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

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**Determination of functional component astaxanthin in
cosmetics-High performance liquid chromatography**

化妆品中功效组分虾青素的测定 高效液相色谱法

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1 Scope

This document describes the principle, reagents and materials, instruments and equipment, test procedures, test data processing, recovery, precision, and other aspects of the high performance liquid chromatography method for the determination of astaxanthin in cosmetics.

This document is applicable to the determination of astaxanthin in various cosmetics, including aqueous solutions, emulsions, creams, and facial masks.

The method described in this document has a limit of detection of 0.15 mg/kg and a limit of quantitation of 0.50 mg/kg.

2 Normative References

GB/T 6682

3 Terms and Definitions

This document contains no terms or definitions that require clarification.

4 Principle

After the analyte in the specimen undergoes saponification, converting the astaxanthin

esters into free-form astaxanthin, it is separated using high performance liquid chromatography and detected using a diode array detector. Qualitative analysis is performed based on retention times and chromatograms, while quantitative analysis is conducted using the internal standard method.

5 Reagents and Materials

5.1 Water. GB/T 6682, Grade 1.

5.2 Astaxanthin reference material. Purity. $\geq 95\%$; CAS No.. 472-61-7.

5.3 beta-Apo-8'-carotenal reference material. Purity. $\geq 95\%$; CAS No.. 1107-26-2.

5.4 Acetone. Guaranteed reagent grade.

5.5 Methanol. Chromatographic grade.

5.6 Dichloromethane. Guaranteed reagent grade.

5.7 Sodium hydroxide. Analytical reagent grade.

5.8 Butylated hydroxytoluene (BHT). Analytical reagent grade.

5.9 Phosphoric acid. Analytical reagent grade.

5.10 Methanol-dichloromethane solution. Measure 750 mL of methanol and 250 mL of

dichloromethane; add 0.2 g of BHT and mix thoroughly.

5.11 Sodium hydroxide-methanol solution (0.1 mol/L). Weigh 2.0 g of sodium hydroxide,

dissolve it in methanol, dilute to 500 mL, and mix thoroughly.

5.12 2% phosphoric acid-methanol solution. Measure 1 mL of phosphoric acid and dilute to 50 mL with methanol.

5.13 Astaxanthin standard stock solution. Accurately weigh approximately 10 mg of

astaxanthin reference material (weighed to the nearest 0.1 mg) into a 100 mL amber volumetric flask. Dissolve the material in acetone (5.4) and dilute to the mark to prepare an astaxanthin standard stock solution with a mass concentration of 100 $\mu\text{g/mL}$. Purge with nitrogen, seal tightly, and store in a refrigerator at -18°C , protected from light; the solution is valid for 1 month.

5.14 Internal standard stock solution. Accurately weigh approximately 10 mg of beta-apo-8'-

carotenal reference material (weighed to the nearest 0.1 mg) into a 100 mL amber volumetric flask. Dissolve the material in acetone (5.4) and dilute to the mark to prepare an internal standard stock solution with a mass concentration of 100 µg/mL. Purge with nitrogen, seal tightly, and store at -18°C, protected from light; the solution is valid for 1 month.

5.15 Microporous filter membrane. Nylon or polytetrafluoroethylene (PTFE) material, with a

pore size of 0.22 µm.

6 Instruments and Equipment

6.1 High performance liquid chromatograph. Equipped with a diode array detector.

6.2 Analytical balances. With sensitivities of 0.01 g and 0.1 mg, respectively. 6.3 Vortex mixer.

6.4 Refrigerated centrifuge. With a rotational speed of no less than 5 000 r/min and a temperature control accuracy of $\pm 1^{\circ}\text{C}$. 6.5 Precision pH meter.

6.6 Stoppered graduated centrifuge tube. 15 mL.

7.1.1 Prior to sampling, the specimen shall be mixed thoroughly. For aqueous specimens,

sample after shaking the specimen thoroughly; for emulsion and cream specimens, extrude and mix the specimen thoroughly before sampling; for non-woven fabric facial masks, squeeze out the liquid absorbed by the non-woven fabric, then mix thoroughly before sampling.

7.1.2 Accurately weigh 1 g (weighed to the nearest 0.001 g) of the specimen into a 15 mL stoppered graduated centrifuge tube. Add an appropriate volume of the internal standard stock solution (5.14) with a mass concentration of 100 µg/mL such that the final on-column mass concentration of the internal standard reaches 2.0 µg/mL. Mix thoroughly using a vortex mixer for 30 s. Add 2.0 mL of the methanol-dichloromethane solution (5.10), and vortex and oscillate for 30 s to ensure complete dissolution. Subsequently, add 0.7 mL of the sodium hydroxide- methanol solution (5.11) and vortex for 1 min. Add 1.0 mL of methanol-dichloromethane solution (5.10) to the centrifuge tube, mix thoroughly using a vortex mixer, purge with nitrogen and seal, then incubate at a constant temperature of 0°C to 4°C for 12 h under light-shielded conditions. Remove the centrifuge tube and allow it to return to room temperature. Adjust the pH to 7.0 ± 0.2 using a 2% phosphoric

acid-methanol solution (5.12), and dilute to a final volume of 5.0 mL using a methanol-dichloromethane solution (5.10). Centrifuge at 5 000 r/min at 4°C for 5 min. Collect the supernatant, filter it through a 0.22 µm microporous filter membrane, and use the resulting filtrate as the specimen solution for testing.

7.2 Reference Conditions for High Performance Liquid Chromatography

Given that analytical instruments and equipment of different models may exhibit certain variations, the following instrumental conditions have been validated as suitable for meeting the testing requirements specified in this document. The reference conditions for high performance liquid chromatography are as follows.

- a) Chromatographic column. C18 column, 150 mmx2.0 mm, 1.6 µm in particle size, or one of equivalent performance;
- b) Column temperature. 40°C;