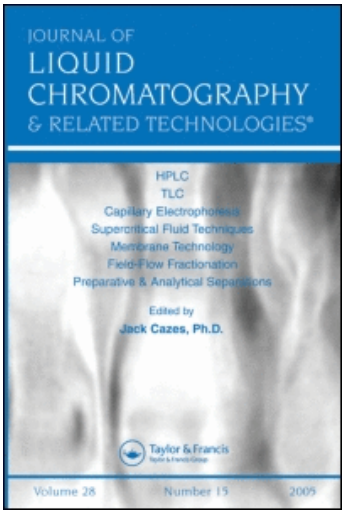

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HIGHLY SENSITIVE DETERMINATION OF 10 DYES IN FOOD WITH COMPLEX MATRICES USING SPE FOLLOWED BY UPLC-DAD-TANDEM MASS SPECTROMETRY

Chao Ji[ab]; Feng Feng[b]; Zhengxing Chen[a]; Xiaogang Chu[b]
[a] State Key Laboratory of Food Science and Technology, Jiang Nan University, Wuxi, China [b] Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing, China

Online publication date: 07 January 2011

To cite this Article Ji, Chao , Feng, Feng , Chen, Zhengxing and Chu, Xiaogang(2011) 'HIGHLY SENSITIVE DETERMINATION OF 10 DYES IN FOOD WITH COMPLEX MATRICES USING SPE FOLLOWED BY UPLC-DAD-TANDEM MASS SPECTROMETRY', Journal of Liquid Chromatography & Related Technologies, 34: 2, 93 — 105
To link to this Article: DOI: 10.1080/10826076.2010.526876
URL: http://dx.doi.org/10.1080/10826076.2010.526876

Journal of Liquid Chromatography & Related Technologies, 34:93–105, 2011

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2010.526876

Taylor & Francis
Taylor & Francis Group

HIGHLY SENSITIVE DETERMINATION OF 10 DYES IN FOOD WITH COMPLEX MATRICES USING SPE FOLLOWED BY UPLC-DAD-TANDEM MASS SPECTROMETRY

Chao Ji,[1,2] Feng Feng,[2] Zhengxing Chen,[1] and Xiaogang Chu[2]

[1]*State Key Laboratory of Food Science and Technology, Jiang Nan University, Wuxi, China*

[2]*Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing, China*

□ *A rapid and highly sensitive determination of 10 water-soluble dyes (E102, E110, E122, E123, E124, E127, E129, E131, E132, and E133) in food has been developed by combining HLB SPE column pretreatment with ultra-performance liquid chromatography-diode array detector-tandem mass spectrometry (UPLC-DAD-MS/MS). The developed method displayed a fast analyzing speed (less than 3 min), low limits of detection (LOD < 0.04 μg/mL, S/N = 3) and excellent recoveries (ranging from 92.1 to 105.4%) for 10 dyes. This work also demonstrated that the signal responses of different dyes to DAD detector and MS/MS detector were inconsistent, thereby using DAD and MS/MS detector simultaneously could improve the sensitivity and accuracy of determination. This method has been successfully applied to determining the concentration of the 10 dyes in shrimp flake and drinks, and it is valuable for analytical control of dyes to ensure food safety.*

Keywords DAD, dyes, ESI-MS/MS, solid-phase extraction, UPLC

INTRODUCTION

Synthetic dyes are widely used to compensate for the loss of natural colors of food which are destroyed during processing and storage and to provide the desired colored appearance.[1] Although the allowable amount of synthetic dyes is reduced for consumer health reasons in recent years, many kinds of synthetic food dyes are still widely used all over the world due to their low price, high effectiveness, and excellent stability.[2] In China, the strict restriction list and maximum permissible concentration of synthetic

Address correspondence to Zhengxing Chen, State Key Laboratory of Food Science and Technology, Jiang Nan University, 1800 Lihu Road, Wuxi, China. E-mail: zxchen2007@126.com

food dyes had been established by the Ministry of Health.[3] However, some illegal producers probably add some disallowed dyes or excessive quantity of dyes in their products. Thus, it is necessary to develop a rapid and accurate method for the determination of synthetic dyes in foods to ensure the food safety.

