Journal of Chromatography B, 879 (2011) 1813–1818

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Highly sensitive and accurate screening of 40 dyes in soft drinks by liquid chromatography–electrospray tandem mass spectrometry

Feng Feng[a], Yansheng Zhao[a], Wei Yong[a], Li Sun[a], Guibin Jiang[b], Xiaogang Chu[a,*]

[a] Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing 100123, China
[b] State Key laboratory of Environmental Chemistry and Eco-toxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Article history:
Received 13 December 2010
Accepted 12 April 2011
Available online 20 April 2011

Keywords:
Dyes
Solid-phase extraction
HPLC
ESI-MS/MS
Soft drink

ABSTRACT

A method combining solid phase extraction with high performance liquid chromatography–electrospray ionization tandem mass spectrometry was developed for the highly sensitive and accurate screening of 40 dyes, most of which are banned in foods. Electrospray ionization tandem mass spectrometry was used to identify and quantify a large number of dyes for the first time, and demonstrated greater accuracy and sensitivity than the conventional liquid chromatography–ultraviolet/visible methods. The limits of detection at a signal-to-noise ratio of 3 for the dyes are 0.0001–0.01 mg/L except for Tartrazine, Amaranth, New Red and Ponceau 4R, with detection limits of 0.5, 0.25, 0.125 and 0.125 mg/L, respectively. When this method was applied to screening of dyes in soft drinks, the recoveries ranged from 91.1 to 105%. This method has been successfully applied to screening of illegal dyes in commercial soft drink samples, and it is valuable to ensure the safety of food.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Organic aromatic dyes are often added to food to compensate for the loss of natural colors, which are destroyed during processing and storage, and to provide the desired colored appearance [1]. Although more and more evidence in recent years indicates that the abuse of dyes may cause cancer [2], many kinds of dyes are still widely used because of their low price, high effectiveness and excellent stability [3].

To protect public health, many countries have established strict regulations for the allowable kinds and concentrations of dyes [4,5]. However, some food producers may still add banned dyes to their products putting sensitive population in health risk. Therefore, it is necessary to develop a sensitive and accurate method to screen banned dyes in foods to ensure food safety.

Various methods for the determination of dyes in foods have been reported, including capillary electrophoresis [6–10], thin-layer chromatography [11], ion-pair chromatography [12,13], high performance liquid chromatography (HPLC) with ultraviolet/visible (UV/Vis) or diode-array detector (DAD) detection [14–25] and liquid chromatography–mass spectrometry (LC–MS) [26–32]. HPLC coupled with UV/Vis or DAD detection is the most commonly used technique because dyes absorb strongly at the ultraviolet and/or visible wavelength. However, these methods are not suitable for simultaneous screening large number of dyes because the multiple isomers and structural analogs of dyes are difficult to separate. Besides, false positives caused by complex food matrices are frequently encountered [6,18]. To solve these problems, the selective detection by liquid chromatography tandem mass spectrometry (LC–MS/MS) has been used [26–32] for it can provide detailed structural information. In the selective reaction monitoring (SRM) mode, the specific MS transition (precursor ion → product ion) can exclude the presence of interference substances, improving the accuracy of the quantification. In spite of the potential value of the application, to our knowledge, no method based on tandem mass spectrometry has been applied to simultaneous screening of large numbers of dyes in foods.

In this work, we developed a highly sensitive and accurate HPLC-MS/MS method to simultaneously screen 40 illegal dyes in soft drinks. The composition of mobile phases and the mass spectrometric parameters for each dye were optimized in detail. This method has been successfully applied to screening of illegal dyes in soft drink samples from local market.

2. Experimental

2.1. Chemicals and reagents

Tartrazine, Amaranth, Ponceau 4R, Indigo Carmine, Carminic Acid, Sunset Yellow FCF, Allura Red AC, Acid Red 1, Acid Yellow 17, Wool Green S, Acid Red 13, Light Green SF, Ponceau 2R, Azorubine, Guinea Green B, Acid Green 25, Acid Violet 17, Erythrosine, Ben-

* Corresponding author. Tel.: +86 10 85791012; fax: +86 10 85770775.
E-mail address: fengf2006@hotmail.com (X. Chu).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.04.014

Table 1
The optimum parameters and selected typical fragment ions for 40 dyes determination.

