, 80% B, 18 min, 80% B, 20 min, 40% B.

precursor ions were sodium-deducted molecules of $[M - Na]^-$. As for basic orange dyes, ions of $[M - Cl]^+$ were characterized as precursor ions. This study indicated that the ionization behavior of ESI-MS was determined by the polar functional groups in dye molecules. To ensure the precursor ion produces the best response, in-source fragmentation voltage was optimized from 100 to 160 V for each dye in selective reaction monitoring scan mode. The response of precursor ions would be decreased because a relatively lower fragmentation voltage would not make precursor ions transfer into the capillary adequately and precursor ions would be destroyed if the fragmentation voltage was too high. The product ions were studied in product ion scan mode under different collision energies, and the results of the daughter ion mass spectra, which revealed the fragmentation patterns of dyes, are shown in Figure 2. Subsequently, to make the product ions reach the best abundance, the collision energy for each product ion was optimized by comparing peak areas. The optimum MS parameters for each dye are summarized in Table 2.

Optimization of HPLC Conditions. To establish the best HPLC conditions of the seven dyes, the pH and composition of the mobile phase, gradient elution program, and column temperature effects on the response and separation of the seven dyes were studied. In this study, when the mobile phase was methanol and water, both acid orange dyes and basic orange dyes had a better response than when the mobile phase was acetonitrile and water. Therefore, methanol and water were chosen as mobile phase.

The pH of the mobile phase has a significant effect on the response, peak shape, and separation of analytes, so pH values from 3 to 9 were studied. At pH 9, acid orange dyes reached the highest response (10^4), and the responses of basic orange dyes were 10^5.

To baseline separate the seven dyes, the gradient program was researched. Because the MS spectrometer cannot achieve negative and positive ion mode switch instantaneously and frequently, the gradient programs of acid orange dyes and basic orange dyes were optimized separately. For acid orange dyes, four kinds of gradients were studied, and the results are shown in Figure 3. For gradient b, the elution ability of 45% acetonitrile was too strong so that A20 and A52 were eluted together. On the contrary, for gradient c, the elution ability of 35% methanol was relatively poor, leading to long elution time. Four acid orange dyes were baseline separated within 6 min in gradients a and d; therefore, the initial proportion of methanol was 40%, and it should be increase to above 80% in 4.5 min. Considering this, four kinds of gradients for three basic orange dyes were studied. As can be seen in Figure 4, three basic orange dyes had a better resolution and response in a shorter time with gradient a.

To shorten the retention time, the temperature of the column was optimized. Results indicated that the responses of the seven dyes were fortified and the retention time was shortened as the temperature increased. However, silica gel in the column would easily dissolve out, and the life of the column would shorten with the temperature increasing when the pH was >6. The temperature would better be <40 °C at a mobile phase pH of 9, and 35 °C was chosen as the column temperature.

Solvents of the working solution mainly have an influence on the separation and peak shape of analytes, which are eluted earlier. In this work, the initial mobile phase (60% water/40%

dx.doi.org/10.1021/jf400619y | J. Agric. Food Chem. 2013, 61, 3834−3841

Table 3. Recoveries of Seven Dyes in Actual Samples with Different Concentrations (%, RSD, n = 3)

