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Method of Test for Preservatives in Cosmetics (5)

1. Scope

This method is applicable to the determination of benzyl alcohol, phenol, phenoxyethanol, phenoxyisopropanol, chlorphenesin, dichlorobenzyl alcohol, *p*-chloro-*m*-cresol, *o*-phenylphenol, *o*-cymen-5-ol, chloroxylenol, chlorophene, triclosan, triclocarban, and bromochlorophene in cosmetics.

2. Method

After extraction, preservatives are determined by high performance liquid chromatography (HPLC).

2.1. Equipment

2.1.1. High performance liquid chromatograph.

2.1.1.1. Detector: photodiode array detector.

2.1.1.2. Column: ACQUITY UPLC® BEH Shield RP18, 1.7 μm, 3 mm i.d. × 50 mm, or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Isopropanol, HPLC grade;
Formic acid, HPLC grade;
Deionized water, resistivity ≧ 18 MΩ·cm (at 25°C);
Benzyl alcohol, phenol, phenoxyethanol, phenoxyisopropanol, chlorphenesin, dichlorobenzyl alcohol, *p*-chloro-*m*-cresol, *o*-phenylphenol, *o*-cymen-5-ol, chloroxylenol, chlorophene, triclosan, triclocarban, and bromochlorophene, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL, 20 mL.

2.3.2. Membrane filter: 0.22 μm, Nylon.

2.4. Mobile phase

2.4.1. Solvent A:

Dilute 1 mL of formic acid with deionized water to 1000 mL, then filter with a membrane filter.

2.4.2. Solvent B: Isopropanol.

2.4.3. Solvent C: Acetonitrile.

2.5. Standard solution preparation

Transfer about 10 mg of reference standards (including benzyl alcohol, etc.) accurately weighed into each 10-mL volumetric flask, dissolve and dilute with methanol to the volume as the standard stock solutions. Store in a refrigerator. When to use, mix appropriate volume of each standard stock solution, and dilute with methanol to concentrations as 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 10 mL of methanol, and ultrasonicate for 30 mins. Dilute to volume with methanol, and filter with a membrane filter. 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 HPLC separately, and operate according to the following conditions. Identify each preservative based on the retention time and the UV absorption spectrum. Calculate the amount of each preservative in the sample by the following formula (%):

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

where,

C: the concentration of each preservative 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)

HPLC operating conditions:

Photodiode array detector:

Analyte	Quantitative wavelength (nm)
benzyl alcohol, phenoxyethanol	260
phenol, phenoxyisopropanol, chlorphenesin, dichlorobenzyl alcohol, *p*-chloro-*m*-cresol, *o*-phenylphenol, *o*-cymen-5-ol, chloroxylenol, chlorophene, triclosan, triclocarban, bromochlorophene	280

Column: ACQUITY UPLC® BEH Shield RP18, 1.7 μm, 3 mm i.d. × 50 mm.

Column oven temperature: 50℃.

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

Time (min)	A (%)	B (%)	C (%)
0 → 0.5	93 → 93	3 → 0	4 → 7
0.5 → 2.0	93 → 93	0 → 0	7 → 7
2.0 → 16.5	93 → 76	0 → 19	7 → 5
16.5 → 25.5	76 → 40	19 → 20	5 → 40
25.5 → 27.0	40 → 10	20 → 20	40 → 70
27.0 → 28.0	10 → 10	20 → 20	70 → 70
28.0 → 29.0	10 → 93	20 → 3	70 → 4
29.0 → 30.0	93 → 93	3 → 3	4 → 4

Flow rate: 1.2 mL/min.

Injection volume: 3 μL.

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

Remark

1. Limits of quantification (LOQs) are 0.02% for benzyl alcohol, 0.008% for phenoxyisopropanol, 0.01% for dichlorobenzyl alcohol and phenoxyethanol, and 0.002% for phenol, chlorphenesin, *p*-chloro-*m*-cresol, *o*-phenylphenol, *o*-cymen-5-ol, chloroxylenol, chlorophene, triclosan, triclocarban and bromochlorophene.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. Cha, N. R., Lee, J. K., Jeong, H. J., Cho, J. C., Kim, M. J. and Lee, S.Y. 2012. Determination of 19 preservatives in various matrices by high-performance liquid chromatography. Anal. Lett. 45: 2148-2160.
2. Hauri, U., Lütolf, B., Wagmann, M. and Hohl, C. 2002. Determination of preservatives in finger paints with HPLC. Mitt. Lebensm. Hyg. 93: 447-458.
3. Lecce, R., Regazzoni, L., Mustazza, C., Incarnato, G., Porrà, R. and Panusa, A. 2016. Screening of preservatives by HPLC-PDA-ESI/MS: a focus on both allowed and recently forbidden compounds in the new EU cosmetics regulation. J. Pharm. Biomed. Anal. 125: 260-269.

4. Long, W. J., Wu, H. L., Wang, T., Dong, M. Y. and Yu, R. Q. 2021. Interference-free analysis of multi-class preservatives in cosmetic products using alternating trilinear decomposition modeling of liquid chromatography diode array detection data. Microchem. J. 162: 105847.
5. Wu, T., Wang, C., Wang, X. and Ma, Q. 2008. Simultaneous determination of 21 preservatives in cosmetics by ultra performance liquid chromatography. Int. J. Cosmet. Sci. 30: 367-372.