No	Analyte	Molecular formula	Color index number	E number	Precursor ion (*m/z*)	Product ion (*m/z*)	DP (V)	CE (V)	ESI mode
1	Tartrazine	$C_{16}H_9N_4Na_3O_9S_2$	19,140	E102	467.2	198.1[a] 423.1	−80	−43 −22	ESI⁻
2	New Red	$C_{18}H_{12}N_3Na_3O_{11}S_3$			544.2	359.2[a] 464.2	−80	−39 −35	ESI⁻
3	Amaranth	$C_{20}H_{11}N_2Na_3O_{10}S_3$	16,185	E123	537.2	317.0[a] 457.1	−160	−45 −35	ESI⁻
4	Ponceau 4R	$C_{20}H_{11}N_2Na_3O_{10}S_3$	16,255	E124	537.2	302.0[a] 429.2	−131	−34 −28	ESI⁻
5	Indigo Carmine	$C_{16}H_8N_2Na_2O_8S_2$	73,015	E132	421.1	341.1[a] 261.1	−145	−42 −54	ESI⁻
6	Carminic Acid	$C_{22}H_{20}O_{13}$	75,470	E120	491.2	447.3[a] 327.1	−80	−30 −37	ESI⁻
7	Sunset Yellow FCF	$C_{16}H_{10}N_2Na_2O_7S_2$	15,985	E110	407.1	207.1[a] 327.1	−152	−45 −30	ESI⁻
8	Allura Red AC	$C_{18}H_{14}N_2Na_2O_8S_2$	16,035	E129	451.2	207.1[a] 371.1	−80	−47 −32	ESI⁻
9	Acid Red 1	$C_{18}H_{13}N_3Na_2O_8S_2$	18,050	E128	232.1	179.0[a] 291.2	−65	−15 −22	ESI⁻
10	Wool Green S	$C_{27}H_{25}N_2NaO_7S_2$	44,090	E142	553.3	511.3[a] 496.3	−80	−34 −45	ESI⁻
11	Acid Red 13	$C_{20}H_{12}N_2Na_2O_7S_2$	16,045		457.1	206.8[a] 377.2	−130	−44 −34	ESI⁻
12	Light Green SF	$C_{37}H_{34}N_2Na_2O_9S_3$	42,095		373.2	497.4[a] 170.0	−80	−34 −35	ESI⁻
13	Ponceau 2R	$C_{18}H_{14}N_2Na_2O_7S_2$	16,150		435.2	302.1[a] 355.1	−80	−40 −35	ESI⁻
14	Azorubine	$C_{20}H_{12}N_2Na_2O_7S_2$	14,720	E122	457.1	377.2[a] 171.0	−145	−33 −37	ESI⁻
15	Fast Green FCF	$C_{37}H_{34}N_2Na_2O_{10}S_3$	42,053		763.3	683.5[a] 421.6	−80	−50 −66	ESI⁻
16	Ponceau SX	$C_{18}H_{14}N_2Na_2O_7S_2$	14,700		435.2	355.1[a] 171.0	−80	−28 −35	ESI⁻
17	Brilliant Blue FCF	$C_{37}H_{34}N_2Na_2O_9S_3$	42,090	E133	747.4	170.1[a] 561.2	−80	−79 −61	ESI⁻
18	Quinoline Yellow	$C_{18}H_9NNa_2O_8S_2$	47,005	E104	352.2	288.2[a] 244.2	−60	−35 −35	ESI⁻
19	Ponceau 3R	$C_{19}H_{16}N_2Na_2O_7S_2$	16,155		449.2	369.2[a] 302.1	−80	−37 −39	ESI⁻
20	Uranine	$C_{20}H_{10}Na_2O_5$	45,350		331.1	286.1[a] 243.2	−80	−30 −34	ESI⁻
21	Orange II	$C_{16}H_{11}N_2NaO_4S$	15,510		327.2	171.1[a] 156.1	−80	−34 −40	ESI⁻
22	Sulforhodamine B	$C_{27}H_{29}N_2NaO_7S_2$	45,100		557.2	513.2[a] 433.4	−80	−58 −62	ESI⁻
23	Acid Black 1	$C_{22}H_{14}N_6Na_2O_9S_2$	20,470		571.2	507.3[a] 479.1	−80	−34 −37	ESI⁻
24	Patent Blue V	$C_{54}H_{62}CaN_4O_{14}S_4$	42,051	E131	559.2	435.3[a] 479.5	−60	−62 −45	ESI⁻
25	Alizarin Yellow GG	$C_{13}H_8N_3NaO_5$	14,025		286.0	242.2[a] 156.1	−57	−24 −31	ESI⁻
26	Guinea Green B	$C_{37}H_{35}N_2NaO_6S_2$	42,085		667.4	170.1[a] 497.4	−80	−65 −54	ESI⁻
27	Metanil Yellow	$C_{18}H_{14}N_3NaO_3S$	13,065		352.2	156.0[a] 260.2	−80	−42 −36	ESI⁻
28	Eosin Y	$C_{20}H_6Br_4Na_2O_5$	45,380		646.9	523.2[a] 443.1	−60	−44 −45	ESI⁻
29	Acid Green 25	$C_{28}H_{20}N_2Na_2O_8S_2$	61,570		577.3	497.3[a] 417.4	−80	−52 −56	ESI⁻
30	Acid Violet 17	$C_{41}H_{44}N_3O_6S_2Na$	42,650		738.6	170.0[a] 568.4	−60	−67 −55	ESI⁻
31	Erythrosine	$C_{20}H_6I_4Na_2O_5$	45,430	E127	834.8	663.0[a] 537.0	−60	−52 −54	ESI⁻
32	Bengal Rose B	$C_{20}H_2Cl_4I_4Na_2O_5$	45,440		972.7	674.8[a] 893.0	−80	−50 −37	ESI⁻
33	Acid Yellow 9	$C_{12}H_{11}N_3O_6S_2$	13,015		358.4	157.0[a] 109.0	80	37 52	ESI⁺
34	Acid Yellow 17	$C_{16}H_{10}Cl_2N_4Na_2O_7S_2$	18,965		507.0	108.1[a] 173.0	160	60 48	ESI⁺
35	Chrysoidine	$C_{12}H_{13}ClN_4$	11,320		213.3	121.1[a] 196.2	80	30 28	ESI⁺
36	Basic Flavine O	$C_{17}H_{22}N_3Cl$	41,000		268.5	147.1[a] 252.3	80	42 44	ESI⁺
37	Patent Green	$C_{37}H_{34}ClN_2NaO_6S_2$	42,100		703.4	517.2[a] 533.3	80	70 66	ESI⁺
38	Phloxine B	$C_{20}H_2Br_4Cl_4Na_2O_5$	45,410		786.7	742.8[a] 563.8	60	73 88	ESI⁺
39	Rhodamine B Chloride	$C_{28}H_{31}ClN_2O_3$	45,170		443.4	399.3[a] 355.3	40	60 83	ESI⁺
40	Methyl Yellow	$C_{14}H_{15}N_3$	11,020		226.3	77.1[a] 120.1	80	32 46	ESI⁺

