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**Determination of 20 kinds of prohibited
and restricted dyestuff in hair dyes - High
performance liquid chromatography**

染发类化妆品中 20 种禁限用染料成分的测定
高效液相色谱法

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Determination of 20 kinds of prohibited and restricted dyestuff in hair dyes - High performance liquid chromatography

1 Scope

This standard specifies the high-performance liquid chromatography method for the determination of 20 kinds of prohibited and restricted dyestuff in hair dyes.

This standard applies to the determination of 20 kinds of prohibited and restricted dyestuff in hair dyes.

The detection limits and quantification limits for the dyestuff components of this standard are as given in Table A.1 of Appendix A.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) are applicable to this standard.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

3 Summary of method

After the specimen is extracted by solvent, it is separated by high-performance liquid chromatography, detected by diode array detector, quantified by external standard method.

4 Reagents and materials

Unless otherwise stated, the water used is the grade I water as specified in GB/T 6682.

4.1 Standard substances: The Chinese names, INCI names, CAS numbers, molecular formulas, structural formulas, relative molecular masses of the 20

kinds of prohibited and restricted dyestuff are as listed in Table B.1 of Appendix B.

4.2 Methanol: Chromatographically pure.

4.3 Ethanol: Analytically pure.

4.4 Potassium dihydrogen phosphate: Analytically pure.

4.5 Disodium hydrogen phosphate: Analytically pure.

4.6 Sodium bisulfite: Analytically pure.

4.7 Sodium hydrogen sulfite aqueous solution (1 g/L): Weigh 1.00 g (accurate to 0.01 g) of sodium bisulfite (4.6) into a volumetric flask. Add water to make its volume reach to 1000 mL.

4.8 Aqueous ethanol solution: [Ethanol + sodium hydrogen sulfite aqueous solution (4.7)] = 1 + 1 (volume ratio).

4.9 0.05 mol/L phosphate buffer solution: Accurately weigh 6.8045 g (accurate to 0.0001 g) of potassium dihydrogen phosphate (4.4) and 17.907 g (accurate to 0.0001 g) of disodium hydrogen phosphate (4.5) into the volumetric flask. Add water to make its volume reach to 1000 mL.

4.10 Ethanol + 0.05 mol/L phosphate buffer solution = 1 + 49 (volume ratio).

4.11 Ethanol + 0.05 mol/L phosphate buffer solution = 1 + 1 (volume ratio).

4.12 Ethanol + 0.05 mol/L phosphate buffer solution = 5 + 95 (volume ratio).

4.13 Standard stock solution A: Respectively, accurately weigh 50 mg of each standard substance (1,4-diaminobenzene, 1,2-diaminobenzene, 1,3-diaminobenzene, 4-aminophenol, 3-aminophenol, 1,3-dihydroxylbenzene, 4-amino-2-hydroxytoluene, 2-methyl-5-N-hydroxyethylaminophenol, 2-methyl-5-amino-6-chlorophenol, 6-hydroxyindole, 4-chlororesorcinol, hydroxybenzomorpholine, o-aminophenol, 6-amino-m-cresol, 1-naphthol, 1,5-naphthalenediol, 2,7-dihydroxynaphthalene, a total of 17 kinds) (accurate to 0.1 mg) in a brown volumetric flask. Use the ethanol aqueous solution (4.8) to dissolve it to make its volume reach to 10 mL. Prepare it into a standard stock solution which has a concentration of 5 mg/mL. Preserve it at 4 °C ~ 6 °C in the dark. The preservation period is 3 days.

Standard stock solution B: Respectively, accurately weigh 50 mg of each standard substance (2-methyl-1,4-benzenediamine sulfate, N,N-bis(2-hydroxyethyl)-p-phenylenediamine sulfate, a total of 2 kinds) (accurate to 0.1 mg) in a brown volumetric flask. Use the sodium bisulfite aqueous solution (4.7) to dissolve it to make its volume reach to 10 mL. Prepare it into a standard stock

ethanol + 0.05 mol/L phosphate buffer solution = 5 + 95 (volume ratio) (4.12) to dilute it to make its volume reach to 5 mL. Vortex it for 1 min. Use the 0.45 μ m filter membrane to filter the supernatant, to obtain the determination solution. Use the high-performance liquid chromatography for determination.

Method 2: Accurately weigh 1.0 g of sample (accurate to 0.001 g) in a 25 mL graduated plastic centrifuge tube with stopper. Add the mixed solution of ethanol + 0.05 mol/L phosphate buffer solution = 1 + 49 (volume ratio) (4.10) to make its volume reach to 25 mL. Vigorously shake it to disperse the sample. Vortex it for 1 min. Ultrasonic it for 15 min. Centrifuge it at 5000 r/min for 10 min. Use the 0.45 μ m filter membrane to filter the supernatant, to obtain the determination solution. Use the high-performance liquid chromatography for determination.

Note: Is it suitable for the separation of the following 13 dyestuffs: 1,4-diaminobenzene, 1,2-diaminobenzene, 1,3-diaminobenzene, 4-aminophenol, m-aminophenol, 1,3-dihydroxylbenzene, 4-amino-2-hydroxytoluene, hydroxybenzomorpholine, o-aminophenol, 6-amino-m-cresol, 2-methyl-1,4-benzenediamine sulfate, N,N-bis(2-hydroxyethyl)-p-phenylenediamine sulfate, 4-nitro-o-phenylenediamine.

Method 3: Accurately weigh 1.0 g of sample (accurate to 0.001 g) in a 25 mL graduated plastic centrifuge tube with stopper. Add the mixed solution of ethanol + 0.05 mol/L phosphate buffer solution = 1 + 1 (volume ratio) (4.11) to make its volume reach to 25 mL. Vigorous shake it to disperse the sample. Vortex it for 1 min. Ultrasonic it for 15 min. Centrifuge it at 5000 r/min for 10 min. Use the 0.45 μ m filter membrane to filter the supernatant, to obtain the determination solution. Use the high-performance liquid chromatography for determination.

Note: It is suitable for the separation of the following 7 dyestuffs: 2-methyl-5-N-hydroxyethylaminophenol, 2-methyl-5-amino-6-chlorophenol, 6-hydroxyindole, 4-chlororesorcinol, 1-naphthol, 1,5-naphthalenediol, 2,7-dihydroxynaphthalene.

7 Analytical procedures

7.1 Reference conditions for liquid chromatography

The reference conditions for liquid chromatography are as follows:

- a) Column: Poroshell HPH C18, 2.7 μ m, 3.0 mm $\times$ 100 mm (or equivalent);
- b) Column's temperature: 30 $^{\circ}$ C;
- c) Detector: Diode array detector;

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