

Method of Test for Hair Dyes in Cosmetics (3)

1. Scope

This method is applicable to the determination of 31 hair dyes (Basic blue 7, etc. listed in Table 1) in cosmetics.

2. Method

After extraction, hair dyes are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: CORTECS UPLC C18⁺, 1.6 μm , 2.1 mm i.d. $\times$ 10 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Methanol, HPLC grade;

Acetonitrile, HPLC grade;

Formic acid, GR grade;

Ammonium formate, GR grade;

Ascorbic acid, GR grade;

Deionized water, resistivity $\geq 18 \text{ M}\Omega\cdot\text{cm}$ (at 25°C);

Basic blue 7 and other hair dyes listed in Table1, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL and 20 mL.

2.3.2. Membrane filter: 0.22 μm , PTFE.

2.4. Reagents

2.4.1. 50% methanol solution

Mix equal volume of methanol and deionized water.

2.4.2. 0.1% ascorbic acid in 50% methanol solution

Dissolve and dilute 1 g of ascorbic acid with 50% methanol solution to 1000 mL.

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 0.63 g of ammonium formate with deionized water to 1000 mL. Adjust pH to 3.5 with formic acid, and filter with a membrane filter.

2.5.2. Solvent B

Mix methanol and acetonitrile well at the ratio of 1:1 (v/v). Filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 10 mg of reference standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute with 0.1% ascorbic acid in 50% methanol solution to volume as the standard stock solutions. Store in a refrigerator. Upon use, mix appropriate volume of each standard stock solution, and dilute with 0.1% ascorbic acid in 50% methanol solution as the standard solutions. The concentration of each standard solution is listed in the attached table 1.

2.7. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 20-mL volumetric flask. Add 15 mL of 0.1% ascorbic acid in 50% methanol solution, and sonicate for 30 min. Add 0.1% ascorbic acid in 50% methanol solution to volume. Filter with a membrane filter, and take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 3 µL of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Identify each hair dye based on the retention time and the relative ion intensities ^(note 1). Calculate the amount of each hair dye in the sample by the following formula:

$$\text{The amount of each hair dye in the sample (\%)} = \frac{C \times V}{M} \times 10^{-4}$$

where,

C: the concentration of each hair dye in the sample solution calculated by the standard curve (µg/mL)

V: the final make-up volume of sample (mL)

M: the weight of the sample (g)

LC-MS/MS operating conditions^(note 2):

Column: CORTECS UPLC C18⁺, 1.6 µm, 2.1 mm i.d. × 10 cm.

Column temperature: 30°C.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	Solvent A (%)	Solvent B (%)
0 → 1	98 → 98	2 → 2
1 → 3	98 → 95	2 → 5
3 → 4	95 → 90	5 → 10
4 → 8.5	90 → 20	10 → 80
8.5 → 10	20 → 5	80 → 95
10 → 12.5	5 → 5	95 → 95
12.5 → 13	5 → 98	95 → 2
13 → 15	98 → 98	2 → 2

Flow rate: 0.3 mL/min.

Injection volume: 3 µL.

Capillary voltage: 2.85 KV.

Ion source temperature: 150°C.

Desolvation temperature: 500°C.

Cone gas flow rate: 30 L/hr.

Desolvation flow rate: 650 L/hr.

Detection mode: multiple reaction monitoring (MRM). Selected ion pair, cone voltage (CV) and collision energy (CE) are shown Table 2.

Note: 1. Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions ($\leq 100\%$). Maximum permitted tolerances for relative ion intensities by LC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

2. All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. Limits of quantitation (LOQs) are listed in Table 2.
2. Further validation should be performed when interference compounds appear in samples.
3. In the List of Specific Purpose Ingredients in Cosmetic Products, if the

maximum concentration for a hair dye as free base or salt form in cosmetics is required, the content should be converted according to the conversion factors in Table 3.

Reference

1. Fu, R., Cao, M. and Wang, Y. 2012. Fast analysis of hair dyes using an Agilent Poroshell 120 Bouns-RP column by UHPLC and LC/MS/MS. Application Note, Agilent Technologies Inc., Publication number 5991-1289EN.
2. Feng, F., Zhao, Y., Yong, W., Sun, L., Jiang, G. and Chu, X. 2011. Highly sensitive and accurate screening of 40 dyes in soft drinks by liquid chromatography–electrospray tandem mass spectrometry. J. Chromatogr. B 879: 1813-1818.

