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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

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**Protocol of the real-time PCR for detecting genetically modified
plants and their derived products
(English Translation)**

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1 Scope

This Standard specifies the liquid-chromatography determination for the methylisothiazolinone (MIT) and chloromethylisothiazolinone (CIT) in cosmetics. This

Standard is applicable to the determination of the methylisothiazolinone and chloromethylisothiazolinone in skin-cleaning cosmetics and skin-care cosmetics.

2 Principle

Use ultrasound to extract the methylisothiazolinone and chloromethylisothiazolinone in cosmetics with methanol; Perform centrifugal filtration; adopt the high-performance liquid chromatography to conduct separation and determination. Use the retention time for qualitiveness; use the external standard method for quantification.

3 Reagents and materials

Unless otherwise stated, the reagents used shall be the analytical reagent; the water shall be the secondary deionized water.

3.1

Methanol. chromatographically pure reagent.

3.2

Methylisothiazolinone (2-methyl-4-isothiazolone-3-ketone). the purity is $\geq 98\%$.

3.3 Chloromethylisothiazolinone (5-chlorine-2-methyl-4-isothiazolone-3-ketone). the purity

is $\geq 98\%$.

3.4 Standard-stock mixed solution of methylisothiazolinone and

chloromethylisothiazolinone. accurately weigh (3.2) 0.025g of methylisothiazolinone and (3.3) 0.1g of chloromethylisothiazolinone respectively. The accuracy is 0.0001 g. Place them in the 50 mL beaker; add appropriate amount of methanol to dissolve it. Transfer quantitative solution in to the 100mL volumetric flask; dilute it with methanol to the scale; mix it uniformly. In this solution, the concentration of methylisothiazolinone is 250mg/L; the concentration of chloromethylisothiazolinone is 1000mg/L. The stock solution shall be stored in the refrigerator for cold storage.

5.2.2 Drawing of the standard working curve

Accurately and respectively absorb the standard working solution (3.5) for 10 μ L; inject them into the liquid chromatograph in the order of the concentration from the dilute to the concentrated; perform the determination according to the chromatographic condition (5.2.1); use the peak area of the chromatographic peak as the ordinate AND use the corresponding concentration as the abscissa to draw the diagram; draw the standard working curve. The liquid chromatogram of the standard substance of methylisothiazolinone and chloromethylisothiazolinone is shown in Appendix A.

5.2.3 Sample Determination

Use micro syringes to accurately absorb 10 μ L of sample solution (5.1); inject into the liquid chromatograph; perform the determination according to the chromatographic condition (5.2.1); record the retention time and peak area of the chromatographic peak. From the peak area of the chromatographic peak, it can obtain the concentration of the corresponding tested substance from the standard curve. In the sample solution, both of the response value of methylisothiazolinone and chloromethylisothiazolinone shall be within the linear range of instrumental determination. For the samples in which the content of tested substance is high, it can take appropriate sample solution to perform determination after diluting with methanol.

5.3 Blank test

In addition to not-weigh the sample, it shall be performed according to the above procedure.

6 Results calculation

The results shall be calculated as formula (1).

Where.

W - the content of methylisothiazolinone or chloromethylisothiazolinone in sample, %;

c - the concentration of methylisothiazolinone or chloromethylisothiazolinone in the sample solution, which is obtained in the standard working curve. The unit is milligram per liter (mg/L);

V - final constant volume of the sample solution. The unit is liter (L);

m - the mass of the sample. The unit is gram (g);

The calculation results shall be accurate to 0.001.

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