Currently, capillary electrophoresis (CE),[4–6] thin-layer chromatography,[7] reversed-phase liquid chromatography (RPLC),[8–14] and ion-pair RPLC[15–17] have been employed for the analysis of water-soluble synthetic dyes. Among these methods, HPLC coupled with ultraviolet/visible (UV/Vis) or diode-array detectors (DAD) are the most commonly used methods.[9,10] However, the complex matrices in foods often influence the determination.[6,18] Under this condition, the selective detection by tandem mass spectrometry may partially solve this dilemma. In the mode of multiple-reaction monitoring (MRM) when a triple quadrupole mass spectrometry is adopted, the specific MS transition (parent ions → product ions) can preclude the presence of interference substances, thus the quantified accuracy could be improved. Unfortunately, our result demonstrated that the ionization efficiencies of some water-soluble dyes are very low, and the LOD of some dyes obtained from MS/MS (MRM) detector are even 10 times higher than it obtained from DAD detector. Therefore, the ultraviolet/visible (UV/Vis) or DAD detector is still necessary for the sensitive determination of dyes. In this work, we developed a DAD plus MS/MS dual quantification mode for rapid and accurate determination of 10 water-soluble dyes, tartrazine (E102), amaranth (E123), indigo carmine (E132), ponceau 4R (E124), sunset yellow FCF (E110), allura red AC (E129), brilliant blue FCF (E133), azorubine (E122), patent blue V (E131), and erythrosine (E127) in shrimp flakes and drinks. The developed method not only improved working efficiency (less than 3 min for one separation), but also enhanced the accuracy and sensitivity.

EXPERIMENTAL

Chemicals and Reagents

HPLC grade methanol was purchased from Fisher (Pittsburgh, PA, USA). The ultrapure water was prepared by the Milli-Q water system (Millipore, Bedford, MA, USA). Analytical grade ammonium formate and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). The dyes of E102, E110, E123, E124, E129, and E133 were obtained from Fluka (Buchs, Switzerland), E122, E132, E131, E127, and E120 were obtained from Sigma-Aldrich (St. Louis, MO, USA). The chemical structure of these dyes can be seen in Figure 1.

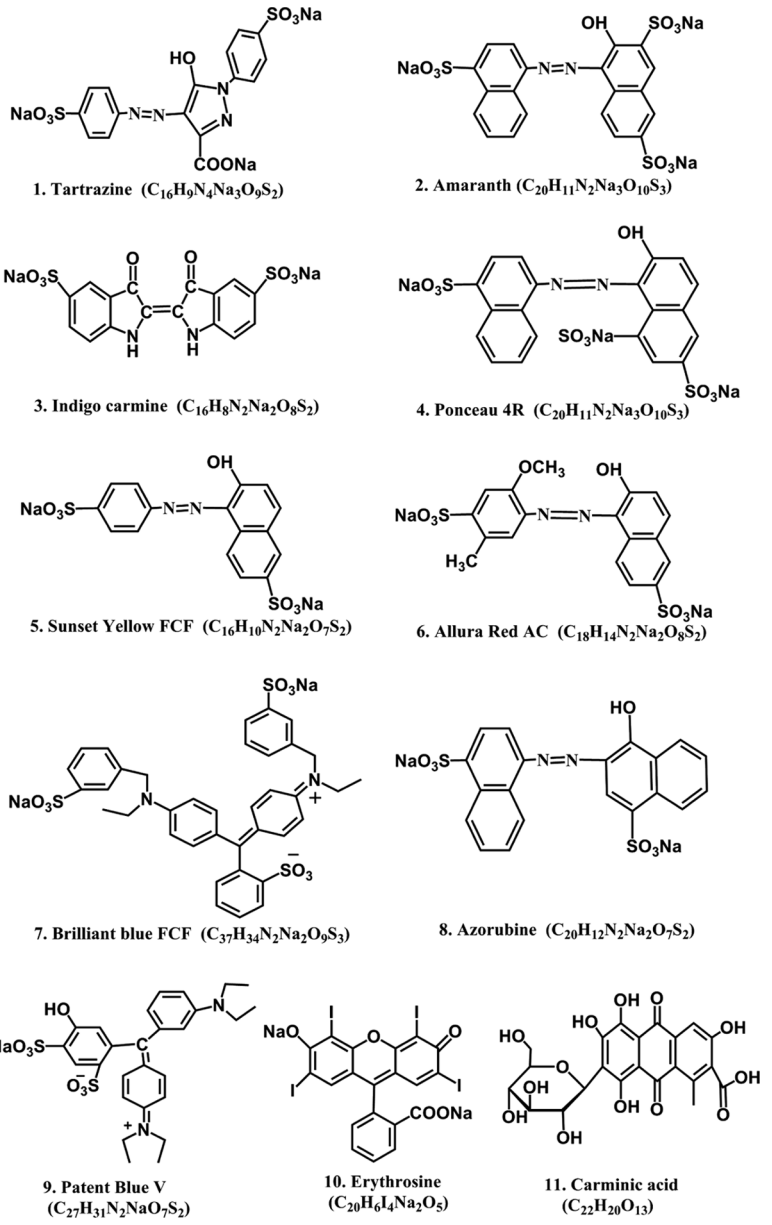

FIGURE 1 Chemical structures of investigated water-soluble dyes.

