

Method of Test for Hair Dyes in Cosmetics

1. Scope

This method is applicable to the determination of 54 hair dyes (2,6-diaminopyridine, 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: ACQUITY UPLC HSS T3, 1.8 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;

Ammonium formate, GR grade;

Ammonium solution (25%), GR grade;

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

2,6-Diaminopyridine and other hair dyes listed in Table 1, 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. Preparation of 50% methanol

Mix equal volume of methanol and deionized water.

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 9 with ammonium solution, and filter with a membrane filter.

2.5.2. Solvent B:

Mix methanol and acetonitrile well at the ratio of 1:1 (v/v), and 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 separately, dissolve and dilute with 50% methanol to volume as the standard stock solutions. Store in a freezer. Upon use, mix appropriate volume of each standard stock solution, and dilute with 50% methanol as the standard solutions. Each standard solution's concentration is listed in the Table 1.

2.7. Sample solution preparation

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

2.8. Identification and quantification

Accurately inject 3 μ L 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 ^(note1) by multiple reaction monitoring. 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 (μ g/mL)

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

M: the weight of the sample (g)

LC-MS/MS operating conditions ^(note2):

Column: ACQUITY UPLC HSS T3, 1.8 μ m, 2.1 mm i.d. $\times$ 10 cm.

Column temperature: 30°C.

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

Time (min)	A (%)	B (%)
0 $\rightarrow$ 3	95 $\rightarrow$ 95	5 $\rightarrow$ 5
3 $\rightarrow$ 8	95 $\rightarrow$ 70	5 $\rightarrow$ 30
8 $\rightarrow$ 10	70 $\rightarrow$ 5	30 $\rightarrow$ 95
10 $\rightarrow$ 13	5 $\rightarrow$ 5	95 $\rightarrow$ 95
13 $\rightarrow$ 13.5	5 $\rightarrow$ 95	95 $\rightarrow$ 5
13.5 $\rightarrow$ 15	95 $\rightarrow$ 95	5 $\rightarrow$ 5

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: 900 L/hr.

Detection mode: multiple reaction monitoring (MRM). Selected ion pair, cone voltage (CV) and collision energy (CE) are shown in the attached 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 quantification (LOQs) of hair dyes 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 product, 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. Ma, Q., Bai, H., Ding, Y., Chen, W., Zhang, Q., Wang, C., Dong, Y. Y. and Wang, B. L. 2009. Determination of 8 water-soluble colorants in cosmetics by HPLC and verified by LC-MS/MS. J. Chin. Mass Spectrom. Soc. 30: 216-218.

Table 1. The concentration ranges of 54 hair dye standard solutions

Item	Analyte	Concentration range ($\mu\text{g/mL}$)
1	2,6-Diaminopyridine	0.005~0.1
2	Phenyl methyl pyrazolone	0.01~0.5
3	4-Amino-2-hydroxytoluene	0.02~0.25
4	<i>N,N</i> -bis(2-Hydroxyethyl)- <i>p</i> -phenylenediamine	
5	Dihydroxyindoline	
6	<i>m</i> -Phenylenediamine	0.05~0.5
7	1-Hydroxyethyl-4,5-diamino pyrazole	
8	2-Amino-3-hydroxypyridine	
9	Hydroxyethyl- <i>p</i> -phenylenediamine	
10	2-Amino-4-hydroxyethylaminoanisole	
11	2,6-Dihydroxyethylaminotoluene	
12	Hydroxyethyl-3,4-methylenedioxyaniline	
13	<i>N,N</i> -Dimethyl- <i>p</i> -phenylenediamine	
14	<i>N,N</i> -bis(2-Hydroxyethyl)-2-nitro- <i>p</i> -phenylenediamine	
15	2-Methyl-5-hydroxyethylaminophenol	
16	4-Hydroxypropylamino-3-nitrophenol	
17	Disperse violet 1	
18	Hydroxyethyl-2-nitro- <i>p</i> -toluidine	
19	2,6-Dihydroxy-3,4-dimethylpyridine	
20	Isatin	
21	2,4-Diaminophenoxyethanol	0.1~1.0
22	2-Nitro-1,4-diaminobenzene	
23	<i>m</i> -Aminophenol	0.2~2.0
24	<i>N</i> -Phenyl- <i>p</i> -phenylenediamine	
25	<i>p</i> -Phenylenediamine	0.5~5.0
26	<i>p</i> -Aminophenol	
27	<i>o</i> -Phenylenediamine	
28	<i>p</i> -Methylaminophenol	
29	4-Amino-3-nitrophenol	
30	4-Chlororesorcinol	
31	1-Naphthol	