DP: declustering potential; CE: collision energy.
[a] Quantification ion.

gal Rose B, Fast Green FCF and Ponceau SX were purchased from Fluka (Buchs, Switzerland). Basic Flavine O, Patent Green, Phloxine B, Rhodamine B Chloride, Methyl Yellow, Brilliant Blue FCF, Quinoline Yellow, Ponceau 3R, Uranine, Orange II, Chrysoidine and Sulforhodamine B were obtained from Sigma–Aldrich (St. Louis, MO, USA). Acid Black 1, Patent Blue V, Alizarin Yellow GG, Metanil Yellow, Eosin Y and Acid Yellow 9 were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). New Red was purchased from Dr. Ehrenstorfer (Augsburg, Germany). All of the stock solutions (1000 μg/mL) were dissolved in water except Alizarin Yellow GG, Acid Yellow 9, Chrysoidine, Basic Flavine O, Metanil Yellow, Methyl Yellow and Quinoline Yellow which were dissolved in methanol.

HPLC grade methanol and acetonitrile were 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).

2.2. Sample collection and preparation

Twenty soft drink samples were purchased from local markets. Sample preparation was performed as described by Yoshioka et al. [5] with slight modifications. For each sample, 10 g was weighed accurately. If carbonated, the sample was degassed by sonication (5 min). In the case of alcoholic beverages, ethanol in the sample was evaporated on a hot plate (60 °C) and the evaporated volume was filled with water. The sample solution was adjusted to a pH of approximately 3–3.5 with formic acid prior to solid phase extraction (SPE) on a HLB cartridge (500 mg, Waters, Milford, MA). The cartridges were first preconditioned with 5.0 mL methanol followed by 5.0 mL acidified water. The samples were loaded through the cartridges at a rate of less than 3.0 mL/min. The cartridges were then rinsed with 5.0 mL of 15% (v/v) methanol/water solution (the water contained 0.1% formic acid) and were finally eluted with 5.0 mL methanol containing 0.1% (v/v) ammonia. The eluate was dried under a gentle nitrogen gas flow and was reconstituted to a final volume of 2 mL with water/methanol (9:1, v/v). The solution was filtered through a 0.22 μm nylon membrane prior to LC-MS/MS analysis.

2.3. Instrumentation

LC coupled with electrospray ionization–tandem mass spectrometry (ESI-MS/MS) was used for screening. The LC system was Agilent (Palo Alto, CA, USA) 1200 SL Series equipped with a binary pump, vacuum degasser, autosampler and thermostatic column compartment. The tandem mass spectrometer was an API 5000 triple quadrupole from Applied Biosystems (Darmstadt, Germany). Applied Biosystems Analyst software (version 1.5) was used for system operation and data analysis.

Separations were performed using an Ultimate XB-C18 column (100 × 2.1 mm i.d., 3.0 μm) (Welch Materials, Maryland, USA).