Sample Collection and Preparation

Shrimp flakes and drinks were commercial products purchased from local markets. The shrimp flakes consist of shrimp and starch, and its

original color is straw yellow. The producers often add some dyes to it to make it colorful.

For shrimp flake analysis, about 100 g of shrimp flakes were homogenized before 2 g were taken for extraction. The extraction was carried out using 20 mL of ethanol/ammonia/water (v/v/v, 7/2/1). After being sonicated for 20 min, the extract was centrifuged at 10000 rpm for 15 min. This procedure was replicated three times and all the supernatants were incorporated together in a round-bottom flask. The ethanol and most of the ammonia in the round-bottom flask were eliminated by rotary evaporation. The residual solution was diluted with water to 10 mL, and the pH was adjusted to 3.0–3.5. The extract was fractionated by SPE on a HLB C18 column (500 mg, Waters, Milford, MA) as described in the following. The HLB C18 column was preconditioned with 5.0 mL methanol and then with 5.0 mL water before it was rinsed with 5.0 mL 15% methanol containing 0.1% formic acid (v/v). Then, the dyes were eluted with 5.0 mL methanol containing 0.1% ammonia solution (v/v). The elution was dried under gentle nitrogen gas flow and then reconstituted to a final volume of 2 mL with water. The water solution was filtered through a 0.22 μm nylon membrane prior to UPLC analysis.

For drinks, 10 g samples were accurately weighed. If the sample was carbonated, it was de-gassed by ultrasonication for 5 min. In the case of alcoholic beverages, ethanol in the samples was evaporated on a hot plate and the evaporated volume was increased with water. Ten milliliters of water was added to the sample and mixed. The content was then adjusted to approximately pH 3.0–3.5 with formic acid. Finally, the solution was filtered through a 0.22 μm nylon membrane prior to UPLC analysis.

Instrumentation

A UPLC-DAD-ESI-MS/MS system was used for the separation and quantification of these dyes. It consisted of an ACQUITY ultra performance liquid chromatography system equipped with a DAD detector (detected wavelength ranged from 190 to 800 nm) and an ESI-Quattro Premier XE triple quadrupole mass spectrometer (Waters, Milford, MA, USA). Data acquisition was conducted by MassLynx 4.1 software. Separations were done using an UPLC BEH C18 column (100 × 2.1 mm i.d., 1.7 μm) (Waters, Milford, MA). Detection wavelengths were set at the maximum absorption of these dyes respectively. The mobile phase system consisted of A (20 mM ammonium formate buffer containing 0.1% formic acid (v/v), pH 3.8) and B (methanol) using an gradient elution of 20% B at 0–0.5 min, 35% B at 0.5–1.5 min, 60% B at 1.5–1.8 min, 95% B at 1.8–3.0 min. The flow rate was 0.3 mL/min and the column temperature was controlled at 30°C.

The injection volume was 3 μL. The eluate from the UPLC columns was directly introduced into mass spectrometer without flow-splitting. The mass spectrometer was operated in the negative ion mode. A multiple reaction monitoring (MRM) mode (dwell time of 0.15 s and inter channel delay of 0.05 s) was adopted for detection. Nitrogen was used as the desolvation, cone, and nebulizing gas. The desolvation and cone gas was set at 500 and 50 L/h, respectively. The desolvation and source temperature was 350 and 110°C. The capillary voltage was 3 KV. Inert argon was used as the collision gas. The collision cell pressure was 7.2×10^{-3} mbar. The optimum cone voltages, collision energies, and selected product ions for the determination of 10 dyes were listed in Table 1.

Quantification and Method Validation

The sensitivity of the method was evaluated by estimating the LOD at a signal-to- noise ratio of 3. Quantification was carried out by external standard calibration method. The calibration standard solutions were prepared by appropriate dilution of intermediate mixed standard solutions in water to the concentrations between 0.1 and 50 μg/mL. The intra-day and

TABLE 1 The Optimum Parameters and Selected Typical Product Ions for Dyes Determination

