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Method of Test for Aromatic Amines in Hair Dyes

1. Scope

This method is applicable to the determination of 29 aromatic amines (4,4'-bi-*o*-toluidine, etc. listed in the 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: ZORBAX Eclipse XDB-C18, 3.5 μm, 2.1 mm i.d. × 5 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Ammonium acetate, GR grade;
Formic acid, GR grade;
Ammonia water, 25%, GR grade;
Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);
4,4'-bi-*o*-toluidine and other aromatic amines listed in the 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. Reagent

2.4.1. 0.05% formic acid solution

Transfer 0.5 mL of formic acid into a 1000 mL volumetric flask, and dilute to volume with deionized water.

2.4.2. Extraction solution

Transfer 4 mL of ammonia water (25%) into a 1000 mL volumetric flask, and dilute to volume with deionized water.

2.5. Mobile phase

2.5.1. Solvent A:

Dissolve and dilute 0.385 g of ammonium acetate with 0.05% formic acid

solution to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B: Acetonitrile.

2.6. Standard solution preparation

Transfer about 10 mg of reference standards accurately weighed into each 10 mL volumetric flask, dissolve and dilute with extraction solution to the volume as the standard stock solutions. Store in a refrigerator. Upon use, mix appropriate volume of each standard stock solution, and dilute with extraction solution as the standard solutions. The concentration range of each standard solution is listed in the 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 extraction solution, and sonicate for 30 min. Add extraction solution to the volume. Filter with a membrane filter, and take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 2 μL of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Identify each aromatic amine based on the retention time and the relative ion intensities (note1) by multiple reaction monitoring. Calculate the amount of each aromatic amine in the sample by the following formula:

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

where,

C: the concentration of each aromatic amine 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 (note2):

Column: ZORBAX Eclipse XDB-C18, 3.5 μm, 2.1 mm i.d. × 5 cm.

Column temperature: 30℃.

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

Time (min)	A (%)	B (%)
0 → 4	98 → 80	2 → 20
4 → 8	80 → 70	20 → 30
8 → 10	70 → 3	30 → 97

10 → 12	3 → 3	97 → 97
12 → 12.5	3 → 98	97 → 2
12.5 → 15	98 → 98	2 → 2

Flow rate: 0.5 mL/min.

Injection volume: 2 μL.

Capillary voltage: 2.50 KV.

Ion source temperature: 150℃.

Desolvation temperature: 500℃.

Cone gas flow rate: 30 L/hr.

Desolvation flow rate: 800 L/hr.

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

Note: 1. Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤ 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) of aromatic amines are listed in the 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.