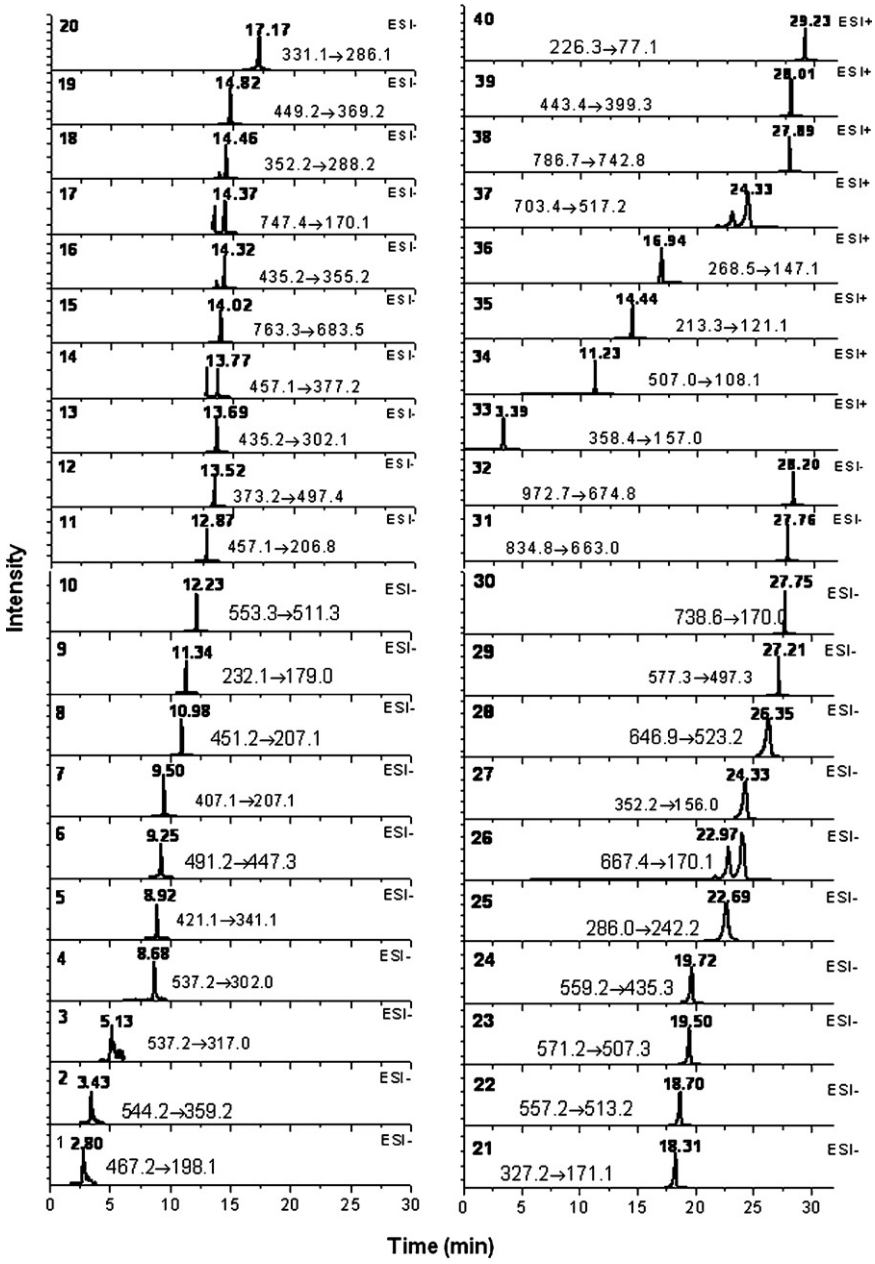

Fig. 1. HPLC-ESI-MS/MS chromatograms from a 40-dye mixed standard solution (each dye at 0.5 μg/mL). The sequence number 1–40 corresponds to dye number in Table 1.

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/acetronitrile, 7/3) using a gradient elution of 10% B at 0–3 min, 10–50% B at 3–12 min, 50% B at 12–25 min, and 85% B at 25–32 min. The flow rate was 0.3 mL/min, and the column temperature was 35 °C. The injection volume was 2 μL. The eluate from the HPLC column was introduced directly into the mass spectrometer without flowsplitting.

The entire eluate was ionized simultaneously in positive and negative ionization mode, and monitored by SRM. Mass selection for the Q1 and Q3 analysers was set on unit resolution. Nitrogen was used as ion source gas 1, ion source gas 2, curtain gas and collision gas, with flow rates controlled at 65, 60, 25 and 6 psi, respectively. Ion electrospray voltage was 5500 V for positive ionization mode and 4500 V for negative ionization mode. The ion source temperature was 500 °C. The optimum declustering potential (DP), collision energy (CE) and representative product ions for these 40 dyes were optimized by flow injection analysis (FIA) using a standard solution of these dyes, and their optimum values are listed in Table 1.

2.4. Method validation

Quantitative analysis was carried out by the external standard calibration method. The calibration solutions were prepared by appropriate dilution of intermediate mixed standard solutions in water to concentrations between 0.0015 and 10 μg/mL. The sensitivity of the method was evaluated by estimating the limit of detection (LOD) at a signal to noise ratio of 3. The intra-day and inter-day variability was utilized to evaluate method precision ($n = 3$).

For extraction recovery calculations, accurate amounts of 40 standards were added to 10 g of blank samples. Each dye was spiked at 50 times of the LOD, then filtrated and analyzed as described above. The matrix effect (ion suppression or enhancement) was investigated by adding the standard mixture into soft drinks that

had been pretreated and filtered; then the peak area was compared with the same concentration of diluted standard solution.