Dyes (MW)	Cone Voltage (V)	Collision Energy (V)	Selected Parent ions	Selected Daughter ions
Tartrazine	25	40	$[M-3Na+2H]^-$	$467.3 > 134.3^*$
534.36		38	m/z 467.3	$467.3 > 198.2^\#$
Amaranth	30	35	$[M-3Na+H]^{2-}$	$268.3 > 142.1^*$
604.47		32	m/z 268.3	$268.3 > 205.9^\#$
Indigo carmine	30	35	$[M-2Na+H]^-$	$421.2 > 340.9^*$
466.35		40	m/z 421.2	$421.2 > 277.1^\#$
Ponceau 4R	30	35	$[M-3Na+2H]^-$	$537.3 > 301.9^*$
604.47		38	m/z 537.3	$537.3 > 237.9^\#$
Sunset Yellow FCF	30	35	$[M-2Na+H]^-$	$407.1 > 207.1^*$
452.36		36	m/z 407.1	$407.1 > 155.9^\#$
Allura Red AC	30	35	$[M-2Na+H]^-$	$451.2 > 206.9^*$
496.42		42	m/z 451.2	$451.2 > 143.2^\#$
Brilliant blue FCF	30	50	$[M-2Na+H]^-$	$747.3 > 170.0^*$
792.84		48	m/z 747.3	$747.3 > 561.2^\#$
Azorubine	30	40	$[M-2Na+H]^-$	$457.2 > 221.2^*$
502.42		42	m/z 457.2	$457.2 > 170.1^\#$
Patent Blue V	30	35	$[M-Na]^-$	$559.3 > 479.3^*$
582.66		34	m/z 559.3	$559.3 > 435.2^\#$
Erythrosine	30	40	$[M-2Na+H]^-$	$834.8 > 662.9^*$
879.86		43	m/z 834.8	$834.8 > 536.9^\#$

*Quantifying ion. #Qualifying ion.

inter-day variability was utilized to evaluate the precision of the developed method (n = 5). For extraction recovery calculations, accurate amounts of 10 standards were added to 2 g of incarnadine shrimp flakes (sample No. 4) at two final concentration levels (5 and 25 μg/g). Then, the spiked samples were extracted and analyzed as described previously.

RESULTS AND DISCUSSION

Sample Pretreatment

The complex matrices in food usually influence the determination of dyes. For example, food that contains high concentrations of proteins or fats or carbohydrates can complicate the extraction and contaminate the chromatographic or mass spectrometric systems, thus deteriorate the dyes determination. Traditional method for dye extraction and cleanup usually uses a polyamide column.[3,13,19] However, the polyamide column could not successfully reserve xanthenes dyes such as erythrosine (E127).[19] Therefore, an HLB SPE column was tested for the capacity of cleanup of shrimp flake matrices. The reason for choosing the HLB column was that its copolymer contained two monomers, the lipophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone. The N-vinylpyrrolidone could provide a special "polar hook" for enhanced capture of polar analytes, and the divinylbenzene could provide a better reservation for weak polar analytes. After optimizing the fraction process, our result demonstrated that all of the dyes, including xanthene-dyes, could be successfully reserved on the column when it was rinsed with 5.0 mL 15% methanol containing 0.1% formic acid (v/v). In addition, these dyes could be eluted completely from the column by 5.0 mL methanol containing 0.1% ammonia solution (v/v).

Optimization of Chromatographic Conditions

Traditional methods for determination of dyes by UV/Vis or DAD detector usually use acetic acid/ammonium acetate buffer (pH 4.0).[20,21] Under this condition, the concentrations of acetic acid and ammonium acetate were usually more than 1% and 20 mM, respectively.[11,12] However, this ion intensity in mobile phase may be too high and not suitable for determination of dyes by mass spectrometer. Therefore, we developed a method to separate 10 dyes using formic acid/ammonium formate buffer. The recorded chromatogram under optimum separation conditions was shown in Figure 2. It can be seen that the 10 dyes could be separated well in less than 3 min. We also separated these 10 dyes using 20 mM acetic