Table 1. The concentration ranges of 54 hair dye standard solutions (Continued)

Item	Analyte	Concentration range ($\mu\text{g/mL}$)
32	Gallic acid	0.5~5.0
33	2-Amino-4-nitrophenol	
34	<i>p</i> -Amino- <i>o</i> -cresol	
35	2-Amino-6-chloro-4-nitrophenol	
36	Picramic acid	
37	Toluene-3,4-diamine	
38	2-Amino-5-nitrophenol	
39	3-Nitro- <i>p</i> -hydroxyethylaminophenol	
40	4,4-Diaminodiphenylamine	
41	5-Amino-6-chloro- <i>o</i> -cresol	
42	2,4-Diaminodiphenylamine	1~10
43	2,2'-Methylenebis-4-aminophenol	
44	4-Amino- <i>m</i> -cresol	
45	<i>o</i> -Aminophenol	2~20
46	Toluene-2,5-diamine	
47	<i>o</i> -Chloro- <i>p</i> -phenylenediamine	
48	4-Nitro- <i>o</i> -phenylenediamine	
49	6-Amino- <i>m</i> -cresol	
50	1,5-Naphthalenediol	
51	6-Hydroxyindole	2~25
52	2,7-Naphthalenediol	
53	2-Methylresorcinol	
54	Resorcinol	20~200
		25~250

Table 2. MRM parameters and LOQs of 54 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	2,6-Diaminopyridine	ESI ⁺	110 > 93 [*] 110 > 66		38	15 25	0.00001
2	Phenyl methyl-pyrazolone	ESI ⁺	175 > 65 [*] 175 > 106		44	24 16	0.00002
3	4-Amino-2-hydroxytoluene	ESI ⁺	124 > 77 [*] 124 > 109		35	20 15	0.00004
4	<i>N,N</i> -bis(2-Hydroxyethyl)- <i>p</i> -phenylenediamine	ESI ⁺	197 > 152 [*] 197 > 121		12	12 26	0.00004
5	Dihydroxyindoline	ESI ⁺	150 > 122 [*] 150 > 94		34	14 22	0.00004
6	<i>m</i> -Phenylenediamine	ESI ⁺	109 > 92 [*] 109 > 65		36	12 20	0.0001
7	1-Hydroxyethyl-4,5-diamino pyrazole	ESI ⁺	143 > 99 [*] 143 > 126		28	12 10	0.0001
8	2-Amino-3-hydroxy-pyridine	ESI ⁺	111 > 94 [*] 111 > 66		40	14 16	0.0001
9	Hydroxyethyl- <i>p</i> -phenylenediamine	ESI ⁺	153 > 135 [*] 153 > 118		24	20 22	0.0001
10	2-Amino-4-hydroxy-ethylaminoanisole	ESI ⁺	183 > 123 [*] 183 > 95		28	22 36	0.0001
11	2,6-Dihydroxy-ethylaminotoluene	ESI ⁺	211 > 135 [*] 211 > 107		36	24 38	0.0001
12	Hydroxyethyl-3,4-methylenedioxyaniline	ESI ⁺	182 > 137 [*] 182 > 106		28	16 18	0.0001
13	<i>N,N</i> -Dimethyl- <i>p</i> -phenylenediamine	ESI ⁺	137 > 122 [*] 137 > 107		30	20 22	0.0001
14	<i>N,N</i> -bis(2-Hydroxyethyl)-2-nitro- <i>p</i> -phenylene-diamine	ESI ⁺	242 > 146 [*] 242 > 166		28	22 20	0.0001
15	2-Methyl-5-hydroxy-ethylaminophenol	ESI ⁺	168 > 122 [*] 168 > 77		32	18 20	0.0001
16	4-Hydroxypropylamino-3-nitrophenol	ESI ⁺	213 > 123 [*] 213 > 148		26	12 12	0.0001
17	Disperse violet 1	ESI ⁺	239 > 166 [*] 239 > 105		50	28 30	0.0001
18	Hydroxyethyl-2-nitro- <i>p</i> -toluidine	ESI ⁺	197 > 121 [*] 197 > 94		24	14 18	0.0001
19	2,6-Dihydroxy-3,4-dimethylpyridine	ESI ⁺	140 > 67 [*] 140 > 94		40	20 14	0.0001

* The quantitative ion.