3. Results and discussion

3.1. SPE fractionation

It has been proved that carbonated drinks without pulp could be analyzed directly after filtration. However, SPE cleanup was still necessary for some fruit drinks or juices. Traditionally used for dye cleanup, polyamide column, however, does not retain xanthenes dyes such as erythrosine [21,23]. In this study, a HLB SPE column was chosen for its dual functionality: hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene. The former provides a special "polar hook" for enhanced capture of polar dyes, and the latter provides a better retention for weak polar dyes. After optimization, all the dyes including xanthene-dyes were retained well on the column even after the column was rinsed with 5.0 mL of 15% (v/v) methanol/water solution (the water containing 0.1% formic acid), and the dyes were eluted completely with 5.0 mL methanol containing 0.1% (v/v) ammonia.

3.2. LC–MS/MS method development

Traditional methods use HPLC coupled with UV/Vis or DAD detection for determining dyes in foods [20–22]. However, multiple isomers and structural analogs of the dyes are difficult to separate and determine. For instance, Yoshioka et al. used a Zorbax Eclipse XDB-C18 Rapid Resolution HT (50 mm × 4.6 mm, 1.8 μm) column to separate 40 dyes in food, but many dyes were overlapped [5]. Although the overlapped peaks can be quantified by diode-array detectors, similar absorption of overlapped peaks renders quantification inaccurate.

The goal of this study was to develop a highly sensitive and accurate HPLC-ESI-MS/MS method to simultaneously screen 40 illegal dyes. The optimum mass spectrometric parameters for the identification and quantification of the 40 dyes were first obtained after analyzing the dyes by flow injection analysis (FIA) respectively (see Table 1). The FIA results demonstrated that 32 dyes could be determined in the negative ionization mode, and the rest 8 were appropriate for determination in the positive ionization mode.

Three columns were tested to obtain the best resolution for these dyes, including Capcell Pak C18 MG Ш (75 × 2.1 mm, 3 μm), Phenomenex Luna C18 (100 × 4.6 mm, 2.6 μm), and Ultimate XB-C18 (100 × 2.1 mm i.d., 3.0 μm). After optimizing the mobile phase conditions, the results showed that the Ultimate XB-C18 column achieved the best resolution when a mixture of acetonitrile-methanol-ammonium formate buffer was used as the mobile phase. An acetonitrile-methanol mixture was chosen as the organic phase because this mixture achieved a better resolution than methanol [19]. Two ratios of methanol/acetonitrile (7:3 vs. 3:7, v/v) were tested. The former resulted in better resolution. Fig. 1 shows adequate separation of the 40 dyes under the optimum condition in 30 min.

Each dye was analyzed using two SRM transitions in order to improve accuracy. One transition was used for qualification and quantification while the other was used as a supplemental data for qualification. Some isomers with the same SRM transitions could be identified and quantified by the difference in another SRM transition. As shown in Fig. 2A, the retention times of Azorubine and Acid Red 13 were similar, and one of their SRM transitions was identical (m/z 457.1 → 377.2). It was difficult to distinguish the two dyes if we chose only the transition of m/z 457.1 → 377.2 as the identified and quantified ion. However, because of different locations of the hydroxyl moiety in the dye structure, the product ion spectra were different (m/z 457.1 → 171.0 vs. m/z 457.1 → 206.8) (see Fig. 2B). Although it is uncertain why Azorubine produced fragment ion of m/z 171.0 but not m/z 206.8 or why Acid Red 13 could produce fragment ion of m/z 206.8 but not m/z 171.0, the different SRM transitions provided a simple and reliable distinction. Guinea Green B and Patent Green showed two peaks in their extracted ion chromatograms (see Fig. 1, transitions No. 26 and No. 37). The peak area ratios of each dye in two SRM transitions were similar (data not shown). These observations suggest that both Guinea Green B and Patent Green are composed of a mixture of isomers. The two dyes were quantified using the sum of two peaks.