analyte	spike level (μg/kg)	dried beancurd sticks	fried tofu skin	spiced corned eggs
A6	0	ND	ND	ND
	10	77 (11.9)	74 (2.22)	79 (12.0)
	50	89 (13.4)	80 (8.29)	83 (9.55)
	100	87 (11.1)	83 (14.1)	85 (9.93)
A20	0	0.74	ND	3.15
	10	91 (19.4)	92 (25.4)	94 (23.0)
	50	95 (12.9)	92 (12.2)	90 (11.0)
	100	91 (9.43)	95 (7.76)	91 (7.24)
A52	0	1.19	0.08	2.66
	10	104 (15.5)	97 (20.3)	99 (19.3)
	50	103 (14.9)	92 (10.6)	97 (11.4)
	100	91 (12.91)	98 (6.63)	94 (7.33)
A7	0	2.14	ND	2.75
	10	103 (10.3)	102 (15.2)	95 (17.2)
	50	91 (19.0)	97 (11.0)	93 (12.2)
	100	98 (10.3)	92 (11.7)	91 (11.7)
B2	0	1.64	ND	0.97
	10	126 (18.2)	92 (12.4)	92 (17.1)
	50	107 (11.6)	103 (12.6)	94 (12.2)
	100	81 (9.42)	85 (8.30)	83 (8.92)
B21	0	2.66	0.72	5.44
	10	109 (21.1)	111 (18.6)	118 (17.6)
	50	98 (10.5)	91 (13.3)	91 (15.5)
	100	87 (9.42)	90 (8.65)	80 (6.45)
B22	0	2.60	0.84	4.24
	10	107 (15.3)	117 (15.1)	107 (9.97)
	50	86 (9.02)	89 (8.40)	95 (9.41)
	100	79 (7.61)	89 (5.98)	77 (5.66)

methanol), ultrapure water, and methanol were chosen as the solvents of the working solution, and their influence on the response of the seven dyes was researched. The results showed that the response and separation of the seven dyes were best when the solvent was methanol. This was attributed to methanol being more volatile than water and promoted the nebulization efficiency of the solvent.

Optimization of the SPE Procedure. X-AW SPE columns possess both nonpolar and weak anion exchange forces. Acid orange dyes could be adsorbed on the X-AW SPE column by the force of weak ionic exchange because of their sulfonic groups. Basic orange dyes were adsorbed by the nonpolar force owing to their possessing benzene rings. Therefore, X-AW SPE columns are chosen for the purification of extaction mix. In the process of purification, ionic and nonionic impurities in the matrix could be scoured off without losing targets through pH adjustment. The pH of the extraction mix was evaluated in the range of 3−9, and the seven dyes achieved maximum adsorption at pH 6. This is because most of the acid orange dyes existed as an ionic state and most basic orange dyes existed as neutral at pH 6, and the seven dyes interact adequately with adsorbents.

To remove the impurities and improve the recoveries of the seven dyes, a washing solution that consists of different proportions of water and methanol was researched. Results showed that basic orange dyes were lost as the methanol proportion increased because methanol could destroy the nonpolar force. Therefore, water/methanol (8:2, v/v) was used to eliminate the impurities with the recoveries of the seven dyes ranging from 75 to 110%.

To destroy the force between dyes and adsorbents, the presence of ammonia in the elution was studied. The results indicated that 10% of ammonia (v/v) in the elution could maximally elute the seven dyes. If the content of ammonia was lower than 10%, it would not destroy the force between dyes and absorbents. In contrast, it would destroy the structures of dyes and lead to low recoveries.

Organic solvents have different elution abilities, so the choice of elution solvents was important. In this study, six common solvents (including n-hexane, ethyl acetate, dichloromethane, acetonitrile, methanol, and ethanol) with different polarities and selectivities were studied, and ethanol was chosen as elution solvent according to the results, because ethanol not only has a good solubility for the seven dyes but also can destroy the force between the adsorbent and dyes.

To shorten the time of the SPE procedure and desolvation, the elution volume should be as small as possible under the condition of eluting the seven dyes completely. Results showed that 6 mL of elution could elute 85% of the acid orange dyes, but the basic orange dyes were under 80% until the volume was 18 mL.

Optimization of the Extraction Procedure. As dyes mainly bond with proteins, sample extraction plays an important role in real sample analysis. In this work, ultrasonic extraction, a simple and convenient method, was chosen as sample extraction method. In the ultrasonic extraction, extraction solvent and its pH were mainly factors of extraction efficiency. The influences of ethanol and acetonitrile that contained 10% ammonia (v/v) on the extraction efficiency were studied. Results indicated that acetonitrile could make protein easier to precipitate, and the extraction efficiency of acetonitrile ranged from 79 to 115%. The content of ammonia in the acentrile on the influence of extraction has been studied, and the results showed that 10% ammonia (v/v) in the acetonitrile reached the highest extraction efficiency with recoveries of 78−109%.