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Reference chromatogram

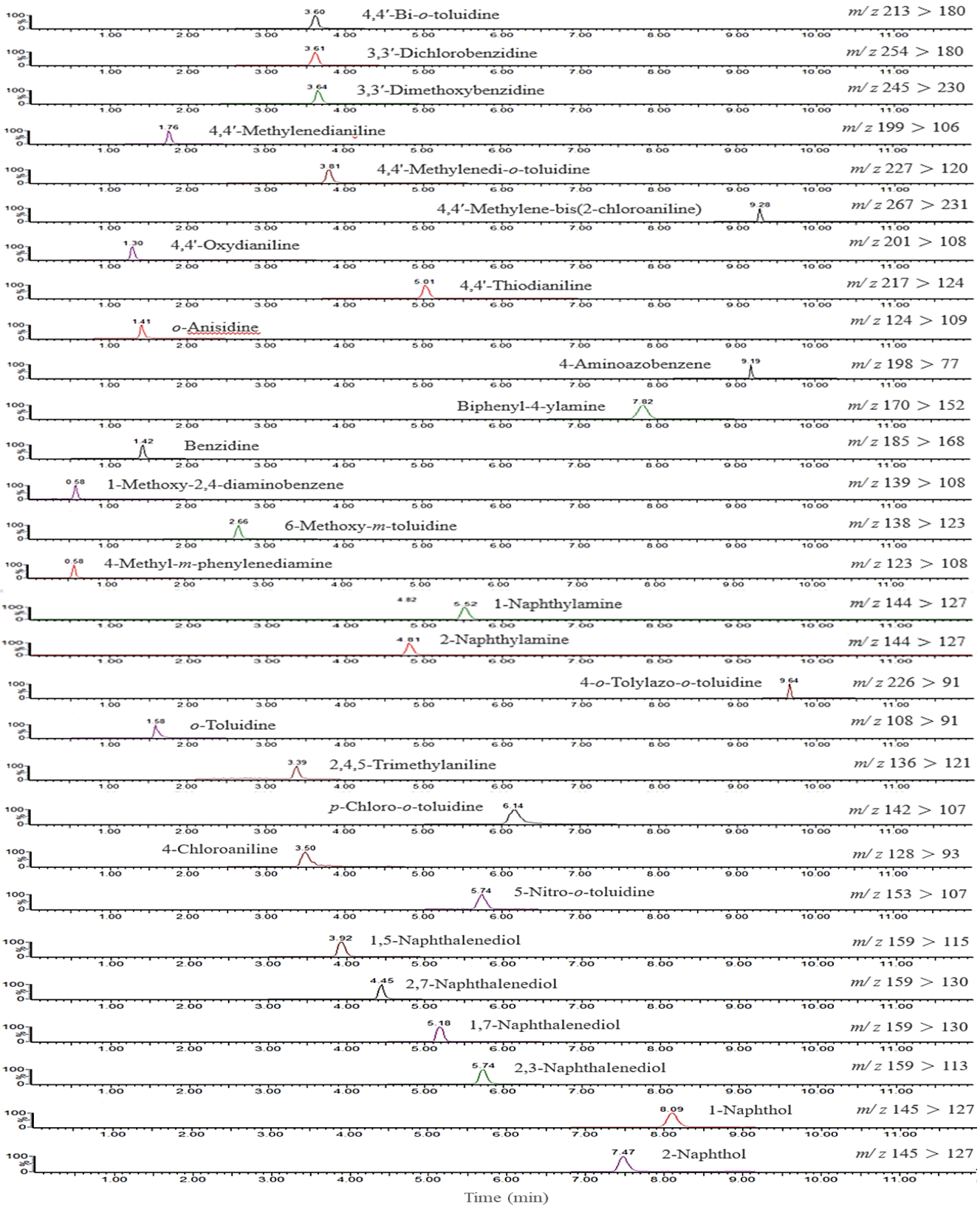

Figure. MRM chromatograms of 29 aromatic amines analyzed by LC-MS/MS

Table 1. Concentration ranges of 29 aromatic amine standard solutions

Item	Analyte	Concentration range (μg/mL)
1	4,4'-Bi-*o*-toluidine	0.001-0.02
2	3,3'-Dichlorobenzidine	
3	3,3'-Dimethoxybenzidine	
4	4,4'-Methylenedianiline	
5	4,4'-Methylenedi-*o*-toluidine	
6	4,4'-Methylene-bis(2-chloroaniline)	
7	4,4'-Oxydianiline	
8	4,4'-Thiodianiline	
9	*o*-Anisidine	0.01-0.2
10	4-Aminoazobenzene	
11	Biphenyl-4-ylamine	
12	Benzidine	
13	1-Methoxy-2,4-diaminobenzene	
14	6-Methoxy-*m*-toluidine	
15	4-Methyl-*m*-phenylenediamine	
16	1-Naphthylamine	
17	2-Naphthylamine	
18	4-*o*-Tolylazo-*o*-toluidine	
19	*o*-Toluidine	
20	2,4,5-Trimethylaniline	
21	*p*-Chloro-*o*-toluidine	0.1-2
22	4-Chloroaniline	
23	5-Nitro-*o*-toluidine	1-10
24	1,5-Naphthalenediol	
25	2,7-Naphthalenediol	
26	1,7-Naphthalenediol	
27	2,3-Naphthalenediol	
28	1-Naphthol	5-100
29	2-Naphthol	