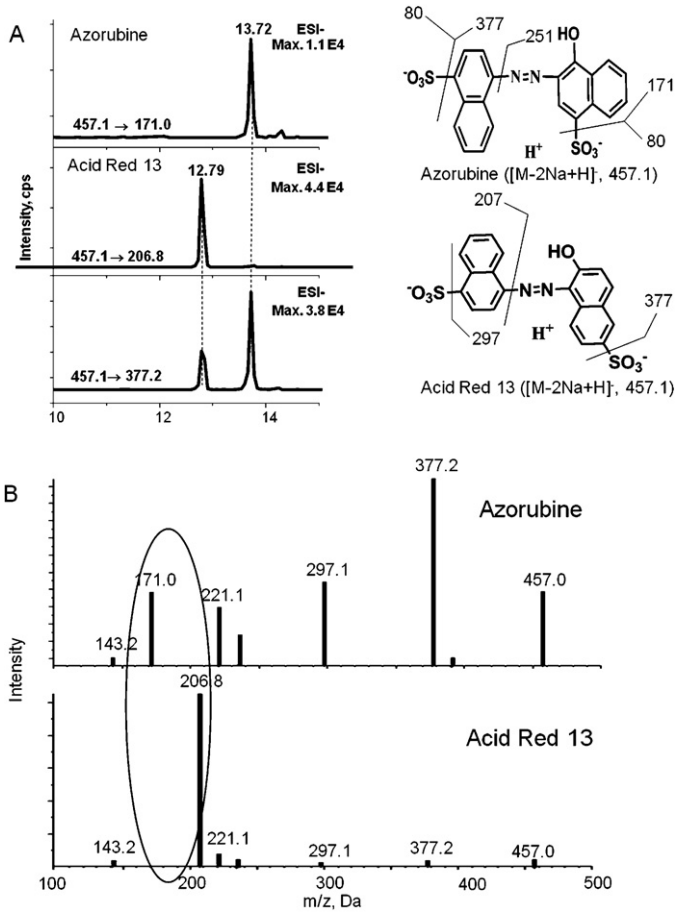

Fig. 2. (A)HPLC-ESI-MS/MS chromatograms of Azorubine (m/z 457.1 → 171.0, m/z 457.1 → 377.2) and Acid Red 13 (m/z 457.1 → 206.8, m/z 457.1 → 377.2) monitored in SRM mode. (B) Product ion spectra of Azorubine and Acid Red 13 obtained in product ion scan mode.

3.3. Method validation

Method precision was examined by intra-day and inter-day peak area variation (less than 5%). The matrix effect was investigated by comparing the peak areas of standards dissolved in water/methanol (9:1, v/v) to standards spiked into matrices at the same concentration. Our results demonstrated that peak areas varied less than 5%, suggesting a negligible matrix effect on quantification.

Linear dynamic range, correlation coefficient (r), limit of detection and recovery for the method are listed in Table 2. Excellent linearity for each dye was achieved with a linear regression coefficient of $r \geq 0.9990$ (Table 2). The recoveries were in the range of 91.1–105%.

Table 2
Linear range, correlation coefficients, limits of detection, recoveries and relative standard deviations (RSDs) of dyes were determined. The recoveries were evaluated by controlling the fortification level of each dye in negative soft drink samples at 50 times the limit of detection ($n = 3$).

Peak	Analyte	RT (min)	Linear range (mg L^{-1})	R	LOD (mg L^{-1})	Recovery (%)	RSD (%)
1	Tartrazine	2.80	1.25–10	0.9990	0.5	97.7	5.9
2	New Red	3.43	1.25–10	0.9997	0.125	91.3	7.1
3	Amaranth	5.13	1.25–10	0.9992	0.25	96.5	7.3
4	Ponceau 4R	8.68	1.25–10	0.9996	0.125	99.8	3.4
5	Indigo Carmine	8.92	0.031–0.5	0.9997	0.010	94.9	5.4
6	Carminic Acid	9.25	0.015–0.50	0.9995	0.003	92.1	6.1
7	Sunset Yellow FCF	9.50	0.030–0.50	0.9993	0.010	95.9	3.2
8	Allura Red AC	10.98	0.015–0.50	0.9993	0.005	92.4	6.7
9	Acid Red 1	11.34	0.015–0.50	0.9998	0.006	96.3	2.1
10	Wool Green S	12.23	0.0030–0.50	0.9990	0.001	105	4.3
11	Acid Red 13	12.87	0.031–0.50	0.9990	0.008	101	5.4
12	Light Green SF	13.52	0.0015–0.50	0.9995	0.0005	96.8	3.5
13	Ponceau 2R	13.69	0.007–0.50	0.9992	0.002	99.4	2.7
14	Azorubine	13.77	0.030–0.50	0.9997	0.010	102	4.3
15	Fast Green FCF	14.02	0.0075–0.50	0.9997	0.002	96.6	3.5
16	Ponceau SX	14.32	0.0015–0.50	0.9999	0.0004	91.5	5.4
17	Brilliant Blue FCF	14.37	0.0075–0.50	0.9997	0.002	96.6	4.2
18	Quinoline Yellow	14.46	0.0075–0.50	0.9997	0.001	91.1	2.1
19	Ponceau 3R	14.82	0.0015–0.50	0.9998	0.0005	96.5	4.6
20	Uranine	17.17	0.0015–0.50	0.9999	0.0001	94.8	5.2
21	Orange II	18.31	0.0015–0.25	0.9999	0.0001	93.7	5.1
22	Sulforhodamine B	18.70	0.0015–0.50	0.9995	0.0004	98.9	3.1
23	Acid Black 1	19.50	0.015–0.50	0.9995	0.003	103	1.2
24	Patent Blue V	19.72	0.0015–0.50	0.9999	0.0003	99.9	0.5
25	Alizarin Yellow GG	22.69	0.0015–0.063	0.9996	0.0001	98.5	2.3
26	Guinea Green B	22.97	0.0075–0.50	0.9998	0.002	102	3.1
27	Metanil Yellow	24.33	0.0015–0.25	0.9997	0.0001	103	2.4
28	Eosin Y	26.35	0.015–0.50	0.9992	0.004	104	4.6
29	Acid Green 25	27.21	0.0015–0.50	0.9999	0.0002	91.8	6.7
30	Acid Violet 17	27.75	0.015–0.500	0.9990	0.0005	92.4	6.2
31	Erythrosine	27.76	0.0015–0.50	0.9995	0.0004	105	2.1
32	Bengal Rose B	28.20	0.0075–0.5	0.9998	0.002	91.2	3.4
33	Acid Yellow 9	3.39	0.015–0.5	0.9998	0.005	95.4	4.5
34	Acid Yellow 17	11.23	0.0075–0.50	0.9992	0.002	93.2	3.4
35	Chrysoidine	14.44	0.0015–0.031	0.9996	0.0001	92.1	4.3
36	Basic Flavine O	16.94	0.0015–0.063	0.9990	0.0001	101	3.2
37	Patent Green	24.33	0.0015–0.5	0.9990	0.0004	99.4	2.3
38	Phloxine B	27.89	0.0030–0.5	0.9998	0.0008	99.1	3.4
39	Rhodamine B Chloride	28.01	0.0015–0.031	0.9990	0.0001	97.8	3.2
40	Methyl Yellow	29.23	0.0075–0.125	0.9996	0.001	93.4	2.1