Matrix Effect. The matrix effect[27,28] can suppress or enhance the signal and severely interfere with quantitative analysis of targets in trace levels and method reproducibility. Matrix effects were studied by comparing the slopes of solvent calibration curves and matrix-matched calibration curves at 10 concentration levels (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 50, and 100 μg L^{-1}). Matrix-matched calibration curves were constructed by adding different concentrations of standards into the matrix. Marinated eggs and bean products were examined. It was revealed[29] that the signal of the target was enhanced if the percentage of the difference between these two slopes was positive, whereas if this percentage was negative, the signal was suppressed. According to the value of this percentage, different matrix effects[9,25] could be observed: if the values were −20 to 0% and 0−20%, respectively, weak signal suppression or enhancement had happened; it was considered to be of medium matrix effect if the values were between −50 and −21% or between 21 and 50%; and if the value were below −51% or above +51%, a strong signal

dx.doi.org/10.1021/jf400619y | J. Agric. Food Chem. 2013, 61, 3834−3841

Table 4. Reported Detection Limit of the Acid Orange Dyes and Basic Orange Dyes

detection method	sample	sample treatment	analyte	LOD
HPLC-UV−vis[17]	tap water	C_{18} SPE	A7	7 μg/L
			A12	21 μg/L
			A52	7 μg/L
HPLC-DAD[20]	drinks, sugars, jam	polyamide column	A6	36 μg/L
			A7	40 μg/L
			A10	38 μg/L
			A12	39 μg/L
			A20	17 μg/L
LC-MS-MS[9]	spice	LLE	A7	6.6−61.2 μg/kg
CE-DAD[30]	river water	SPE Oasis HLB	A7	900 μg/L
			A12	1820 μg/L
			A52	430 μg/L
HPLC-DAD-MS[7]	drinks, jam, salted vegetables	LLE	A7	10 μg/L
			A20	10 μg/L
HPLC-DAD[8]	drinks, chilli powder, bean products	LLE	B2	10 μg/kg
			B21	10 μg/kg
			B22	10 μg/kg
UPLC-MS[31]	drinks, wine	LLE	B2	7.5 μg/kg
			A7	5 μg/kg
LC-MS-MS (this paper)	bean product, spiced corned eggs	X-AW SPE	A6	2.0−3.0 μg/kg
			A20	3.0 μg/kg
			A52	0.5−1.0 μg/kg
			A7	1.0−2.0 μg/kg
			B2	0.5−1.0 μg/kg
			B21	0.5−1.0 μg/kg
			B22	0.5−1.0 μg/kg

suppression or enhancement could be observed. In this study, the matrix effects of bean products were 1.13%, implying weak enhancement. The matrix effects of egg products were 12.90%, which indicated that signals of the targets were enhanced slightly. As a result, the matrix effects were weak and could be ignored. Therefore, solvent calibrations could be directly used for quantification of the seven dyes.

Performance of the LC-MS/MS Method. To evaluate the overall performance of the method, selectivity, linearity, sensitivity, and repeatability were studied, and the results are shown in Tables 3 and 4. Selectivity was verified by comparing the TIC of 100 μg L^{-1} dye standards in pure solvents and in matrix, respectively, and the results show in Figure 5 that no interferences were observed in corresponding retention times of the seven dyes. Therefore, this method had high selectivity for both acid orange dyes and basic orange dyes. The linearity of the method was built by using a 10 mL spiked deionized water solution at 10 levels (ranging from 0.005 to 100 μg L^{-1}), which were purified by the optimum SPE procedure first. All seven dyes displayed good linearity over the range of 5−500 μg L^{-1} with satisfactory correlation coefficients (R^2) exceeding 0.9998. The accuracy of the seven dyes in spiked blank bean products and egg products (six replicates) at three levels (10, 50, and 100 μg L^{-1}) were 79−112 and 79−116% with RSDs ranging from 4.74 to 18.1% and from 5.10 to 19.9%, respectively, which showed great stability and repeatability. The limits of detection (LODs) and limits of quantification (LOQs) of the seven dyes for bean products and egg products were detected in MRM mode; the LODs and LOQs were based on the lowest spiked level able to produce a signal-to-noise ratio (S/N) of 3 or 10, respectively. LODs and LOQs of the seven dyes were 0.5−3.0 and 2.0−6.0 μg kg^{-1}, respectively, which indicated that this method had a high sensitivity for the seven dyes.