Reference chromatograms

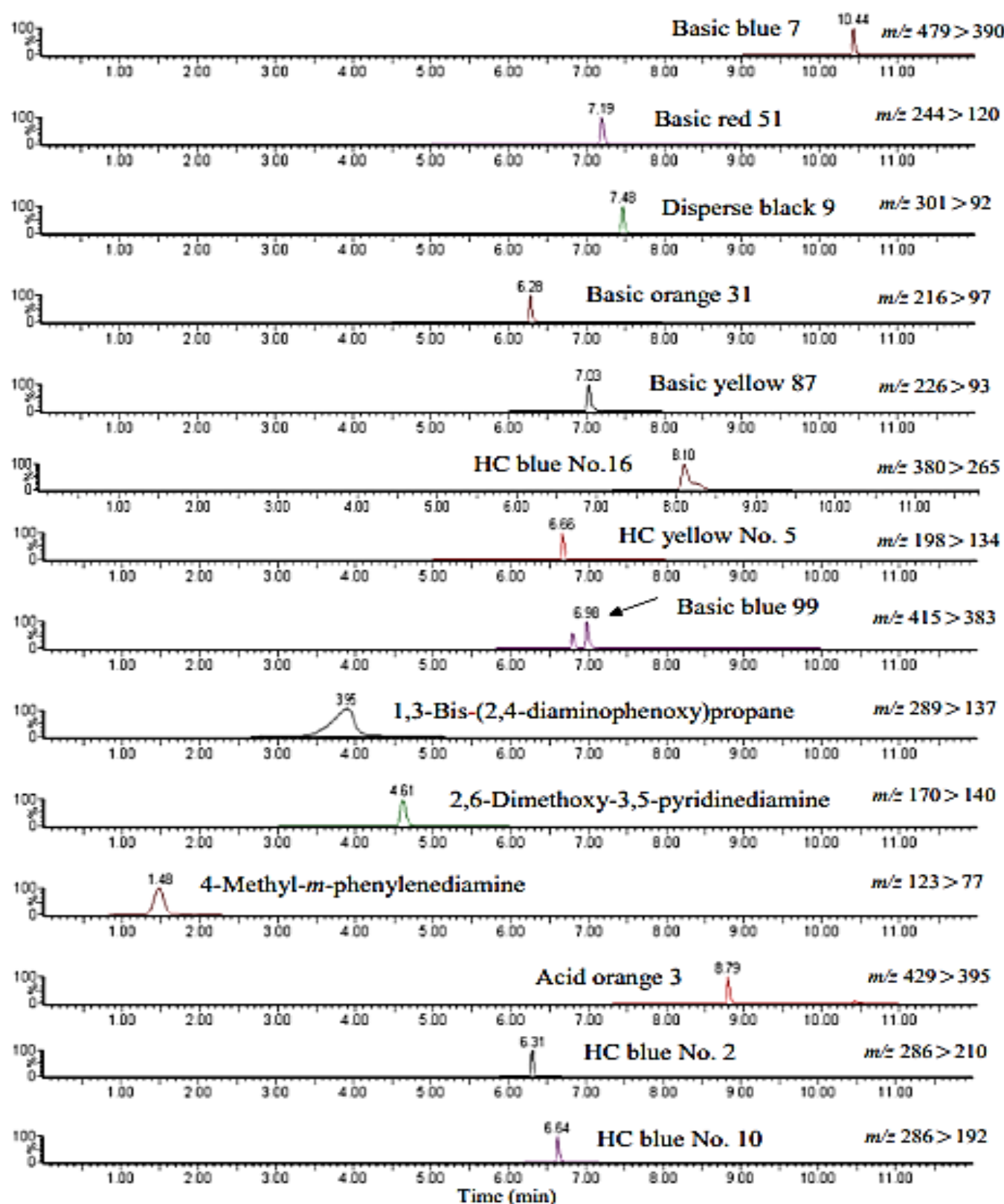


Figure. MRM chromatograms of 31 hair dye standards analyzed by LC-MS/MS.