Table 2. MRM parameters and LOQs of 54 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)			
20	Isatin	ESI ⁺	148	102 [*] 92	44	20 20	0.0001
21	2,4-Diamino-phenoxyethanol	ESI ⁺	169	124 [*] 108	38	10 14	0.0002
22	2-Nitro-1,4-diaminobenzene	ESI ⁺	154	91 [*] 119	34	22 14	0.0002
23	<i>m</i> -Aminophenol	ESI ⁺	110	65 [*] 93	38	18 15	0.0004
24	<i>N</i> -Phenyl- <i>p</i> -phenylenediamine	ESI ⁺	185	93 [*] 108	40	30 20	0.0004
25	<i>p</i> -Phenylenediamine	ESI ⁺	109	92 [*] 65	36	12 20	0.001
26	<i>p</i> -Aminophenol	ESI ⁺	110	65 [*] 93	38	20 15	0.001
27	<i>o</i> -Phenylenediamine	ESI ⁺	109	92 [*] 65 [*]	36	14 20	0.001
28	<i>p</i> -Methylaminophenol	ESI ⁺	124	109 [*] 80	38	16 40	0.001
29	4-Amino-3-nitrophenol	ESI ⁻	153	123 [*] 118	22	6 8	0.001
30	4-Chlororesorcinol	ESI ⁻	143	79 [*] 107	32	14 8	0.001
31	1-Naphthol	ESI ⁻	143	115 [*] 41	52	18 55	0.001
32	Gallic acid	ESI ⁻	169	125 [*] 79	32	12 20	0.001
33	2-Amino-4-nitrophenol	ESI ⁻	153	123 [*] 107	36	14 16	0.001
34	<i>p</i> -Amino- <i>o</i> -cresol	ESI ⁺	124	109 [*] 77	34	16 22	0.001
35	2-Amino-6-chloro-4-nitrophenol	ESI ⁻	187	120 [*] 157	38	20 14	0.001
36	Picramic acid	ESI ⁻	198	181 [*] 138	34	12 16	0.001
37	Toluene-3,4-diamine	ESI ⁺	123	106 [*] 79	34	18 22	0.001
38	2-Amino-5-nitrophenol	ESI ⁻	153	122 [*] 106	38	14 18	0.001

* The quantitative ion.

Table 2. MRM parameters and LOQs of 54 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)			
39	3-Nitro- <i>p</i> -hydroxyethyl-aminophenol	ESI ⁺	199	151 [*] 123	24	10 12	0.001
40	4,4-Diamino-diphenylamine	ESI ⁺	200	107 [*] 80	32	18 34	0.001
41	5-Amino-6-chloro- <i>o</i> -cresol	ESI ⁺	158	123 [*] 104	26	20 16	0.001
42	2,4-Diamino-diphenylamine	ESI ⁺	200	183 [*] 166	28	12 20	0.001
43	2,2'-Methylenebis-4-aminophenol	ESI ⁺	231	122 [*] 94	28	20 32	0.001
44	4-Amino- <i>m</i> -cresol	ESI ⁺	124	109 [*] 77	45	25 35	0.002
45	<i>o</i> -Aminophenol	ESI ⁺	110	65 [*] 93	45	20 30	0.002
46	Toluene-2,5-diamine	ESI ⁺	123	108 [*] 77	34	12 24	0.004
47	<i>o</i> -Chloro- <i>p</i> -phenylenediamine	ESI ⁺	143	80 [*] 108	36	20 12	0.004
48	4-Nitro- <i>o</i> -phenylenediamine	ESI ⁻	152	46 [*] 105	34	30 18	0.004
49	6-Amino- <i>m</i> -cresol	ESI ⁺	124	106 [*] 80	36	20 25	0.004
50	1,5-Naphthalenediol	ESI ⁻	159	115 [*] 131	30	30 25	0.004
51	6-Hydroxyindole	ESI ⁻	132	131 [*] 104	38	20 22	0.004
52	2,7-Naphthalenediol	ESI ⁻	159	130 [*] 102	44	20 22	0.004
53	2-Methylresorcinol	ESI ⁻	123	79 [*] 55	32	12 14	0.04
54	Resorcinol	ESI ⁻	109	41 [*] 65	34	16 8	0.05

* The quantitative ion.