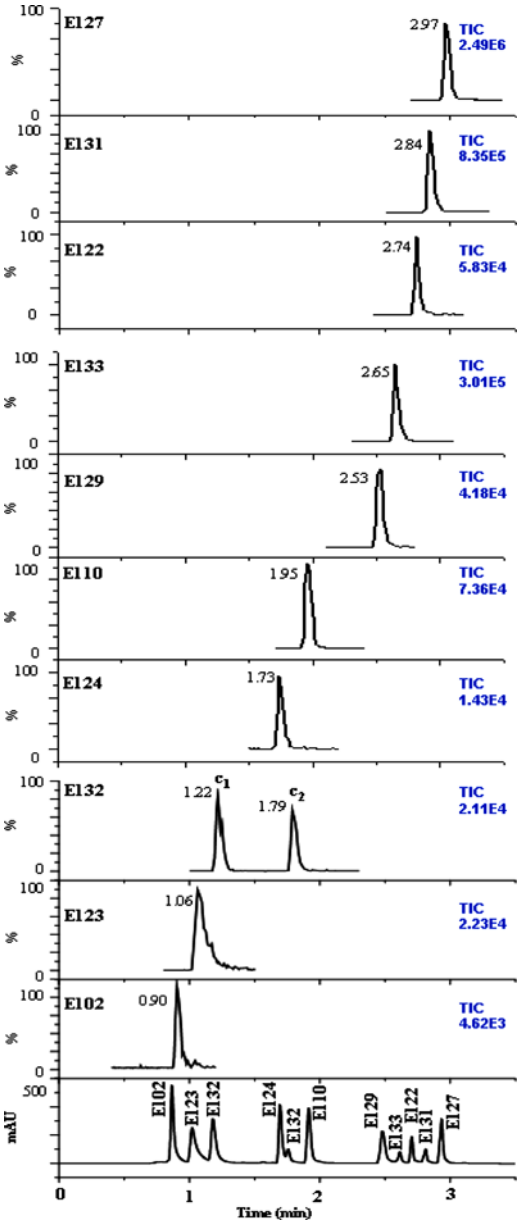

FIGURE 2 UPLC-DAD-MS/MS chromatograms obtained from the standard mixed solution of 10 dyes. These dyes were simultaneously monitored by MS/MS (upper panel) and UV absorption at 254 nm (lower panel). c_1 and c_2 are two isomers of E132. (Figure available in color online.)

acid/ammonium acetate buffer. The result demonstrated that, although their retention times were the same as formic acid/ammonium formate buffer, the signal intensity of each dye was almost 2–3 times lower.

Method Validation

The precision of the method was evaluated by examining the variance of peak areas in intra-day and inter-day. Overall intra-day and inter-day variations were less than 5%. Linear dynamic range, correlation coefficient (r^2), recovery, and LOD of the method were listed in Table 2. The recoveries of the developed method were in the range of 92.1–105.4%. Comparing the LOD which was acquired by DAD and MS/MS detector, it can be seen that the former 5 dyes, including E102, E123, E132, E124, and E110, were suitable for quantifying by DAD detector because the LOD of these dyes obtained from DAD detector was lower, and the latter 5 dyes, including E129, E133, E122, E131, and E127, were suitable for quantifying by MS/MS detector because the LOD of these dyes obtained from MS/MS detector was lower. Obviously, the DAD plus MS/MS dual quantification mode could significantly improve the sensitivity of dyes determination.

Compared with the conventional HPLC-DAD method in which the dye separation usually needs 20–30 min,[22,23] the throughput of the developed UPLC-DAD-MS/MS method is significantly improved 7–10 times; meanwhile, the detection sensitivity is also improved more than 10 times. Because of the high speed and high sensitivity, the developed UPLC-DAD-MS/MS method provided a capability of 12–15 runs/h and 100–120 runs/day (8 working hr per day) for detection and quantification of dyes in foods.

TABLE 2 Linear Ranges, Correlation Coefficients, Limits of Detection and Recoveries of the UPLC-DAD-MS/MS Method for Determination of 10 Synthetic Dyes in Shrimp Flakes

	DAD						Mass Spectrometry				
					Recovery (%)					Recovery (%)	
Analyte	λ_{max} (nm)	Linear range (μg/mL)	r^2	LOD (μg/mL)	5 μg/g	25 μg/g	Linear range (μg/mL)	r^2	LOD (μg/mL)	5 μg/g	25 μg/g
E102	427	0.20–50	0.999	0.05	103.2	97.2	3.12–50.00	0.991	0.75	97.1	96.4
E123	520	0.78–50	0.998	0.15	105.4	97.3	3.12–50.00	0.997	0.67	96.1	97.8
E132	608	0.20–50	0.999	0.01	92.1	95.4	3.12–50.00	0.991	0.78	103.2	101.1
E124	508	0.20–50	0.997	0.04	98.3	98.4	1.56–50.00	0.993	0.40	95.4	98.7
E110	482	0.20–50	0.999	0.01	95.4	97.2	0.39–50.00	0.996	0.11	98.3	103.2
E129	507	0.40–50	0.998	0.04	103.2	101.4	0.39–50.00	0.998	0.06	94.4	93.2
E133	624	0.40–50	0.998	0.05	103.5	101.0	0.20–50.00	0.999	0.02	93.4	96.7
E122	516	0.78–50	0.997	0.17	97.2	98.3	0.40–50.00	0.994	0.08	91.4	96.4
E131	631	1.56–50	0.994	0.13	93.2	97.5	0.20–50.00	0.995	0.04	95.4	97.2
E127	528	1.56–50	0.990	0.39	103.2	99.2	0.20–50.00	0.992	0.03	104.1	94.3