Application to Real Samples. The feasibility of the proposed method was investigated through testing the nonspiked and spiked samples (dried beancurd sticks, fried tofu skin, spiced corned eggs) at three concentration levels (10, 50, and 100 μg kg^{-1}). Results in Table 3 show that the recoveries of the seven dyes were in the range of 74−126% with RSDs in the range of 2.22−25.4%. All of the results indicated that the established method has great potential for the accurate quantification of the seven dyes.

In this study, a sensitive and accurate method for the simultaneous quantitation of acid orange dyes and basic orange dyes in foods was developed, using LC-MS/MS via negative/positive ion switching mode. Samples were purified by X-AW SPE columns. Linearity, reproducibility, and LODs were improved over the previous results shown in Table 4. Furthermore, this method was stable with RSDs in the range of 2.22−25.4%. All of the results showed that this method has

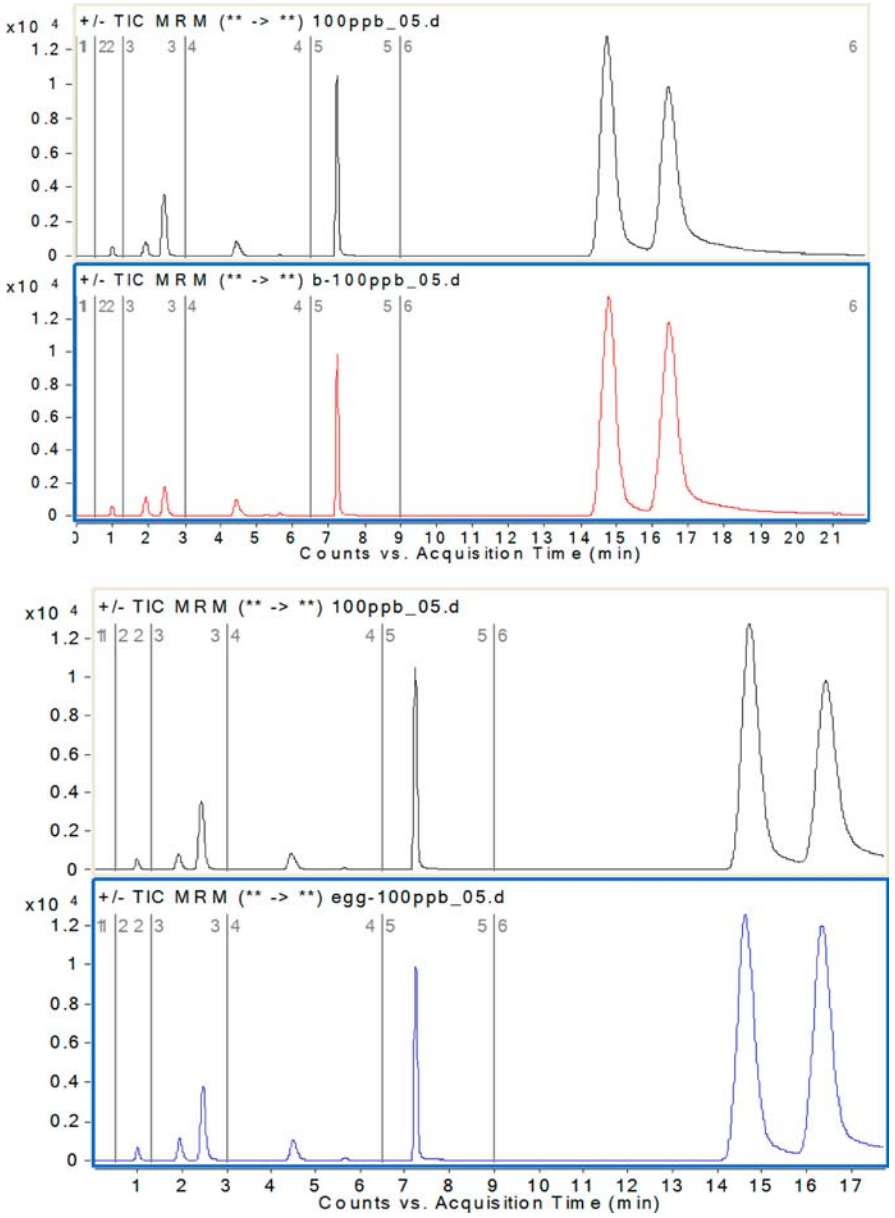

Figure 5. Comparison of TIC of 100 μg L^{-1} dye standards in pure solvents and in matrix, respectively.

great potential for the accurate simultaneous quantification of acid orange dyes and basic orange dyes in trace levels.

AUTHOR INFORMATION

Corresponding Author

*Phone: +86 22 6060 1430. Fax: +86 22 6060 1332. E-mail: s.wang@tust.edu.cn.

Funding