Table 3. Table of Conversion Factors for Hair Dye Ingredients

Item	INCI name	CAS No.	Molecular weight	Maximum concentration		Conversion factor
				Oxidative	Non-oxidative	
10	2-Amino-4-hydroxyethylaminoanisole	83763-47-7	182.22	1.5% (as sulfate)	–	0.650
	2-Amino-4-hydroxyethylaminoanisole sulfate	83763-48-4	280.30			1
21	2,4-Diaminophenoxyethanol	70643-19-5	168.19	2% (as hydrochloride)	–	0.698
	2,4-Diaminophenoxyethanol HCl	66422-95-5	241.12			1
	2,4-Diaminophenoxyethanol sulfate	70643-20-8	266.27			1.104
25	<i>p</i> -Phenylenediamine	106-50-3	108.14	2% (as free base)	–	1
	<i>p</i> -Phenylenediamine HCl	624-18-0	181.06			1.674
	<i>p</i> -Phenylenediamine sulfate	16245-77-5	206.22			1.907
28	<i>p</i> -Methylaminophenol	150-75-4	123.15	0.68% (as sulfate)	–	0.557
	<i>p</i> -Methylaminophenol sulfate (2:1)	55-55-0	344.39			1.557
	<i>p</i> -Methylaminophenol sulfate (1:1)	1936-57-8	221.24			1
46	Toluene-2,5-diamine	95-70-5	122.17	2% (as free base)	–	1
	Toluene-2,5-diamine sulfate	615-50-9	220.25			1.803
46	Toluene-2,5-diamine	95-70-5	122.17	3.6% (as sulfate salt)	–	0.555
	Toluene-2,5-diamine sulfate	615-50-9	220.25			1