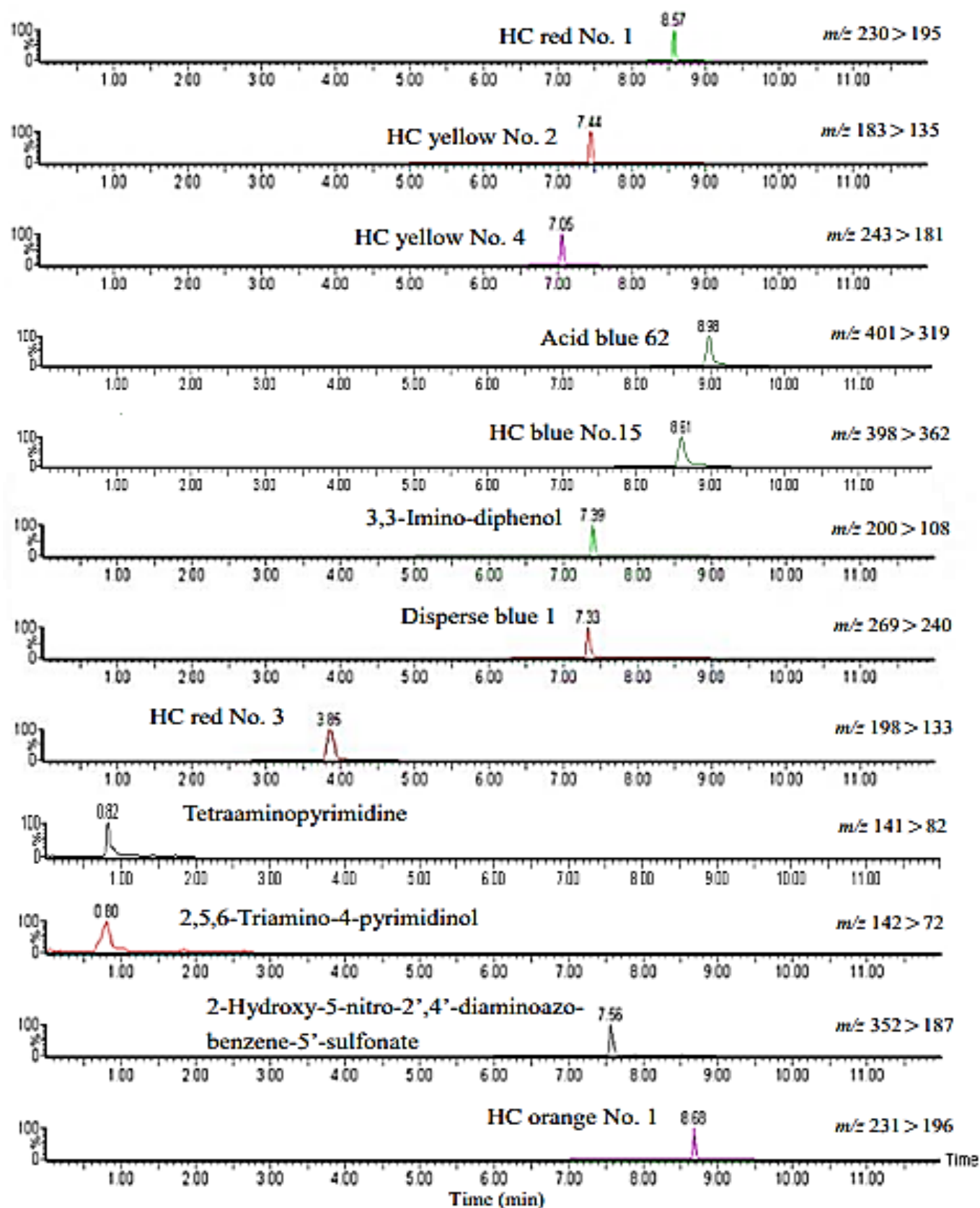


Figure. MRM chromatograms of 31 hair dye standards analyzed by LC-MS/MS (continued).