This work was supported by the Ministry of Science and Technology of China (Projects 2011BAD23B05-4 and 2012BAK17B03), the Special Fund for Agroscientific Research in the Public Interest (Project 201203069), the Tianjin Municipal Science and Technology Commission (Project 10SYSYJC28300), and the Program for Changjiang Scholars and Innovative Research Team in University (Project IRT1166).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Robens, J. F. Thirteen-week subchronic toxicity studies of Direct Blue 6, Direct Black 38, and Direct Brown 95 dyes. *Toxicol. Appl. Pharmacol.* **1980**, *54*, 431−442.

(2) Boeniger, M. *Carcinogenicity and Metabolism of Azo Dyes, Especially Those Derived from Benzidine*; NIOSH (NTIS): Springfield, VA, 1980.

(3) Hueper, W. C. Occupational and environmental cancers of the urinary system. *Br. J. Surg.* **1970**, *57*, 940.

(4) Wang, C.-C. Adsorption of basic dyes onto montmorillonite. *J. Colloid Interface Sci.* **2004**, *273*, 80−86.

(5) Wang, S.; Boyjoo, Y.; Choueib, A.; Zhu, Z. H. Removal of dyes from aqueous solution using fly ash and red mud. *Water Res.* **2005**, *39*, 129−138.

(6) GB 2760-2011, Using standard of food additives, National Standard of People's Republic of China , 2011.

(7) Fuh, M. R.; Chia, K. J. Determination of sulphonated azo dyes in food by ion-pair liquid chromatography with photodiode array and electrospray mass spectrometry detection. *Talanta* **2002**, *56*663−671.