Reference chromatograms

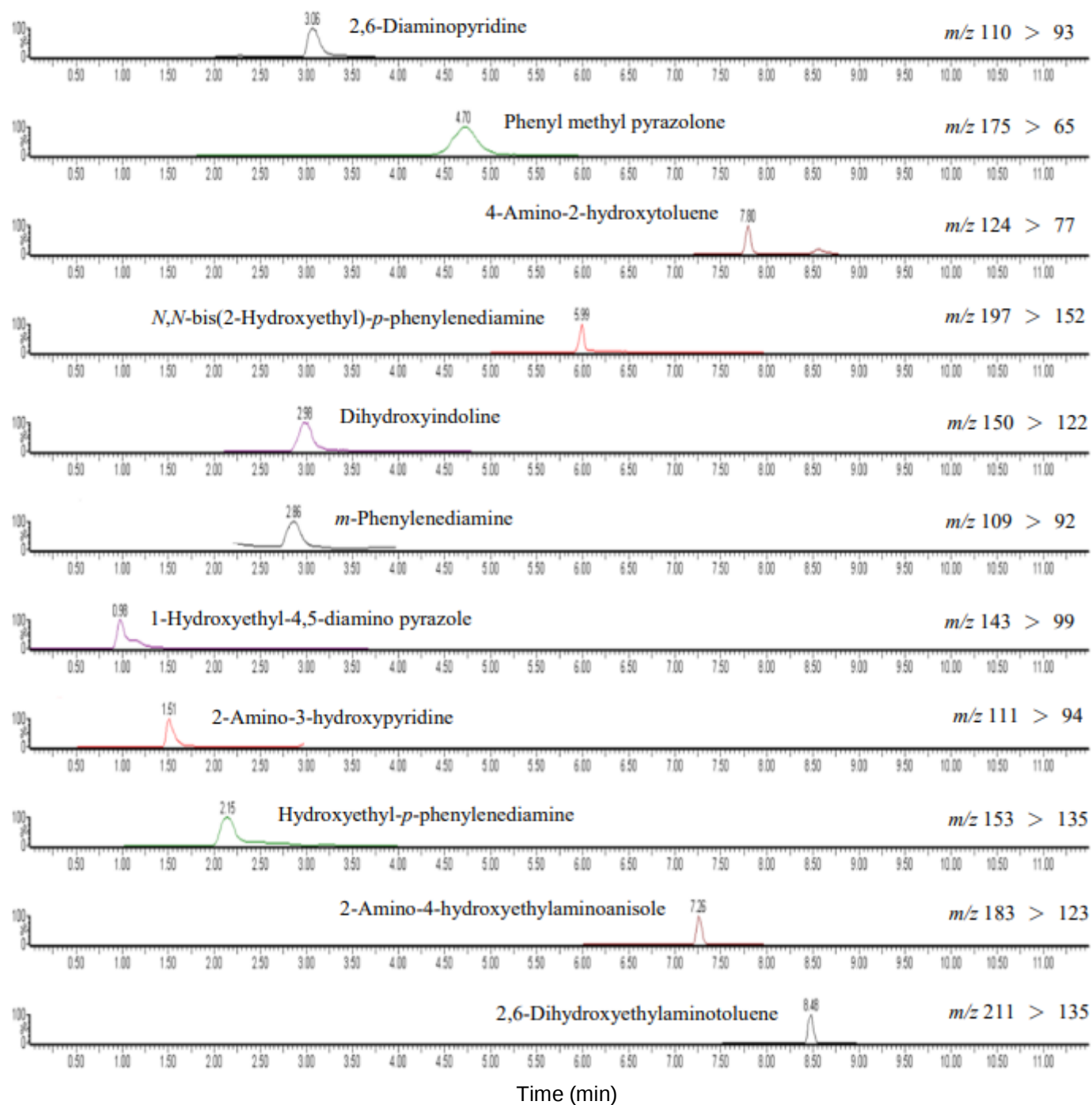


Figure. MRM chromatograms of 54 hair dye standards analyzed by LC-MS/MS.

