

## Identification and Assay for Methylisothiazolinone and Methylchloroisothiazolinone in Cosmetics

### 1. Scope

This method is applicable to the determination of methylisothiazolinone and methylchloroisothiazolinone in cosmetics.

### 2. Method

After extraction, analytes 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: Cosmosil C18-AR-II, 5  $\mu\text{m}$ , 4.6 mm i.d.  $\times$  25 cm, or an equivalent product.

2.1.2. Ultrasonicator.

#### 2.2. Chemicals

Methanol, HPLC grade;

Acetonitrile, HPLC grade;

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

Methylisothiazolinone and methylchloroisothiazolinone, reference standards.

#### 2.3. Apparatus

2.3.1. Volumetric flask: 10 mL and 50 mL.

2.3.2. Membrane filter: 0.45  $\mu\text{m}$ , Nylon.

#### 2.4. Standard solution preparation

Transfer about 50 mg of methylisothiazolinone and methylchloroisothiazolinone 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 volume of each standard stock solution, and dilute with methanol to 0.25 - 10  $\mu\text{g}/\text{mL}$  as the standard solutions.

#### 2.5. Sample solution preparation

Transfer about 1 g of the well-mixed sample accurately weighed into a 10-mL volumetric flask. Add 5 mL of methanol, and sonicate for 30 min. Add methanol to volume. Filter with a membrane filter, and take

the filtrate as the sample solution.

## 2.6. 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 analyte based on the retention time and the UV absorption spectrum. Calculate the amount of each analyte in the sample by the following formula:

The amount of methylisothiazolinone or methylchloroisothiazolinone

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

where,

C: the concentration of methylisothiazolinone or methylchloroisothiazolinone 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<sup>(note)</sup>:

Photodiode array detector: quantitative wavelength 280 nm.

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

Mobile phase: a gradient program of solvent A (deionized water) and solvent B (acetonitrile) is as follows.

Time (min)	Solvent A (%)	Solvent B (%)
0 → 7	92.5 → 92.5	7.5 → 7.5
7 → 14	92.5 → 2.5	7.5 → 97.5
14 → 16	2.5 → 92.5	97.5 → 7.5
16 → 20	92.5 → 92.5	7.5 → 7.5

Flow rate: 1.0 mL/min.

Injection volume: 20 µL

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

### Remark

1. Limits of quantitation (LOQs) are 0.00025% for methylisothiazolinone and methylchloroisothiazolinone.

2. Further validation should be performed when interference compounds appear in samples.

### Reference chromatogram

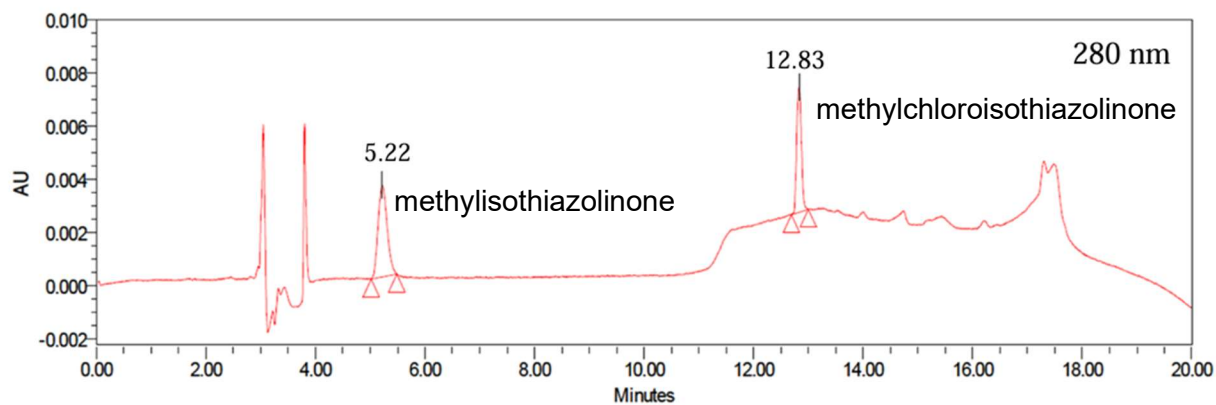


Figure. HPLC chromatogram of standards of methylisothiazolinone and methylchloroisothiazolinone.