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# Determination of Hexamidine, Chlorhexidine and Their Salts as Preservatives in Cosmetics - High Performance Liquid Chromatography

化妆品中防腐剂己脒定和氯己定及其盐类的测定

高效液相色谱法

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# Table of Contents

# Determination of Hexamidine, Chlorhexidine and Their Salts as Preservatives in Cosmetics - High Performance Liquid Chromatography

## 1 Scope

This Standard specifies the method of high performance liquid chromatography of determining hexamidine, chlorhexidine and their salts as preservatives in cosmetics.

This Standard is applicable to the determination of hexamidine, chlorhexidine and their salts as preservatives in aqueous, creamy, shampoo, powdered and lipstick cosmetics.

The detection limit and the limit of quantitation of this Standard: the detection limit of hexamidine, chlorhexidine and their salts: 0.002%; the limit of quantitation: 0.004%.

## 2 Normative References

The following documents are indispensable to the application of this Standard. In terms of references with a specified date, only versions with a specified date are applicable to this Standard. The latest version (including all the modifications) of references without a specified date is applicable to this Standard.

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

## 3 Principle

Sample shall receive solvent extraction, centrifugation and microporous membrane filtration, then, be determined through high performance liquid chromatography; it shall be quantified through external standard method and confirmed through liquid chromatography-mass spectrometry.

## 4 Reagents and Materials

Unless it is otherwise stipulated, all reagents shall be analytical purity. Water shall be Grade-1 water that is stipulated in GB/T 6682.

**4.1** Acetonitrile: chromatographic purity.

**4.2** Methanol: chromatographic purity.

**4.3** Tetrahydrofuran.

# 6 Steps of Determination

## 6.1 Sample Processing

### 6.1.1 Aqueous, creamy, shampoo and powdered sample

Weigh-take 0.5 g (accurate to 0.001 g) of sample, place it into a 50 mL PTFE centrifuge tube with a plug (5.7); add 1 mL of saturated sodium chloride solution (4.6), then, start vortex mixing. Add 9 mL of water; after vortex mixing, start centrifugation at 5,000 r/min for 10 min. Transfer the supernatant into another 50 mL PTFE centrifuge tube with a plug. In the sample residue, continue to add 10 mL of methanol (4.2); in ultrasonic water bath (5.3), start ultrasonic extraction for 20 min. Start centrifugation at 5,000 r/min for 10 min; combine the supernatant, then, filter it through 0.45 μm microporous membrane (5.8) for the determination with high performance liquid chromatography.

### 6.1.2 Creamy sample

Weigh-take 0.5 g (accurate to 0.001 g) of sample, place it into a 50 mL PTFE centrifuge tube with a plug (5.7); add 2 mL of tetrahydrofuran (4.3), start vortex mixing. Add 8 mL of water; after vortex mixing, start centrifugation at 5,000 r/min for 10 min. Transfer the supernatant into another 50 mL PTFE centrifuge tube with a plug. In the sample residue, continue to add 10 mL of methanol (4.2); in ultrasonic water bath (5.3), start ultrasonic extraction for 20 min. Start centrifugation at 5,000 r/min for 10 min; combine the supernatant, then, filter it through 0.45 μm microporous membrane (5.8) for the determination with high performance liquid chromatography.

## 6.2 Conditions of Determination

The determination with high performance liquid chromatography shall be based on the following reference conditions:

a) Chromatographic column: XTerra MS $C_{18}$, 5 μm, 250 mm x 4.6 mm (internal diameter), or others with equivalent performance;

b) Mobile phase: please refer to Table 1;

c) Flow rate: 1.0 mL/min;

d) Column temperature: 30 °C;

e) Detection wavelength: 260 nm;

f) Injection volume: 10 μL.

### 6.5 Blank Test

Follow the above determination conditions and steps, other than the step of weighing-taking sample.

## 7 Result Calculation

The result shall be calculated in accordance with Formula (1). The calculation result shall retain 2 decimals (the calculation result shall deduct the blank value):

$$W = \frac{c \times V}{m \times 10^4} \times 100\% \qquad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (1)$$

Where,

W---the percentage content of hexamidine, chlorhexidine and their salts in the sample, expressed in %;

c---the concentration of hexamidine and chlorhexidine in the sample, which is obtained through the standard working curve, expressed in (μg/mL);

V---the ultimate constant volume of the sample solution, expressed in (mL);

m---sample mass, expressed in (g).

## 8 Detection Limit and Quantitation Limit

The detection limit of hexamidine, chlorhexidine and their salts shall be 0.002%; the quantitation limit shall be 0.004%.

## 9 Recovery Rate and Precision

Within the concentration range of 0.004% ~ 0.3 %, the recovery rate of hexamidine, chlorhexidine and their salts shall be between 84.1% ~ 99.7%, the relative standard deviation shall be 1.1% ~ 7.8%.

## 10 Allowable Difference

The absolute difference of two independent determination results obtained under repeatability conditions shall not exceed 10% of arithmetic mean value.

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