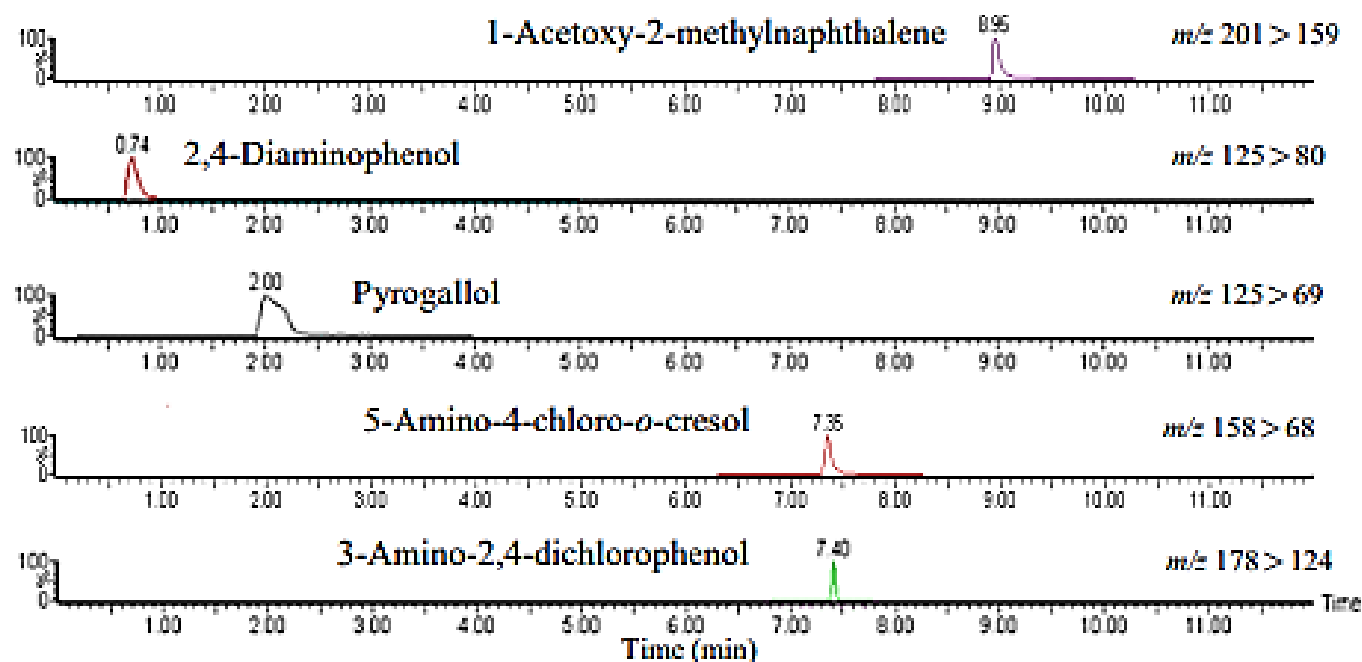


Figure. MRM chromatograms of 31 hair dye standards analyzed by LC-MS/MS (continued).

Table 1. Concentration ranges of 31 hair dye standard solutions

Item	Analyte	Concentration range (μg/mL)
1	Basic blue 7	0.001~0.02
2	Basic red 51	
3	Disperse black 9	
4	Basic orange 31	
5	Basic yellow 87	
6	HC blue No. 16	0.01~0.2
7	HC yellow No. 5	
8	Basic blue 99	0.05~1.0
9	1,3-Bis-(2,4-diaminophenoxy)propane	
10	2,6-Dimethoxy-3,5-pyridinediamine	
11	4-Methyl- <i>m</i> -phenylenediamine	
12	Acid orange 3	
13	HC blue No. 2	
14	HC blue No. 10	
15	HC red No. 1	
16	HC yellow No. 2	
17	HC yellow No. 4	
18	Acid blue 62	
19	HC blue No. 15	
20	3,3-Imino-diphenol	0.1~2.0
21	Disperse blue 1	
22	HC red No. 3	
23	Tetraaminopyrimidine	
24	2,5,6-Triamino-4-pyrimidinol	0.5~10
25	2-Hydroxy-5-nitro-2',4'-diaminoazo-benzene-5'-sulfonate	
26	HC orange No. 1	
27	1-Acetoxy-2-methylnaphthalene	
28	2,4-Diaminophenol	2~25
29	Pyrogallol	
30	5-Amino-4-chloro- <i>o</i> -cresol	10~100
31	3-Amino-2,4-dichlorophenol	25~250