Application of Dual Quantification Mode to Confirming the Determination Results

A special application of the developed method was to confirm the quantification result. That is to say, if the quantification results obtained from DAD detector were similar with those obtained from MS/MS detector, the quantification results could be confirmed. If the quantification results of one or more dyes obtained from DAD detector were different with those obtained from MS/MS detector, we can conclude that there were some interferers or unknown dyes mixed in the UV/Vis peaks of analyzed dyes. In fact, the overlapped peaks often occurred in the determination of multiple dyes simultaneously.[10] For example, if we analyze a sample which contained carminic acid (E120) and E110 simultaneously, these two dyes were difficult to be resolved (Figure 3A). Since both E120 and E110 have light absorption at 482 nm (Figure 3B), the calculated concentration of E110 obtained from UV/Vis or DAD detector would be higher than the result obtained from MS/MS detector. Therefore, the developed method enabled us to confirm the determination results and improve the accuracy of quantification.

Application to Real Samples

The developed UPLC-DAD-MS/MS method was applied to the determination of dyes in shrimp flakes and drinks. According to laws and regulations of the People's Republic of China, four synthetic dyes, E110, E102, E133, and E124, were permitted to be added to shrimp flakes, and eight synthetic dyes, E102, E123, E132, E124, E110, E129, E133, and E127, were permitted to be added to drinks. Our results demonstrated that the concentrations of all of these permitted dyes were not beyond the limits. In addition, an extremely low concentration of E127, which is disallowed in shrimp flakes, was detected (Table 3).

Among the detected dyes in shrimp flakes, E124 was detected only by the DAD detector (Figure 4A). The reason for this phenomenon was that the LOD of E124 obtained from the DAD detector was 10 times lower than it obtained from MS/MS detector (Table 2). An item of note is that the assigned E124 to the peak was validated by a combination of retention time, UV spectrophotometry, and the standard addition test. From the standard addition test, a result of no new peaks appeared when the sample was spiked with E124 standard solution that supported the assigned peak of E124 in the chromatogram. Similar to this, E127 was only detected by the highly sensitive MS/MS detector because the LOD of E127 obtained from DAD detector was 10 times higher than was obtained from MS/MS detector (Figure 4B, Table 2).

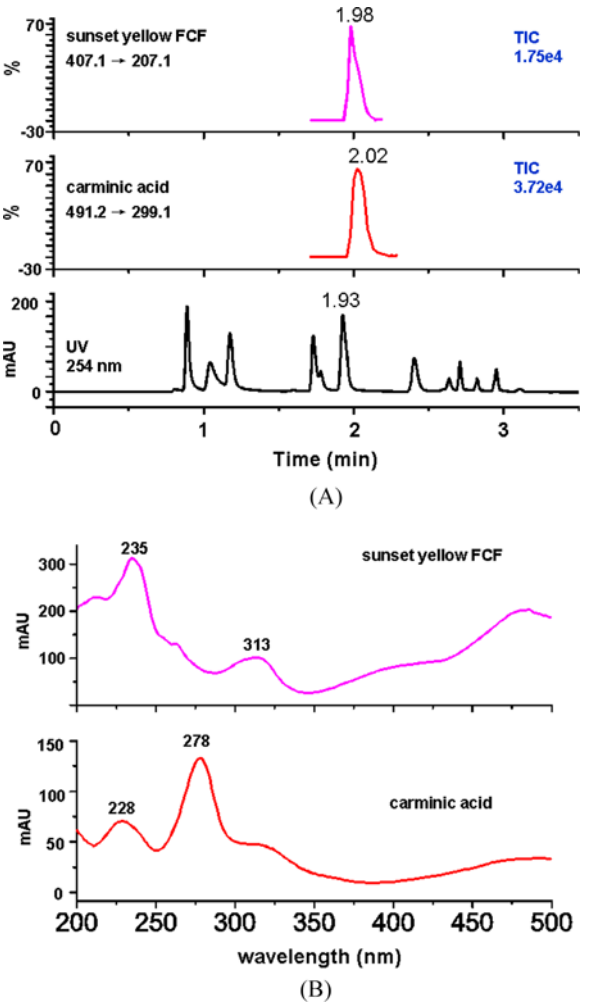

FIGURE 3 UPLC-DAD-MS/MS chromatograms obtained from the standard mixture of 10 dyes and carminic acid. (A) Chromatograms of the 11 dyes monitored by UV absorption at 254 nm (lower trace) and ESI–MS/MS (m/z 407.1 → 207.1 for E110, m/z 491.2 → 299.1 for E120, upper trace). (B) Ultraviolet spectra of carminic acid and sunset yellow FCF. (Figure available in color online.)

