

Method of Test for Hair Dyes in Cosmetics (4)

1. Scope

This method is applicable to the determination of 22 hair dyes (4,4'-methylenedianiline, etc. listed in the attached table 1) in cosmetics.

2. Method

After extraction, analytes 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 BEH Shield RP 18, 1.7 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;

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

4,4'-Methylenedianiline and other hair dyes listed in the attached 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. Mobile phase

2.4.1. Solvent A:

Dissolve and dilute 0.63 g of ammonium formate with deionized water to 1000 mL, and filter with a membrane filter.

2.4.2. Solvent B: Acetonitrile.

2.5. Standard solution preparation

Transfer about 10 mg of reference standards into each 10-mL volumetric flask, dissolve and dilute with methanol to the volume as the standard stock solutions. Store in a freezer. When to use, mix appropriate volume of each standard stock solution, and dilute with methanol as the standard solutions. Each standard solution's concentration is listed in the attached table 1.

2.6. Sample solution preparation

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

2.7. 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:

$$\text{The amount of each hair dye in the sample (ppm)} = \frac{C \times V}{M}$$

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 sample (g)

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

Column: ACQUITY BEH Shield RP 18, 1.7 µm, 2.1 mm i.d. × 10 cm.

Column temperature: 40°C.

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

Time (min)	A (%)	B (%)
0 → 3	95 → 80	5 → 20
3 → 6	80 → 50	20 → 50
6 → 10	50 → 0	50 → 100
10 → 12	0 → 0	100 → 100
12 → 12.5	0 → 95	100 → 5
12 → 15	95 → 95	5 → 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: 800 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection 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 the attached table 2.
2. Further validation should be performed when interference compounds appear in samples.

Reference

Szabó, B. S., Jakab, P. P., Hegedűs, J., Kirchkeszner, C., Petrovics, N., Nyiri, Z., Bodai, Z., Rikker, T. and Eke, Z. 2021. Determination of 24 primary aromatic amines in aqueous food simulants by combining solid phase extraction and salting-out assisted liquid–liquid extraction with liquid chromatography tandem mass spectrometry. *Microchem. J.* 164: 105927.