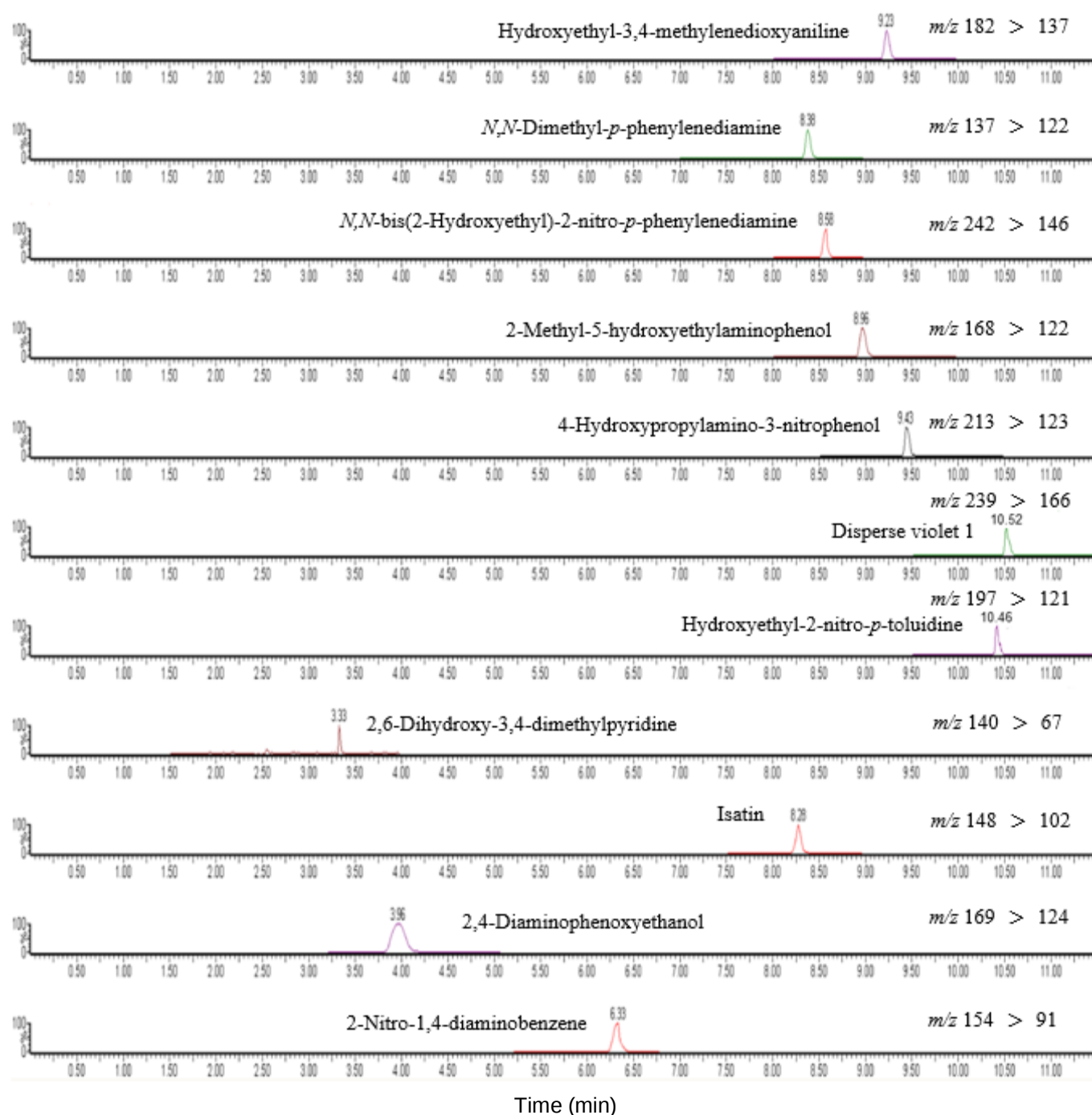


Figure. MRM chromatograms of 54 hair dye standards analyzed by LC-MS/MS.
(Continued).