Based on the dual quantification mode, not only were the low concentration dyes in foods detected, but the accuracies were enhanced. As demonstrated in Table 3, the quantification results of E102, E110, and E129 in shrimp flakes (sample No. 1–5) and drinks (sample No. 6–8) obtained by DAD and MS/MS detector were similar; this suggested that the quantification results of these dyes were accurate, and there were no interferers mixed in the DAD peak. In contrast, the quantification result of E133 (sample No. 5) obtained by DAD detector was significantly

TABLE 3 Quantification Results of Synthetic Dyes in Shrimp Flake (No. 1–5) and Drinks (No. 6–8) by DAD Detector and MS/MS Detector

		Quantification by DAD		Quantification by MS/MS	
Sample	Dye	Concentration (μg/g)	RSD (%)	Concentration (μg/g)	RSD (%)
No. 1	E102	15.2	1.0	15.40	1.3
	E127	–	–	0.56	2.1
No. 2	E110	29.6	1.2	28.80	1.6
	E124	2.6	4.3	–	–
No. 3	E102	18.4	3.2	17.80	3.4
	E133	7.3	0.9	7.20	2.5
	E124	3.7	3.3	–	–
No. 4	E110	4.7	2.6	3.30	4.3
	E127	–	–	1.30	2.1
	E124	3.2	4.3	–	–
No. 5	E133	16.2	0.2	0.17	4.8
	E124	3.4	2.4	–	–
No. 6	E110	35.2	0.6	35.40	0.5
No. 7	E102	155.0	2.3	153.00	2.2
No. 8	E129	54.0	0.2	55.00	0.3
	E133	22.0	0.5	23.00	0.2

different from those obtained by MS/MS detector (16.2 vs. 0.17 μg/g). Since the LOD of E133 obtained by the MS/MS detector was lower (Table 2), which meant that E133 could be detected by the DAD detector but could not be detected by the MS/MS detector and that is impossible;

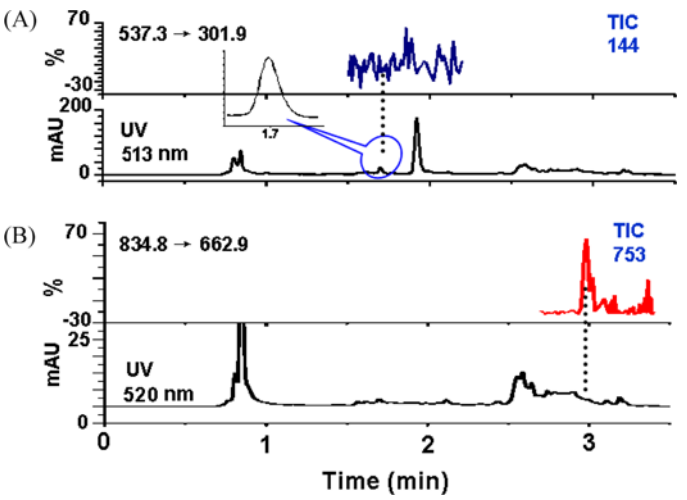

FIGURE 4 Typical chromatograms of real samples analyzed by UPLC-DAD-MS/MS. (A) chromatogram of sample No. 2 for determining ponceau 4R (E124), (B) chromatogram of sample No. 1 for determining erythrosine (E127). (Figure available in color online.)

therefore, we can conclude that the quantification result of E133 obtained from DAD detector was wrong. Obviously, the dual quantification mode could enhance the accuracy of quantification results.

CONCLUSION

In summary, a method for rapid, accurate, and highly sensitive analysis of 10 dyes in foodstuff was developed by combined HLB SPE column pretreatment with UPLC-DAD-MS/MS determination. The HLB SPE column was chosen for the purification of dyes in food samples with complex matrices for the first time and demonstrated excellent performance. The developed UPLC-DAD-MS/MS method not only minimized the analysis time, but also significantly improved the accuracy and sensitivity of quantification by the dual quantification mode.

ACKNOWLEDGMENT

The present research was financially supported by the grants from the project of Beijing Municipal Science and Technology Commission, China (Project number: D08050200310803).