(8) Wen, H. *Studies on the National Standard Method of Determination of the Dyes of Basic Oranges in Food*; Huazhong Agricultural University, 2009.
(9) Ferrer Amate, C.; Unterluggauer, H.; Fischer, R. J.; Fernandez-Alba, A. R.; Masselter, S. Development and validation of a LC-MS/MS method for the simultaneous determination of aflatoxins, dyes and pesticides in spices. *Anal. Bioanal. Chem.* **2010**, *397*, 93−107.
(10) Park, D. L.; Reynolds, H.; Senzel, A. J.; Horwitz, W. *Official Methods of Analysis of the Association of Official Analytical Chemists*; AOAC: Washington, DC, 1975.
(11) Fogg, A. G.; Summan, A. M. Differential-pulse polarographic monitoring of permitted synthetic food colouring matters and ascorbic acid in accelerated light degradation studies and the spectrophotometric determination of the ammonia and simpler amines formed. *Analyst* **1983**, *108*, 691−700.
(12) Graichen, C. Quantitative determination of FD&C colors in foods. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 278.
(13) Young, M. L. Rapid identification of color additives, using the C18 cartridge: collaborative study. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 458−461.
(14) Yang, Y.; Yin, J.; Shao, B. Simultaneous determination of five aluminum lake dyes in chewing gum by HPLC with photodiode array detection. *Food Addit. Contam. Part A: Chem. Anal. Control Exposure Risk Assess.* **2011**, *28*, 1159−1167.
(15) Sun, S. Determination of Sudan dyes in red wine and fruit juice using ionic liquid-based liquid-liquid microextraction and high-performance liquid chromatography. *J. Sep. Sci.* **2011**, *34*, 1730−1737.
(16) Liu, R.; Hei, W.; He, P.; Li, Z. Simultaneous determination of fifteen illegal dyes in animal feeds and poultry products by ultra-high performance liquid chromatography tandem mass spectrometry. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2011**, *879*, 2416−2422.
(17) Perez-Urquiza, M.; Prat, M. D.; Beltran, J. L. Determination of sulphonate dyes in water by ion-interaction high-performance liquid chromatography. *J. Chromatogr., A* **2000**, *871*, 227−234.
(18) Zatar, N. A. Simultaneous determination of seven synthetic water-soluble food colorants by ion-pair reversed-phase high-performance liquid chromatography. *J. Food Technol.* **2007**, *5*, 220−224.
(19) Ma, M.; Luo, X.; Chen, B.; Su, S.; Yao, S. Simultaneous determination of water-soluble and fat-soluble synthetic colorants in foodstuff by high-performance liquid chromatography−diode array detection−electrospray mass spectrometry. *J. Chromatogr., A* **2006**, *1103*, 170−176.
(20) Yoshioka, N.; Ichihashi, K. Determination of 40 synthetic food colors in drinks and candies by high-performance liquid chromatography using a short column with photodiode array detection. *Talanta* **2008**, *74*, 1408−1413.
(21) Chen, G.; Miao, S. HPLC determination and MS confirmation of Malachite Green, Gentian Violet, and their leuco metabolite residues in channel catfish muscle. *J. Agric. Food Chem.* **2010**, *58*, 7109−7114.
(22) Tateo, F.; Bononi, M. Fast determination of Sudan I by HPLC/APCI-MS in hot chilli, spices, and oven-baked foods. *J. Agric. Food Chem.* **2004**, *52*, 655−658.
(23) Ding, Y.; Sun, C.; Xu, X. Simultaneous identification of nine carcinogenic dyes from textiles by liquid chromatography/electrospray ionization mass spectrometry via negative/positive ion switching mode. *Eur. J. Mass Spectrom. (Chichester, Engl.)* **2009**, *15*, 705−713.
(24) Liu, X. Analysis of water-soluble azo dyes in soft drinks by high resolution UPLC-MS. *Food Addit. Contam. A* **2011**, *28*, 1315−1323.
(25) Sun, H. W.; Wang, F. C.; Ai, L. F. Determination of banned 10 azo-dyes in hot chili products by gel permeation chromatography-liquid chromatography-electrospray ionization-tandem mass spectrometry. *J. Chromatogr., A* **2007**, *1164*, 120−128.
(26) Makarov, A.; Scigelova, M. Coupling liquid chromatography to Orbitrap mass spectrometry. *J. Chromatogr., A* **2010**, *1217*, 3938−3945.
(27) Antignac, J. P. The ion suppression phenomenon in liquid chromatography−mass spectrometry and its consequences in the field of residue analysis. *Anal. Chim. Acta* **2005**, *529*, 129−136.
(28) Fernández-Alba, A. R. *Chromatographic-Mass Spectrometric Food Analysis for Trace Determination of Pesticide Residues*; Elsevier Science Limited: Amsterdam, The Netherlands, 2005; Vol. *43*.
(29) Wu, Y. L.; Li, C.; Xia, X.; Liu, Y. J.; Shen, J. Z. Development and validation of a confirmatory HPLC method for simultaneous determination of Sudan dyes in animal tissues and eggs. *J. Chromatogr. Sci.* **2010**, *48*, 63−67.
(30) Perez-Urquiza, M.; Ferrer, R.; Beltran, J. L. Determination of sulfonated azo dyes in river water samples by capillary zone electrophoresis. *J. Chromatogr., A* **2000**, *883*, 277−283.
(31) Zhao, S.; Zhang, J.; Yang, Y.; Shao, B. Determination of 27 industrial dyes in juice and wine using ultra performance liquid chromatography with electrospray ionization tandem quadrupole mass spectrometry. *Se Pu* **2010**, *28*, 356−362.