The limits of detection (S/N = 3) of all analyzed dyes were 0.0001–0.01 mg/L except Tartrazine, Amaranth, New Red and Ponceau 4R which were 0.5, 0.25, 0.125 and 0.125 mg/L respectively (see Table 2). Comparing with the detection limits reported in literatures [5,7,22,25–28], the detection sensitivity was improved more than 10 times (see Table S1, Supporting information).

3.4. Application to real samples

In China, only 10 dyes are permitted to be added to soft drinks (including Tartrazine, Allura Red AC, Erythrosine, Indigo Carmine, Brilliant Blue FCF, Sunset Yellow FCF, Amaranth, Carminic Acid, New Red and Ponceau 4R) [4]. In order to detect illegal dyes, this

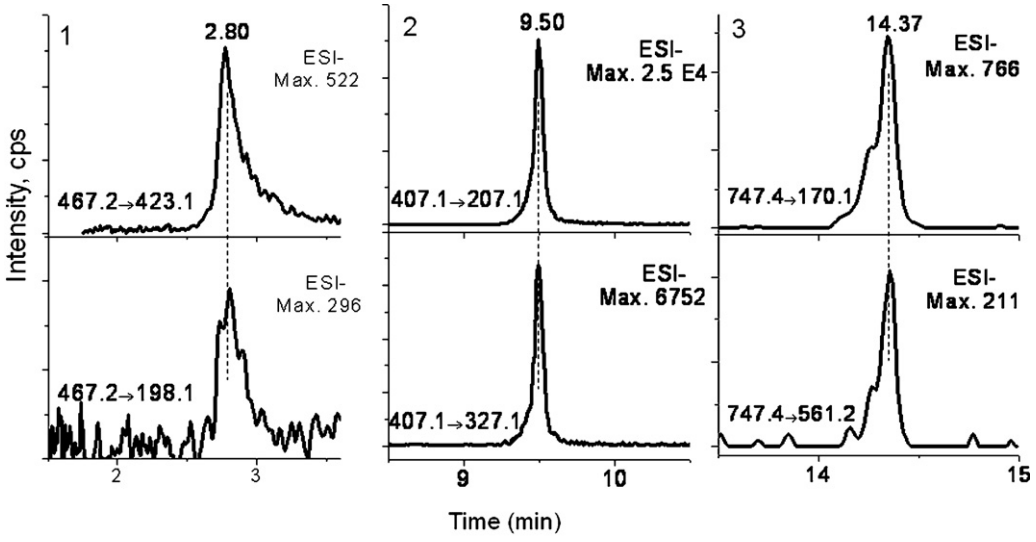

Fig. 3. Examples of typical chromatograms. (1) Tartrazine in sample No. 19. (2) Sunset Yellow FCF in sample No. 19. (3) Brilliant Blue FCF in sample No. 20.

Table 3
Quantification results for synthetic dyes in positive soft drinks samples analyzed by HPLC-MS/MS.

Sample	Dye	Concentration (μg/g)	RSD (%)
No. 1	Brilliant Blue FCF	12.9	0.4
No. 9	Allura Red	0.14	1.4
No. 19	Tartrazine	158	2.8
	Ponceau 4R	2.49	2.4
	Sunset Yellow FCF	13.3	1.5
No.20	Allura Red	0.107	1.8
	Brilliant Blue FCF	0.063	1.6

HPLC-MS/MS method was applied to 20 samples from the local market. No illegal dyes were detected. Tartrazine, Ponceau 4R, Sunset Yellow FCF, Allura Red AC, and Brilliant Blue FCF were identified at levels lower than their legal limits (100, 50, 100, 100 and 25 μg/g) [4]. Table 3 summarizes the screening results of the positive samples.