Reference chromatogram

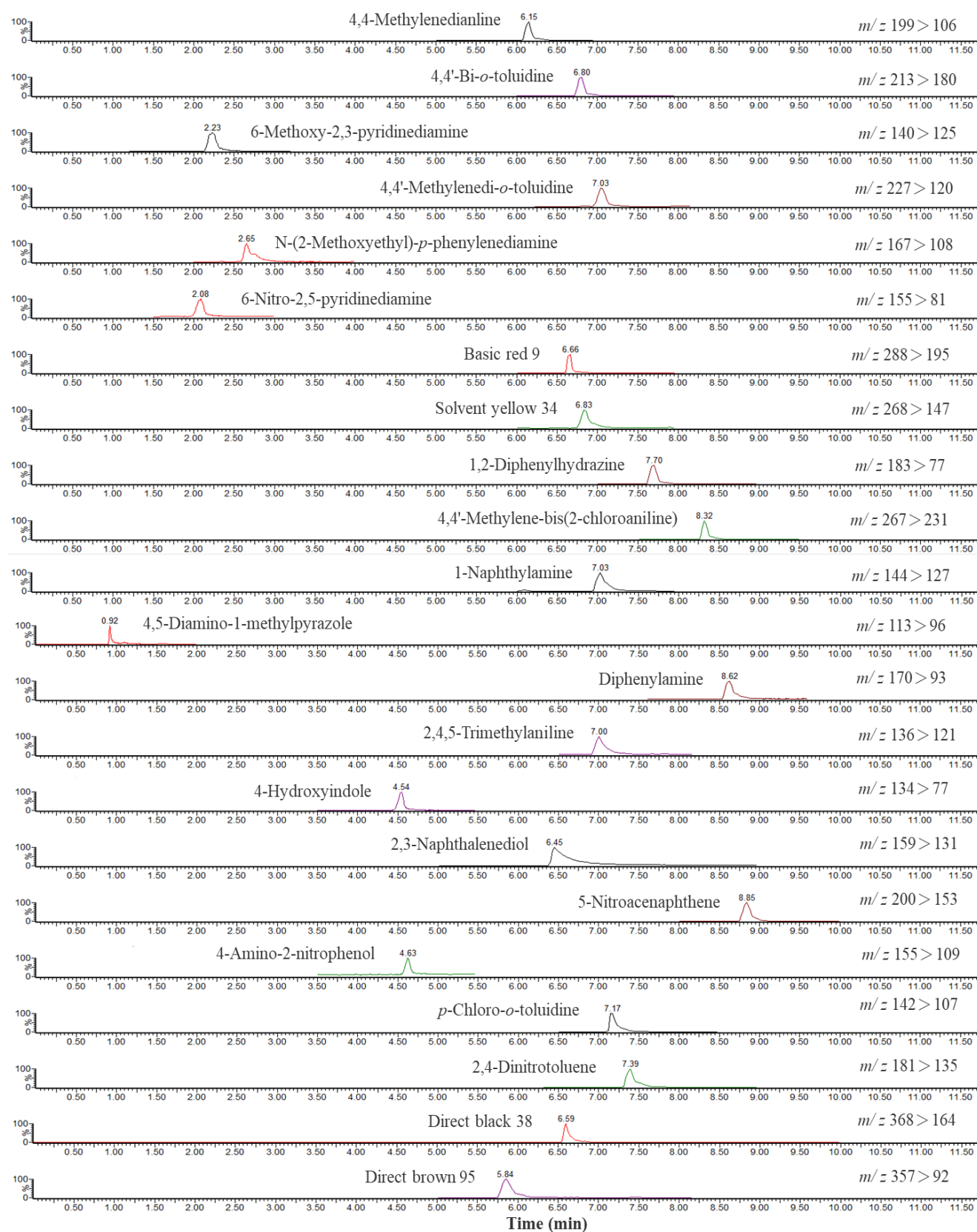


Figure. MRM chromatograms of 22 hair dyes standards analyzed by LC-MS/MS

Table 1. Concentration ranges of 22 hair dyes standard solutions

Analyte	Concentration range (µg/mL)
4,4'-Methylenedianiline	0.001~0.02
4,4'-Bi- <i>o</i> -toluidine	
6-Methoxy-2,3-pyridinediamine	
4,4'-Methylenedi- <i>o</i> -toluidine	
<i>N</i> -(2-Methoxyethyl)- <i>p</i> -phenylenediamine	
6-Nitro-2,5-pyridinediamine	
4,4'-(4-Iminocyclohexa-2,5-dienylidenemethylene) dianiline hydrochloride (Basic red 9)	
4,4'-Carbonimidoylbis[<i>N,N</i> -dimethylaniline] (Solvent yellow 34)	
1,2-Diphenylhydrazine	0.01~0.2
4,4'-Methylene-bis(2-chloroaniline)	
1-Naphthylamine	
4,5-Diamino-1-methylpyrazole	
Diphenylamine	
2,4,5-Trimethylaniline	
4-Hydroxyindole	0.1~2
2,3-Naphthalenediol	
5-Nitroacenaphthene	
4-Amino-2-nitrophenol	1~10
<i>p</i> -Chloro- <i>o</i> -toluidine	
2,4-Dinitrotoluene	
Disodium 4-amino-3-[[4'-[(2,4-diaminophenyl)azo][1,1'-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)naphthalene-2,7-disulphonate (Direct black 38)	
Disodium[5-[[4'-[[2,6-dihydroxy-3-[(2-hydroxy-5-sulphophenyl)azo]phenyl]azo][1,1'-biphenyl]-4-yl]azo]salicylato(4-)]cuprate(2-) (Direct brown 95)	

Table 2. MRM parameters and LOQs of 22 hair dyes

Analyte	Ionization mode	Ion pair		Cone voltage (V)	Collision energy (eV)	Limits of quantitation (ppm)
		Precursor ion (m/z)	Product ion (m/z)			
4,4'-Methylenedianiline	ESI ⁺	199	106* 77	36	22 52	0.02
4,4'-Bi-o-toluidine	ESI ⁺	213	180* 152	30	30 52	0.02
6-Methoxy-2,3-pyridinediamine	ESI ⁺	140	125* 108	8	14 20	0.02
4,4'-Methylenedi-o-toluidine	ESI ⁺	227	120* 77	32	32 60	0.02
N-(2-Methoxyethyl)-p-phenylenediamine	ESI ⁺	167	108* 80	24	16 40	0.02
6-Nitro-2,5-pyridinediamine	ESI ⁺	155	81* 108	16	18 12	0.02
Basic red 9	ESI ⁺	288	195* 151	26	28 54	0.02
Solvent yellow 34	ESI ⁺	268	147* 122	20	22 26	0.02
1,2-Diphenylhydrazine	ESI ⁺	183	77* 51	12	16 38	0.2
4,4'-Methylene-bis (2-chloroaniline)	ESI ⁺	267	231* 195	26	20 32	0.2
1-Naphthylamine	ESI ⁺	144	127* 77	24	22 34	0.2
4,5-Diamino-1-methylpyrazole	ESI ⁺	113	96* 55	25	14 18	0.2

Table 2. MRM parameters and LOQs of 22 hair dyes (Continued)

Analyte	Ionization mode	Ion pair		Cone voltage (V)	Collision energy (eV)	Limits of quantitation (ppm)
		Precursor ion (m/z)	Product ion (m/z)			
Diphenylamine	ESI ⁺	170	93*	28	30	0.2
		170	65		32	
2,4,5-Trimethylaniline	ESI ⁺	136	121*	20	14	0.2
		136	91		22	
4-Hydroxyindole	ESI ⁺	134	77*	14	6	2
		134	107		40	
2,3-Naphthalenediol	ESI ⁻	159	131*	2	16	2
		159	141		20	
5-Nitroacenaphthene	ESI ⁺	200	153*	16	22	2
		200	127		42	
4-Amino-2-nitrophenol	ESI ⁺	155	109*	48	14	20
		155	92		26	
p-Chloro- <i>o</i> -toluidine	ESI ⁺	142	107*	36	16	20
		142	125		20	
2,4-Dinitrotoluene	ESI ⁻	181	135*	12	6	20
		181	116		18	
Direct black 38	ESI ⁻	368	164*	38	14	20
		368	322		10	
Direct brown 95	ESI ⁻	357	92*	38	34	20
		357	186		22	

*Quantitative ion pair.