Reference chromatogram

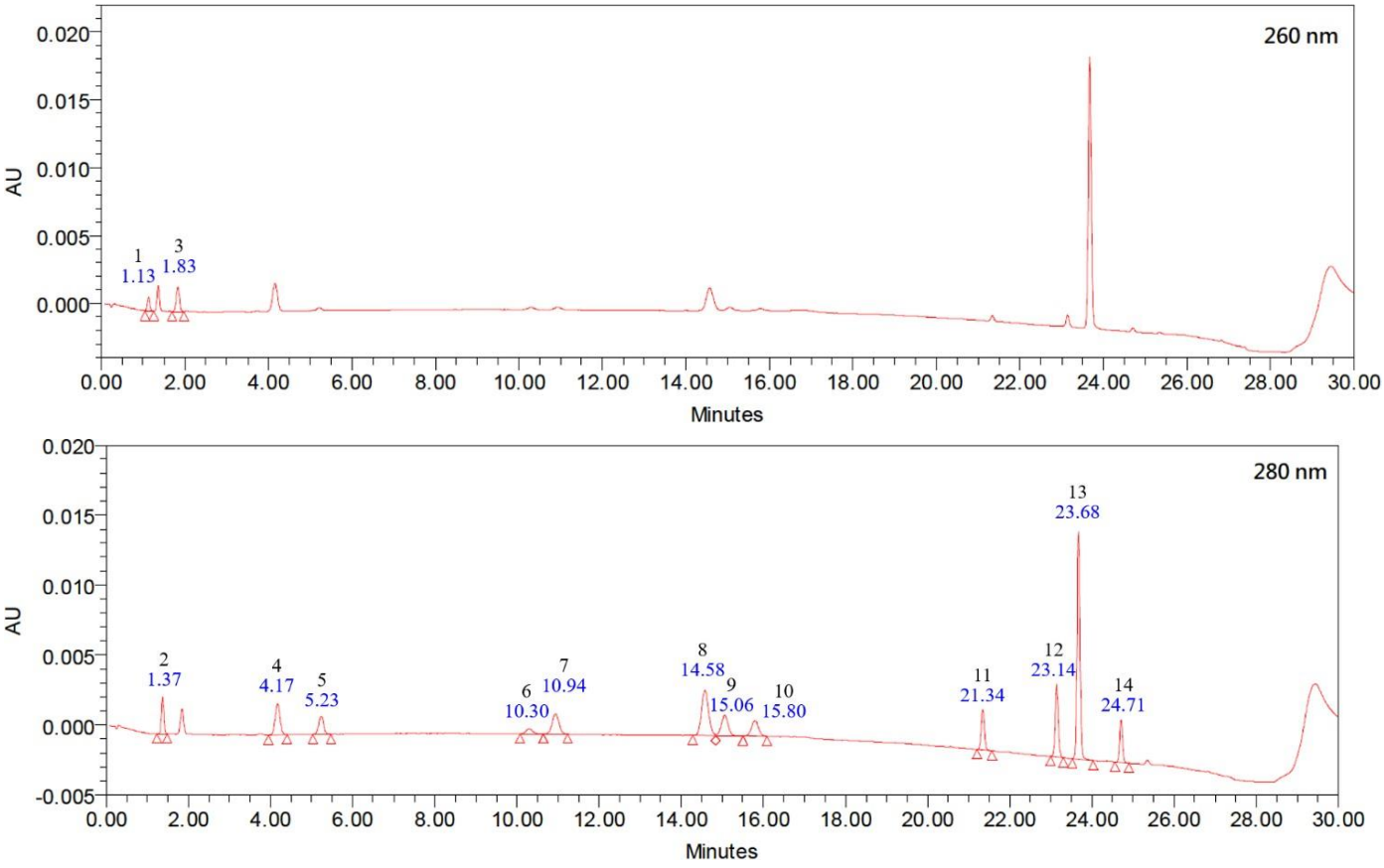

Figure. HPLC chromatograms of 14 preservatives reference standards.
1. benzyl alcohol; 2. phenol; 3. phenoxyethanol; 4. phenoxyisopropanol; 5. chlorphenesin; 6. dichlorobenzyl alcohol; 7. *p*-chloro-*m*-cresol; 8. *o*-phenylphenol; 9. *o*-cymen-5-ol; 10. chloroxylenol; 11. chlorophene; 12. triclosan; 13. triclocarban; 14. bromochlorophene.

Table 1. The concentration ranger of 14 preservative standard solutions.

Analyte	Concentration range (μg/mL)
benzyl alcohol	10 – 200
phenol	1 – 50
phenoxyethanol	5 – 100
phenoxyisopropanol	4 – 200
chlorphenesin	1 – 50
dichlorobenzyl alcohol	5 – 100
p-chloro-*m*-cresol	1 – 50
o-phenylphenol	1 – 50
o-cymen-5-ol	1 – 50
chloroxylenol	1 – 50
chlorophene	1 – 50
triclosan	1 – 50
triclocarban	1 – 50
bromochlorophene	1 – 50