Fig. 3 shows the typical chromatograms of dyes detected in positive samples. With this HPLC-ESI-MS/MS method, not only accuracy was enhanced (identified by two SRM transitions simultaneously), but also the low concentration dye, Brilliant Blue FCF (0.063 μg/g, Table 3), was detected. This suggested that the HPLC-MS/MS method is appropriate for the screening of illegal dyes in foods.

4. Conclusion

In summary, by combining SPE cleanup and HPLC-MS/MS, an accurate and highly sensitive method was developed to screen 40 dyes in foods. Compared with traditional methods, the accuracy was enhanced, and the sensitivity was improved by more than 10 times, leading to a powerful method for screening illegal dyes in foods.

Acknowledgments

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

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2011.04.014.

References

[1] J. Noonan, in: T.E. Furia (Ed.), CRC Handbook of Food Additives, vol. I, 2nd ed., CRC Press, Boca Raton, FL, 1981, p. 587.
[2] IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, vol. 8, IARC, Lyon, 1975, p. 125.
[3] R. Macrae, R.K. Robinson, M.J. Sadler, Food Technology and Nutrition, Academic Press, California, 1993, p. 1232.
[4] Ministry of Health, P.R.C., Hygienic Standards for Uses of Food Additives, National Standard of the P.R.C., Beijing, 2007, p. 90.
[5] N. Yoshioka, K. Ichihashi, Talanta 74 (2008) 1408.
[6] H.Y. Huang, C.L. Chuang, C.W. Chiu, M.C. Chung, Electrophoresis 26 (2005) 867.
[7] M. Jaworska, Z. Szulińska, M. Wilk, E. Anuszewska, J. Chromatogr. A 1081 (2005) 42.
[8] N. Dossi, R. Toniolo, A. Pizzariello, S. Susmel, F. Perennes, G. Bontempelli, J. Electroanal. Chem. 601 (2007) 1.
[9] M. Ryvolová, P. Táborský, P. Vrábel, P. Krásenský, J. Preisler, J. Chromatogr. A 1141 (2007) 206.
[10] H.Y. Huang, Y. Shih, Y. Chen, J. Chromatogr. A 959 (2002) 317.
[11] J.J.B. Nevado, C.G. Cabanillas, A.M.C. Salcedo, Fresenius J. Anal. Chem. 350 (1994) 606.
[12] J.J. Berzas Nevado, C. Guiberteau Cabanillas, A.M. Contento Salcedo, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 3073.
[13] Q.C. Chen, S.F. Mou, X.P. Hou, J.M. Riviello, Z.M. Ni, J. Chromatogr. A 827 (1998) 73.
[14] C. Long, Z. Mai, Y. Yang, B. Zhu, X. Xu, L. Lu, X. Zou, J. Chromatogr. A 1216 (2009) 8379.
[15] M.S. García-Falcón, J. Simal-Gándara, Food Control. 16 (2005) 293.
[16] A. Kósa, T. Cserháti, E. Forgács, H. Morais, T. Mota, A.C. Ramos, J. Chromatogr. A 915 (2001) 149.
[17] L. He, Y. Su, B. Fang, X. Shen, Z. Zeng, Y. Liu, Anal. Chim. Acta 594 (2007) 139.
[18] Y.S. Al-Degs, Food Chem. 117 (2009) 485.
[19] K.S. Minioti, C.F. Sakellariou, N.S. Thomaidis, Anal. Chim. Acta 583 (2007) 103.
[20] J. Zhang, N. Gao, Y. Zhang, Anal. Lett. 40 (2007) 3080.
[21] J. Kirschbaum, C. Krause, H. Brückner, Eur. Food Res. Technol. 222 (2006) 572.
[22] M. Serdar, Z. Knežević, Chromatographia 70 (2009) 1519.
[23] M. Jalón, M.J. Peña, J.C. Rivas, J. Assoc. Off. Anal. Chem. 72 (1989) 231.
[24] M. González, M. Gallego, M. Valcárcel, Anal. Chem. 75 (2003) 685.
[25] M. González, M. Gallego, M. Valcárcel, J. Agric. Food Chem. 51 (2003) 2121.
[26] M. Ma, X. Luo, B. Chen, S. Su, S. Yao, J. Chromatogr. A 1103 (2006) 170.
[27] M.R. Fuh, K.J. Chia, Talanta 56 (2002) 663.
[28] H.W. Sun, F.C. Wang, L.F. Ai, J Chromatogr. A 1164 (2007) 120.
[29] M. Murty, N. Sridhara Chary, S. Prabhakar, N. Prasada Raju, M. Vairamani, Food Chem. 115 (2009) 1556.
[30] T. Storm, T. Reemtsma, M. Jekel, J Chromatogr A 854 (1999) 175.
[31] M. Holčapek, P. Jandera, P. Zderadička, J. Chromatogr A 926 (2001) 175.
[32] M. Holčapek, K. Volná, D. Vaněrková, Dyes and Pigments 75 (2007) 156.