Published, Agu 12, 2014
RA03P005.001

Method of Test for Preservatives in Cosmetics

1. Scope

This method is applicable to the determination of acid preservatives (benzoic acid, salicylic acid, *p*-hydroxybenzoic acid, sorbic acid and dehydroacetic acid) and esters of *p*-hydroxybenzoic acid (methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, isopropyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate, isobutyl *p*-hydroxybenzoate and secbutyl *p*-hydroxybenzoate) in cosmetics.

2. Method

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

2.1. Equipments

2.1.1. High performance liquid chromatograph.

2.1.1.1. Detector: photodiode array detector.

2.1.1.2. Column: ACE C18-AR, 5μm, 4.6 mm i.d. × 25 cm; or an equivalent product.

2.1.2. Ultrasonicator.

2.2. Chemicals

Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Sodium hydroxide, AR grade;
Monopotassium phosphate, AR grade;
Phosphoric acid (85%), AR grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
Benzoic acid, salicylic acid, *p*-hydroxybenzoic acid, sorbic acid, dehydroacetic acid, methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, isopropyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate, isobutyl *p*-hydroxybenzoate and secbutyl *p*-hydroxybenzoate, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 20 mL and 50 mL.

2.3.2. Membrane filter: 0.45 μm, PVDF.

2.4. Reagents

2.4.1. 50% Methanol

Mix deionized water and methanol at the ratio of 1:1 (v/v).

2.4.2. 0.1 N Sodium hydroxide

Dissolve and dilute 0.4 g of sodium hydroxide with deionized water to 100 mL.

2.5. Mobile phase

2.5.1. Solvent A: Dissolve and dilute 0.68 g of monopotassium phosphate with deionized water to 1000 mL, adjust pH with phosphoric acid to 2.5 and filter with a membrane filter.

2.5.2. Solvent B: Methanol.

2.5.3. Solvent C: Acetonitrile.

2.5.4. Solvent D: Mix solvent A, solvent B and solvent C at the ratio of 7:1:2 (v/v/v).

2.5.5. Solvent E: Mix solvent A and solvent B at the ratio of 4:6 (v/v).

2.6. Standard solution preparation

Transfer about 50 mg of acid preservatives (benzoic acid, salicylic acid, *p*-hydroxybenzoic acid, sorbic acid and dehydroacetic acid) reference standards accurately weighed into each 50-mL volumetric flask, dissolve with 5 mL of 0.1 N sodium hydroxide, and then dilute with deionized water to volume as the standard stock solutions. Transfer about 50 mg of esters of *p*-hydroxybenzoic acid (methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, isopropyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate, isobutyl *p*-hydroxybenzoate and secbutyl *p*-hydroxybenzoate) reference standards accurately weighed into each 50-mL volumetric flask, dissolve and dilute with methanol to volume as the standard stock solutions. When to use, mix appropriate amount of each standard stock solution, and dilute with 50% methanol to 0.25 ~ 50 μg/mL as the standard solutions.

2.7. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 20-mL volumetric flask, add 10 mL of 50% methanol, and ultrasonicate for 30 min. Dilute to volume with methanol, and filter with a membrane filter. Take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 20 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:

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

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 the sample (g)

HPLC operating conditions:

Column: ACE C18-AR, 5 μm, 4.6 mm i.d. × 25 cm.

Flow rate: 1.0 mL/min.

Chromatographic parameter 1:

Photodiode array detector: the quantitative wavelength for each preservative is as follows.

Analyte	Wavelength (nm)
Benzoic acid	230
Salicylic acid	235
p-Hydroxybenzoic acid/ esters of *p*-hydroxybenzoic acid	256
Sorbic acid	260
Dehydroacetic acid	306

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

Time (min)	A (%)	B (%)	C (%)
0 → 20	78 → 65	7 → 10	15 → 25
20 → 50	65 → 34	10 → 21	25 → 45
50 → 55	34 → 78	21 → 7	45 → 15
55 → 60	78 → 78	7 → 7	15 → 15

Chromatographic parameter 2:

Photodiode array detector: the quantitative wavelength for each preservative is as follows.

Analyte	Wavelength (nm)
Benzoic acid	230

Salicylic acid	235
p-Hydroxybenzoic acid	256
Sorbic acid	260
Dehydroacetic acid	306

Mobile phase: solvent D.

Chromatographic parameter 3:

Photodiode array detector: 256 nm for esters of *p*-hydroxybenzoic acid.

Mobile phase: solvent E.

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 0.002% for acid preservatives, and 0.0005% for esters of p-hydroxybenzoic acid.
2. Further validation should be performed when interference compounds appear in samples.