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**Determination of 5 kinds of ammonium chloride
antibacterial agents in toothpaste - High performance
liquid chromatography method**

牙膏中 5 种氯铵类抗菌剂的检测方法 高效液相色谱法

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Determination of 5 kinds of ammonium chloride antibacterial agents in toothpaste - High performance liquid chromatography method

1 Scope

This Standard specifies high performance liquid chromatography (HPLC) method for the content of ammonium chloride antibacterial agents - lauryl ammonium chloride, benzethonium chloride, cetylpyridinium chloride, mitanum chloride and sitacium chloride - in toothpaste.

This Standard is applicable to the determination of the content of 5 kinds of ammonium chloride antibacterial agents in toothpaste.

The method detection limit of this Standard is: 0.075g/kg (lauryl ammonium chloride, benzethonium chloride), 0.0125g/kg (cetylpyridinium chloride, methachloride) and 0.025g/kg (sitacium chloride).

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682-2008, *Water for analytical laboratory use - Specification and test methods*

3 Principle

The sample is ultrasonically extracted and filtered by water and ethanol. Use high performance liquid chromatography to separate. Use UV to determine. Use external standard method to quantify.

4 Reagents and standard materials

Unless otherwise specified, it shall only use the confirmed analytically-pure reagents AND water specified as grade one water in GB/T 6682-2008.

6.3 Determination

6.3.1 Reference conditions of liquid chromatography

The reference conditions of liquid chromatography are as follows:

- a) Chromatographic column: CN column (150×4.6mm, 5μm).
- b) Mobile phase: Acetonitrile (A) + 0.1% HClO₄ aqueous solution (add 1mL of HClO₄ into 1L of water) (B). Use isocratic elution: A/B=40:60 (volume ratio).
- c) Flow rate: 1.0mL/min.
- d) Injection volume: 10μL.
- e) Column temperature: 30°C.
- f) Detection wavelength: 215nm (laura ammonium chloride, mitanium chloride and sitacium chloride), 225nm (benzethonium chloride), 260nm (cetylpyridinium chloride).

6.3.2 Drawing of standard working curve

Determine according to liquid chromatography conditions (6.3.1). The linear range of laura ammonium chloride and benzethonium chloride is 0.001 mg/mL~0.2 mg/mL. The linear range of cetylpyridinium chloride and mitanium chloride is 0.0015mg/mL~0.2mg/mL. The linear range of sitacium chloride is 0.003mg/mL~0.2mg/mL. For the liquid chromatograms of 5 mixed standard materials, refer to Figure A.1 ~ Figure A.3 in Annex A.

6.3.3 Quantitative analysis

Determine the test sample solution according to the liquid chromatographic conditions (6.3.1). Quantify by comparing the peak area with the standard series. If the response value of the specimen solution exceeds the response value of the standard series, it is necessary to dilute the specimen by an appropriate multiple before injection.

6.3.4 Qualitative confirmation

Based on the retention time of the tested component in the standard solution, perform preliminary characterization. During the determination, if the specimen solution has a positive result, it shall be compared with the UV absorption spectrum of the standard product, for qualitative confirmation.

6.4 Blank test

Except for not weighing samples, all follow the above steps.

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