Table 2. MRM parameters and LOQs of 31 hair dyes

Item	Analyte	Ionization mode	Ion pair		Cone voltage (V)	Collision energy (eV)	Limits of quantitation (%)
			Precursor ion (m/z)	Product ion (m/z)			
1	Basic blue 7	ESI ⁺	479	> 390*	66	54	0.000002
			479	> 405		52	
2	Basic red 51	ESI ⁺	244	> 120*	28	18	0.000002
			244	> 148		12	
3	Disperse black 9	ESI ⁺	301	> 92*	40	24	0.000002
			301	> 149		22	
4	Basic orange 31	ESI ⁺	216	> 97*	18	12	0.000002
			216	> 92		20	
5	Basic yellow 87	ESI ⁺	226	> 93*	14	22	0.000002
			226	> 120		16	
6	HC blue No.16	ESI ⁺	380	> 265*	50	22	0.00002
			380	> 293		16	
7	HC yellow No. 5	ESI ⁺	198	> 134*	26	18	0.00002
			198	> 150		20	
8	Basic blue 99	ESI ⁺	415	> 383*	58	32	0.0001
			415	> 400		26	
9	1,3-Bis-(2,4-diamino-phenoxy)propane	ESI ⁺	289	> 137*	34	24	0.0001
			289	> 165		14	
10	2,6-Dimethoxy-3,5-pyridiendiamine	ESI ⁺	170	> 140*	24	16	0.0001
			170	> 155		12	
11	4-Methyl- <i>m</i> -phenylenediamine	ESI ⁺	123	> 77*	38	14	0.0001
			123	> 108		18	
12	Acid orange 3	ESI ⁻	429	> 395*	62	30	0.0001
			429	> 382		32	
13	HC blue No. 2	ESI ⁺	286	> 210*	32	26	0.0001
			286	> 190		28	
14	HC blue No. 10	ESI ⁺	286	> 192*	32	24	0.0001
			286	> 209		18	
15	HC red No. 1	ESI ⁺	230	> 195*	40	30	0.0001
			230	> 168		20	
16	HC yellow No. 2	ESI ⁺	183	> 135*	24	12	0.0001
			183	> 107		10	
17	HC yellow No. 4	ESI ⁺	243	> 181*	30	22	0.0001
			243	> 135		16	

*Quantitative ion pair

Table 2. MRM parameters and LOQs of 31 hair dyes (continued)

Item	Analyte	Ionization mode	Ion pair		Cone voltage (V)	Collision energy (eV)	Limits of quantitation (%)
			Precursor ion (m/z)	Product ion (m/z)			
18	Acid blue 62	ESI ⁺	401	319* 237	42	18 28	0.0001
19	HC blue No.15	ESI ⁺	398	362* 367	64	36 32	0.0001
20	3,3-Imino-diphenol	ESI ⁻	200	108* 132	42	22 20	0.0002
21	Disperse blue 1	ESI ⁺	269	240* 169	62	36 42	0.0002
22	HC red No. 3	ESI ⁺	198	133* 163	22	16 12	0.0002
23	Tetraaminopyrimidine	ESI ⁺	141	82* 99	36	18 14	0.0002
24	2,5,6-Triamino-4-pyrimidinol	ESI ⁺	142	72* 97	38	20 16	0.001
25	2-Hydroxy-5-nitro-2',4'-diaminoazobenzene-5'-sulfonate	ESI ⁻	352	187* 200	46	18 26	0.001
26	HC orange No. 1	ESI ⁺	231	196* 168	24	14 30	0.001
27	1-Acetoxy-2-methyl-naphthalene	ESI ⁺	201	159* 131	24	8 20	0.001
28	2,4-Diaminophenol	ESI ⁺	125	80* 107	66	20 24	0.004
29	Pyrogallol	ESI ⁻	125	69* 79	62	16 12	0.004
30	5-Amino-4-chloro- <i>o</i> -cresol	ESI ⁺	158	68* 95	40	20 18	0.02
31	3-Amino-2,4-dichloro-phenol	ESI ⁺	178	124* 143	12	22 18	0.05

*Quantitative ion pair

Table 3. Table of Conversion Factors for Hair Dye Ingredients

Item	INCI name	CAS No.	Molecular weight	Maximum concentration		Conversion factor
				Oxidative	Non-oxidative	
9	1,3-Bis-(2,4-diaminophenoxy)propane	81892-72-0	288.345	1.2%	1.2%	1
	1,3-Bis-(2,4-diaminophenoxy)propane HCl	74918-21-1	434.189	(as free base)	(as free base)	1.506
9	1,3-Bis-(2,4-diaminophenoxy)propane	81892-72-0	288.345	1.8%	1.8%	0.664
	1,3-Bis-(2,4-diaminophenoxy)propane HCl	74918-21-1	434.189	(as tetrahydrochloride)	(as tetrahydrochloride)	1
10	2,6-Dimethoxy-3,5-pyridinediamine HCl	56216-28-5	242.103	0.25%	—	1
	2,6-Dimethoxy-3,5-pyridinediamine	85679-78-3	169.181	(as hydrochloride)		0.699
31	3-Amino-2,4-dichlorophenol	61693-42-3	178.016	1.5%	1.5%	0.830
	3-Amino-2,4-dichlorophenol HCl	61693-43-4	214.48	(as hydrochloride)	(as hydrochloride)	1