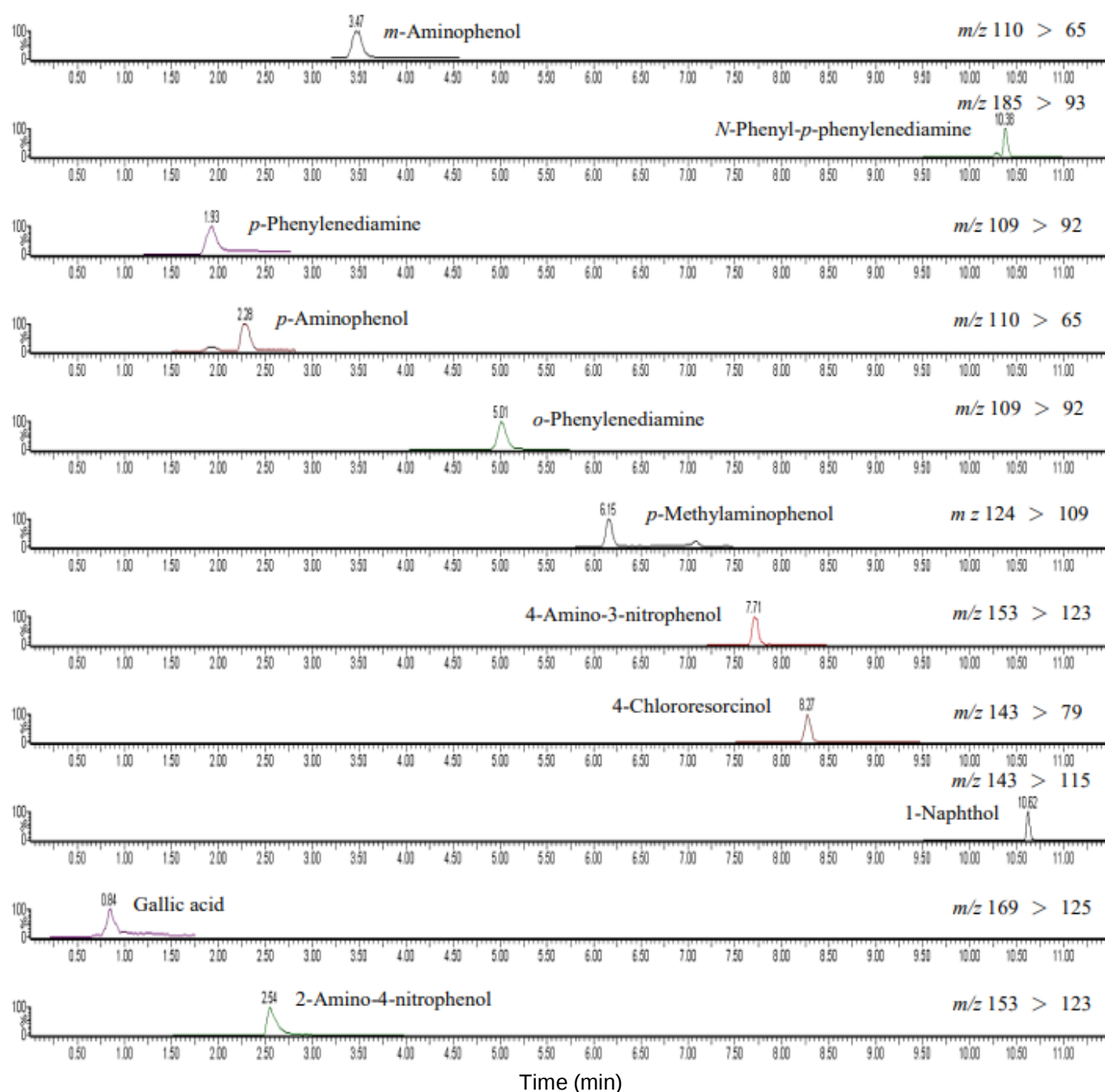


Figure. MRM chromatograms of 54 hair dye standards analyzed by LC-MS/MS (Continued).

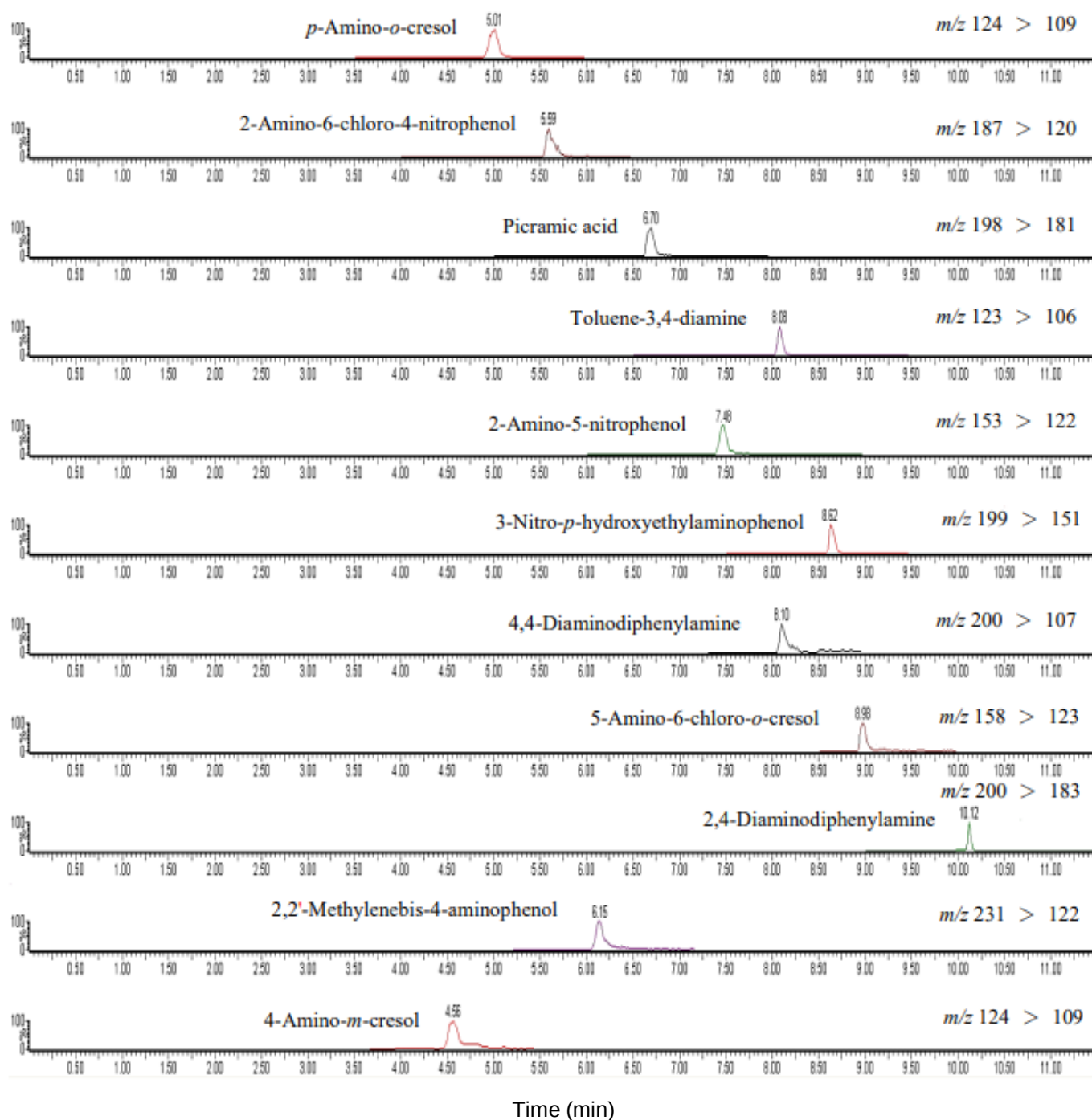


Figure. MRM chromatograms of 54 hair dye standards analyzed by LC-MS/MS (Continued).