REFERENCES

1. Noonan, J. Color Additives in Food. In *CRC Handbook of Food Additives*, 2nd ed.; Furia, T. E., Ed.; CRC Press: Florida, 1981, 587–615.
2. Macrae, R.; Robinson, R. K.; Sadler, M. J. *Encyclopaedia of Food Science, Food Technology and Nutrition*, Academic Press: California, 1993; 1232–1237.
3. Ministry of Health, P.R.C. *Hygienic Standards for Uses of Food Additives*, National Standard *of the* P.R.C.: Beijing, 2007; 90–178.
4. Huang, C. L.; Chuang, C. W.; Chung, M. C. Comparison of Microemulsion Electrokinetic Chromatography and Micellar Electrokinetic Chromatography as Methods for the Analysis of Ten Benzophenones. *Electrophoresis* **2005**, *26*, 867–877.
5. Dossi, N.; Toniolo, R.; Pizzariello, A.; Susmel, S.; Perennes, F.; Bontempelli, G. A Capillary Electrophoresis Microsystem for the Rapid In-Channel Amperometric Detection of Synthetic Dyes in Food. *J. Electroanal. Chem.* **2007**, *601*, 1–2.
6. Ryvolová, M.; Táborský, P.; Vrábel, P.; Krásenský, P.; Preisler, J. Sensitive Determination of Erythrosine and Other Red Food Colorants Using Capillary Electrophoresis with Laser-Induced Fluorescence Detection. *J. Chromatogr. A* **2007**, *1141*, 206–211.
7. Nevado, J. J. B.; Cabanillas, C. G.; Salcedo, A. M. C. Spectrophotometric Resolution of Ternary Mixtures of Amaranth, Carmoisine and Ponceau 4R by the Derivative Ratio Spectrum-Zero Crossing Method. *J. Anal. Chem.* **1994**, *350*, 606–609.
8. Al-Degs, Y. S. Determination of Three Dyes in Commercial Soft Drinks Using HLA/GO and Liquid Chromatography. *Food Chem.* **2009**, *117*, 485–489.
9. Minioti, K. S.; Sakellariou, C. F.; Thomaidis, N. S. Determination of 13 Synthetic Food Colorants in Water-Soluble Foods by Reversed-Phase High-Performance Liquid Chromatography Coupled with Diode-Array Detector. *Anal. Chim. Acta.* **2007**, *583*, 103–110.

10. 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.
11. 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.
12. Zhang, J.; Gao, N.; Zhang, Y. Method Development and Validation for the Determination of Five Synthetic Food Colorants in Alcoholic Beverages by Reversed-Phase High Performance Liquid Chromatography Coupled with Diode-Array Detector. *Anal. Lett.* **2007**, *40*, 3080–3094.
13. Kirschbaum, J.; Krause, C.; Brückner, H. Liquid Chromatographic Quantification of Synthetic Colorants in Fish Roe and Caviar. *Eur. Food Res. Technol.* **2006**, *222*, 572–579.
14. Serdar, M.; Knežević, Z. Simultaneous LC Analysis of Food Dyes in Soft Drinks. *Chromatographia* **2009**, *70*, 1519–1521.
15. Chen, Q. C.; Mou, S. F.; Hou, X. P.; Riviello, J. M.; Ni, Z. M. Determination of Eight Synthetic Food Colorants in Drinks by High-Performance Ion Chromatography. *J. Chromatogr. A* **1998**, *827*, 73–81.
16. 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.
17. Berzas-Nevado, J. J.; Guiberteau Cabanillas, C.; Contento Salcedo, A. M. Separation and Determination of Dyes by Ion-Pair Chromatography. *J. Liq. Chromatogr. Relat. Technol.* **1997**, *20*, 3073–3088.
18. 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.
19. Jalón, M.; Peña, M. J.; Rivas, J. C. Liquid Chromatographic Determination of Carminic Acid in Yogurt. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 231–234.
20. González, M.; Gallego, M.; Valcárcel, M. Determination of Natural and Synthetic Colorants in Prescreened Dairy Samples Using Liquid Chromatography-Diode Array Detection. *Anal. Chem.* **2003**, *75*, 685–695.
21. González, M.; Gallego, M.; Valcárcel, M. Liquid Chromatographic Determination of Natural and Synthetic Colorants in Lyophilized Foods Using an Automatic Solid-Phase Extraction System. *J. Agric. Food Chem.* **2003**, *51*, 2121–2129.
22. García-Falcón, M. S.; Simal-Gándara, J. Determination of Food Dyes in Soft Drinks Containing Natural Pigments by Liquid Chromatography with Minimal Clean-Up. *Food Control* **2005**, *16*, 293–297.
23. González, M.; Gallego, M.; Valcárcel, M. Automatic Screening Method for the Rapid and Simple Discrimination Between Synthetic and Natural Colorants in Foods. *Anal. Chim. Acta.* **2002**, *464*, 237–247.