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Table 2. MRM parameters and LOQs of 29 aromatic amines

Item	Analyte	Ionization mode	Ion pair Precursor ion (*m/z*) > Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Limits of quantitation (μg/g)
1	4,4'-Bi-*o*-toluidine	ESI^+	213 > 180* 213 > 152	30	30 52	0.02
2	3,3'-Dichlorobenzidine	ESI^+	254 > 180* 254 > 198	2	42 24	0.02
3	3,3'-Dimethoxybenzidine	ESI^+	245 > 230* 245 > 187	18	14 28	0.02
4	4,4'-Methylenedianiline	ESI^+	199 > 106* 199 > 77	36	22 52	0.02
5	4,4'-Methylenedi-*o*-toluidine	ESI^+	227 > 120* 227 > 178	26	24 22	0.02
6	4,4'-Methylene-bis-(2-chloroaniline)	ESI^+	267 > 231* 267 > 140	26	20 26	0.02
7	4,4'-Oxydianiline	ESI^+	201 > 108* 201 > 80	32	22 30	0.02
8	4,4'-Thiodianiline	ESI^+	217 > 124* 217 > 139	20	32 18	0.02
9	*o*-Anisidine	ESI^+	124 > 109* 124 > 65	30	14 22	0.2
10	4-Aminoazobenzene	ESI^+	198 > 77* 198 > 105	26	20 14	0.2
11	Biphenyl-4-ylamine	ESI^+	170 > 152* 170 > 127	30	26 32	0.2
12	Benzidine	ESI^+	185 > 168* 185 > 151	54	18 26	0.2
13	1-Methoxy-2,4-diaminobenzene	ESI^+	139 > 108* 139 > 124	14	12 8	0.2
14	6-Methoxy-*m*-toluidine	ESI^+	138 > 123* 138 > 106	32	14 22	0.2
15	4-Methyl-*m*-phenylenediamine	ESI^+	123 > 108* 123 > 79	30	16 20	0.2

*Quantitative ion pair

Table 2. MRM parameters and LOQs of 29 aromatic amines (Continued)

Item	Analyte	Ionization mode	Ion pair Precursor ion (*m/z*) > Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Limits of quantitation (μg/g)
16	1-Naphthylamine	ESI^+	144 > 127* 144 > 77	24	22 34	0.2
17	2-Naphthylamine	ESI^+	144 > 127* 144 > 77	24	22 34	0.2
18	4-*o*-Tolylazo-*o*-toluidine	ESI^+	226 > 91* 226 > 107	16	16 12	0.2
19	*o*-Toluidine	ESI^+	108 > 91* 108 > 65	16	16 24	0.2
20	2,4,5-Trimethylaniline	ESI^+	136 > 121* 136 > 91	30	14 22	0.2
21	*p*-Chloro-*o*-toluidine	ESI^+	142 > 107* 142 > 125	30	8 26	2
22	4-Chloroaniline	ESI^+	128 > 93* 128 > 111	32	22 18	2
23	5-Nitro-*o*-toluidine	ESI^+	153 > 107* 153 > 90	28	14 18	20
24	1,5-Naphthalenediol	ESI^-	159 > 115* 159 > 131	30	30 25	20
25	2,7-Naphthalenediol	ESI^-	159 > 130* 159 > 158	30	15 10	20
26	1,7-Naphthalenediol	ESI^-	159 > 130* 159 > 103	30	18 22	20
27	2,3-Naphthalenediol	ESI^-	159 > 113* 159 > 130	18	32 18	20
28	1-Naphthol	ESI^+	145 > 127* 145 > 91	50	12 25	100
29	2-Naphthol	ESI^+	145 > 127* 145 > 115	50	12 16	100