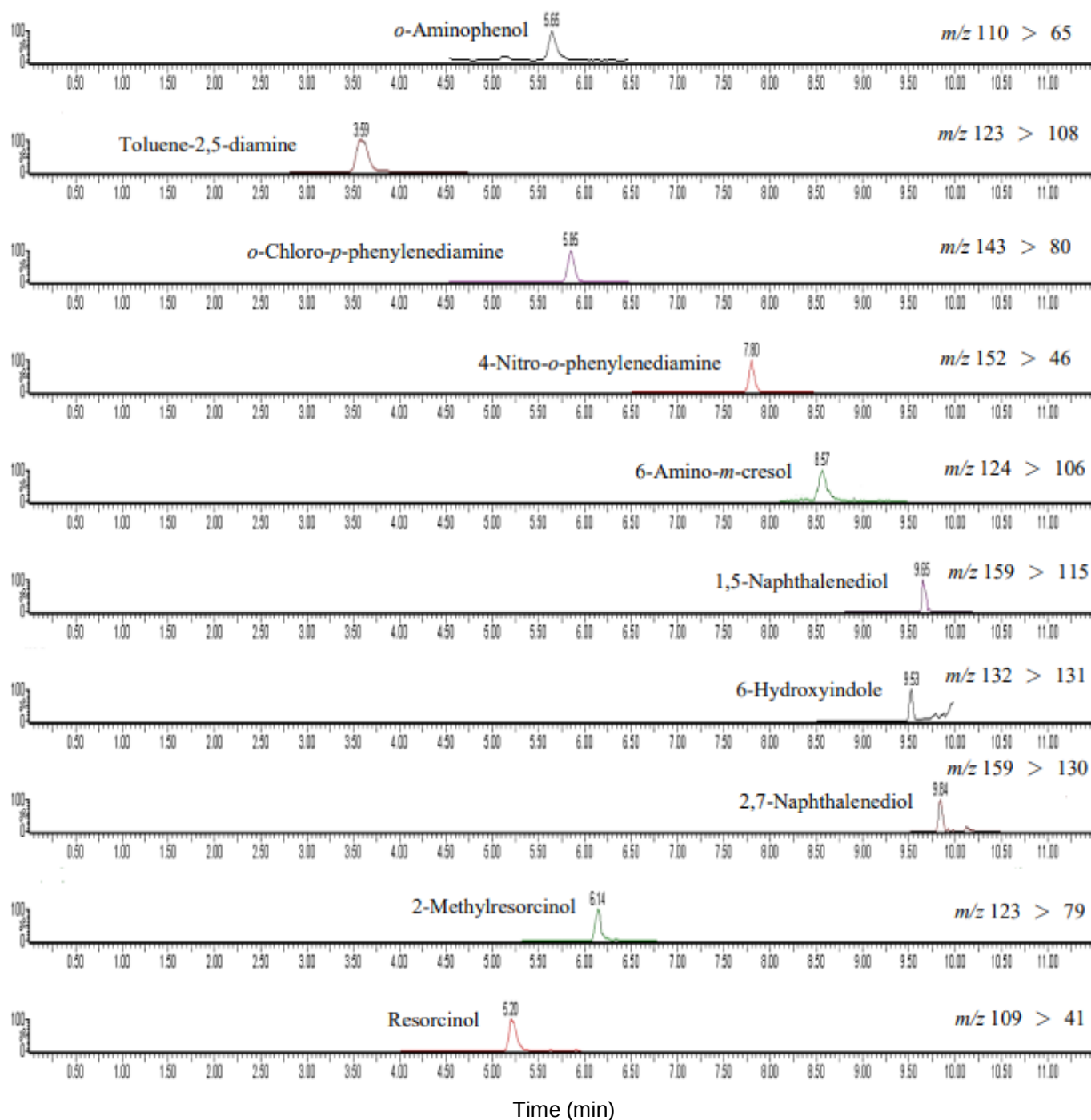


Figure. MRM chromatograms of 54 hair dye standards analyzed by LC-MS/MS (Continued).